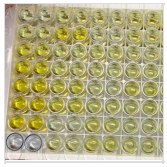

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Primate Hair Cortisol Processing I

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Matthew Lopez

DISCLAIMER

ABSTRACT

This is a protocol to quantify cortisol concentration from hair. Cortisol is a glucocorticoid hormone which is prominent in primates and can be used as a retrospective biomarker of hypothalamic-pituitary-adrenal (HPA) axis activity.

Our method is adapted from a protocol used in the Meyer Lab (Meyer et al. 2014).

Reference:

Meyer, J., Novak, M., Hamel, A., & Rosenberg, K. (2014). Extraction and analysis of cortisol from human and monkey hair. *Journal of visualized experiments : JoVE*, (83), e50882. https://doi.org/10.3791/50882

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KEYWORDS

Non-Human Primates, Hair, Cortisol, HPA activity

LICENSE

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GUIDELINES

Helpful tips:

Steps 26–37 are time sensitive so plan accordingly. Once begun, the timeline for these steps should not be altered.

When performing the cortisol assay for a new NHP species, parallelism tests of serially diluted extracts should be performed.

MATERIALS TEXT

- Hair clippers
- Masking tape
- Envelopes
- Analytical scale (Brand: Mettler Toledo)
- Isopropyl alcohol wipes
- Forceps
- Spatula
- Printer paper
- Scissors
- Ruler
- 15 mL or 50mL polypropylene tubes
- Motorized pipette controller
- 10 mL serological pipettes
- Adjustable volume pipettes
- 200 mL, 300mL pipette tips
- HPLC grade isopropanol
- Multi-Purpose Tube rotator (Brand: Fisher Scientific)
- Chemical waste container
- Funnel
- Ball mill and jars (Brand: Retsch, Model: Mixer Mill MM 400)
- 2 mL microcentrifuge tube (flat bottom)
- HPLC grade methanol
- Microcentrifuge (Brand: VWR, Model: High Speed Centrifuge)
- Labels
- PBS
- Parafilm
- -20°C freezer
- Nitrogen evaporator (Brand: Organomation MICROVAP, Model number: 11824-O)
- Salimetrics cortisol assay #1-3002
- Plate rocker (Brand: Fisher Scientific)
- Plate washer (Brand: BioTek, Model number: 50 TS)

SAFETY WARNINGS

Proper PPE (lab coat, gloves, eye protection) should be worn while implementing this protocol. This protocol involves chemicals (isopropanol and methanol); proper storage of these chemicals is required. Hazardous waste must be stored and disposed of according to university policy.

DISCLAIMER:

> DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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Preparation before obtaining shaved hair 1m

1 Hair clippers should be cleaned with alcohol before and after each use. 1m

Obtaining shaved hair 12m

2 Shave the hair from the upper back of the animal using electric clippers.

2.1 The hair should be shaved close without nicking the skin, as this can cause falsely elevated concentration of cortisol due to blood being present in the sample. It is essential that hair be collected from the same body region across all animals for a given study. 1m

3 The distal end of each sample is then wrapped in masking tape, leaving 1–1.5 cm of the proximal hair exposed. The sample can be placed in an envelope and stored at -20 °C (-4 °F) or room temperature out of direct sunlight until further processing. 10m

4 Envelopes should be labelled with species name, subject ID, sex, DOB (date of birth), DOC (date of collection), location of hair sample, and project affiliation code. 1m

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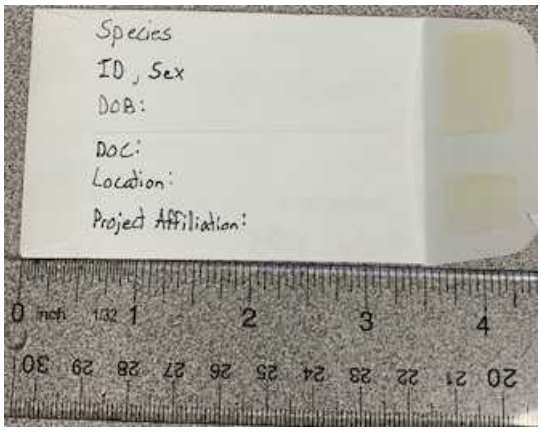

Preparation of weigh and cutting samples 3m 30s

5 Calibrate the analytical scale. 30s

6 Clean instruments (forceps, spatula) with isopropyl alcohol wipes before and after each use. 30s

7 Cut a 7cm x 7 cm square from printer paper for each hair sample. 1m

8 Create 4–5 labels for each sample with the information supplied in step 4. 1m 30s

Weighing and cutting samples 6m

9 Weigh the 7cm x 7cm paper on the analytical scale and record the weight. 30s

10 Tare the piece of paper before adding the hair.

11 Carefully remove the hair sample from the container while wearing laboratory gloves. Identify the shaved end of the sample. 10s

