Version 1

Dec 31, 2020

Hair cortisol analysis protocol V.1

Katy Parker[1], Matt Bristow[1]

[1]ARU Biomarker Laboratory, Anglia Ruskin University

2 Works for me dx.doi.org/10.17504/protocols.io.bqevmte6

katy.parker

ABSTRACT

There has been considerable interest in the measurement of cortisol in hair as a biomarker of stress since work by Davenport et al. (2006). The methodology developed by our laboratory is intended to allow for a scalable method for hair analysis that can be undertaken for large numbers of hair samples and has the minimum number of steps to reduce the chance of error and reduce costs. Our method reduces the number of steps where the sample is transferred to another container as it is kept in the same tube from initial weighing through to end of the methanol incubation. The procedure is also very scalable. The 'rate limiting' step for our hair analysis procedure is the grinding of the hair and this has been partially automated using the MP Biological Fast Prep grinder which is highly scalable. Final quantification of cortisol is performed via an ELISA assay, an assay which is rapid and cost effective.

References:

Davenport, M. D., Tiefenbacher, S., Lutz, C. K., Novak, M. a., & Meyer, J. S. (2006). Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology*, *147*(3), 255–261. https://doi.org/10.1016/j.ygcen.2006.01.005

DOI

dx.doi.org/10.17504/protocols.io.bqevmte6

PROTOCOL CITATION

Katy Parker, Matt Bristow 2020. Hair cortisol analysis protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqevmte6

KEYWORDS

Hair Cortisol Concentration, HCC, Cortisol

CREATED

Dec 04, 2020

LAST MODIFIED

Dec 31, 2020

PROTOCOL INTEGER ID

45237

GUIDELINES

Procedure for the hair analysis method: For each hair sample 25mg of the hair is placed in a 4.5ml polypropylene copolymer (PPCO) vial. The hair samples are then washed twice in 2ml of isopropanol to remove external contaminants, the isopropanol is removed from the vial and the hair allowed to dry in a clean air environment for 48 hours. Once fully dry, three ceramic balls are added to each tube (previous versions of our method used five ceramic balls but we have reduced this to three balls as we found no difference between the two methods) and the hair samples are ground to a powder using a Fast Prep-24 (MP Biomedicals, LLC).To extract cortisol, we add 2ml of methanol to each sample, then incubate the samples for 24 hours rotating constantly at room temperature. The hair, methanol and ceramic balls are decanted into a polypropylene tube (Sarstedt AG & Co, Germany) and centrifuged at 1500RCF to separate the ceramic balls from the rest of the mixture. The tube is then centrifuged at 3000 RCF to separate the ground hair and methanol and 1.40ml of the clear methanol supernatant is decanted into a 2ml polypropylene cryovial. The methanol is then removed using a vacuum centrifuge (Scan Speed 40, ScanVac) and the tubes frozen at -80°C until required for the cortisol ELISA. Cortisol levels are determined using a commercially available competitive ELISA (Salimetrics, US). Samples are thawed and reconstituted with 0.15ml of Salimetrics cortisol assay diluent and the samples then assayed in accordance with the manufacturer's protocol.

The steps are broken down into the following sections

Timings are not provided as this depends on the number of samples being processed.

1. Hair cutting and weighing:
2. Hair washing
3. Sample grinding
4. Extraction into methanol
5. Transfer of clear supernatant
6. Sample dry down
7. Reconstitution and assay for cortisol

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6

MATERIALS TEXT

General laboratory equipment e.g. centrifuges, calibrated air displacement pipettes, fume cupboard or similar to safely handle methanol and isopropanol, Microbiological Safety Cabinet or other source of clean air for drying hair samples, tweezers and hair dressing scissors for handling hair, analytical balance, ultra pure water for the ELISA etc.