12 Using scissors and a ruler cut 1-1.5cm of hair from the exposed hair. Depending upon the rate of hair growth for the specific species and individual, this will reflect hair cortisol from the most recent 1–1.5 months. 3m

13 Transfer the hair on the square paper and weigh the cut sample. Record the weight. 30s

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14 Use forceps to grab the hair and store it in the polypropylene tube. 1m

14.1 Clean the forceps with isopropanol before using it again for the next hair sample. 30s

15 Place the label for that subject on the polypropylene tube. 10s

15.1 Repeat steps 9–15 for each sample.

Washing the hair 5d 0h 21m

16 Use the motorized pipette controller and 10 mL serological pipette to add 5 mL of HPLC grade isopropanol to each 15 mL polypropylene tube. 1m

17 3m

Invert the sample for 3 minutes on the rotator, using speed 12.

18 Use a glass funnel to decant the isopropanol into a waste container. 3m

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18.1 Carefully decant the isopropanol and use a spatula to catch any hair that maybe traveling down the tube as you decant.

19 Repeat steps 16–18 twice. 14m

20 Set the tubes on a rack in the fume hood with the lids off allowing the hair to completely dry. This will take 4 - 5 days. 5d

Preparation for grinding the hair 1m

21 Place the sample ID label (step 8) on a 2 mL microcentrifuge tube.

22 Weigh the microcentrifuge tube (with the label affixed) , then tare.

Grinding the hair 30m

23 Separate each washed and dried hair sample in half and place each half into a mill jar. Grind the hair to a fine powder using the ball mill, for 6 minutes at 25 Hz.

24 With the scapula scrape the sides of the jar to get all powdered hair free from the walls. Collect the hair and put it in the appropriate labeled microcentrifuge tube and weigh it.

24.1 The weight of powdered hair in the microcentrifuge tube needed for the species is indicated below:

- Capuchins, marmosets and tamarins, 50mg (0.05g)
- Chimpanzees, 40mg (0.04g)

It is essential to keep careful records of the subject ID and the weight of each sample.

25 Once the appropriate amount has been placed in the microcentrifuge tube, store any remaining powdered hair in another labelled 2 mL microcentrifuge tube and place it in the refrigerator or at room temperature.

Methanol Extraction 1d

26 Use the motorized pipette controller and a serological pipette to add 1 mL HPLC grade methanol to each microcentrifuge tube.

27 Place the tube on a multi-purpose tube rotator for 24 hours. Set the time for 24:00 (24 hours) and run at speed 12.

27.1 Plan accordingly to be available to extract the methanol at the conclusion of the 24 hours.

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28 At the end of the 24 hours the samples must immediately be centrifuged at 14,000 RPM for 3 minutes.

29 Collect 0.6 mL (600 microliters) of supernatant into a 1.5 mL microcentrifuge tube. Do not disturb the pellet of hair.

Drying down the methanol 1h 30m

30 Steps 30–37 will take approximately 45 minutes - 1 hour.

Using the MicroVap underneath the fume hood turn on the nitrogen tank.

31 Place each sample onto the bed noting where each sample is placed.

32 Position the nitrogen apparatus over the top of the samples so the tips will be in the center of each microcentrifuge tube.

33 Carefully lower until stop and lock into place.

34 Turn the unit on.

Make small turns on the nitrogen gas knob on the side of the panel of the hood, also adjust the gauge of the MicroVap to be sure it is open.

35 Slowly increase the amount of nitrogen being released using the knob on the hood until you see the gauge at approximately 10.

35.1
- Turning on the gas too fast will cause the methanol in each sample to splash out.
- Going slower is better in order to prevent any samples from splashing methanol.

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36 Once the nitrogen steam is at the desired intensity, wait for the evaporation to occur and keep monitoring the sample. Increase the flow of nitrogen as needed.

37 The evaporation takes about 45 to 1 hour depending on the intensity of the nitrogen steam.
After use clean the metal pins with alcohol.

38 Reconstitute each sample with PBS.

38.1 For capuchins, marmosets and tamarins, use 200 micrometers of PBS buffer.
For chimpanzees, use 100 micrometers of PBS buffer.

38.2 Cover the top of each microcentrifuge tube with parafilm and store at -20 C until ready to perform assay.

Preforming cortisol assay 5h

39 Allow samples to thaw before diluting for the assay.

39.1 Dilute the sample.

- Capuchins, marmosets, and tamarins, dilution is 1:40
- Chimpanzees, no dilution is necessary

40 Follow the procedure in the Salimetrics Manual for Salivary Cortisol.

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