Reagents:

- Isopropanol (Propan-2-ol, >=99.8%, CAS:67-63-0)
- Methanol (Analytical reagent grade, CAS:67-56-1)

Consumables

- Ceramic balls (MP Biomedicals Lysing Matrix M cat number: 116959050)
- Extra long 1250µl tip (Sarstedt 70.1186.100) for removal of isopropanol
- 4.5ml sample tube (ThermoFisher Scientific 342800-0045). This tube is used for the washing, drying, grinding and methanol incubation stages of the assay.
- Salivette tube (Sarstedt 51.1534). This tube is used to extract the ceramic balls from the methanol and hair mixture
- 2ml tube (VWR 211-0093). This tube us used to contain the clear methanol supernatant that is then dried down in the vacuum centrifuge.

Equipment

- Fast Prep-24 (MP Biomedicals). This is used to grind up to 24 hair samples at time to a powder.
- ScanVac vacuum centrifuge to remove the methanol from the tubes & ScanVac -110ºC Coolsafe freezer dryer to safely condense methanol (rather than allowing it to pass back into the lab air).
- -80ºC freezer to store fully dried down extract prior to ELISA assay.

ELISA equipment : Cortisol ELISA kit (our laboratory uses Salimetrics ELISA kit #1-3002) and ELISA specific equipment, such as microplate shaker and microplate spectrophotometer with appropriate primary and secondary filters. (Our laboratory automates the ELISA assay using a Tecan 150 Evo liquid handler using Tecan Hydroflex plate washer, Tecan monitored incubator bays, Tecan Sunrise plate reader, 8 Teflon coated tips (washed with fast wash pump option) on the Liquid Handler arm (LiHa) and robotic plate manipulator (RoMa)).

SAFETY WARNINGS

The protocol involves methanol and isopropanol which need to be risk assessed using your institutional protocols. All work with isopropanol or methanol in our laboratory takes place in a fume cupboard to remove any dangerous fumes with the exception of the vacuum centrifuge which uses a -110ºC ScanVac coolsafe condenser to safely contain all of the methanol evaporated from the tubes.

Working with human hair, especially during the current coronavirus pandemic, needs to be to risk assessed.

DISCLAIMER:

ABSTRACT

There has been considerable interest in the measurement of cortisol in hair as a biomarker of stress since work by Davenport et al. (2006). The methodology developed by our laboratory is intended to allow for a scalable method for hair analysis that can be undertaken for large numbers of hair samples and has the minimum number of steps

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6

to reduce the chance of error and reduce costs. Our method reduces the number of steps where the sample is transferred to another container as it is kept in the same tube from initial weighing through to end of the methanol incubation. The procedure is also very scalable. The 'rate limiting' step for our hair analysis procedure is the grinding of the hair and this has been partially automated using the MP Biological Fast Prep grinder which is highly scalable. Final quantification of cortisol is performed via an ELISA assay, an assay which is rapid and cost effective.

References:

Davenport, M. D., Tiefenbacher, S., Lutz, C. K., Novak, M. a., & Meyer, J. S. (2006). Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *General and Comparative Endocrinology*, *147*(3), 255–261. https://doi.org/10.1016/j.ygcen.2006.01.005

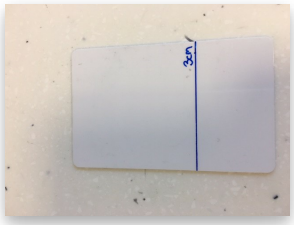
BEFORE STARTING

The timings for the hair assay have not been provided as they depend on the number of samples processed at the same time. *Note that at the hair washing stage the batch size depends on the speed of the laboratory technician - ensure samples are not left soaking in isopropanol for extended length of time, aim for no longer than 5 minutes - 10 minutes maximum.*

The results are expressed as the picograms of cortisol per milligram of hair.

Hair cutting/weighing

1 Create a sample length measure: Measure out required sample length on plastic card, marking length with permanent pen.



2 Ensure analytical balance is calibrated.

3 Place clean beaker on balance.

4 Scan sample id into spreadsheet.

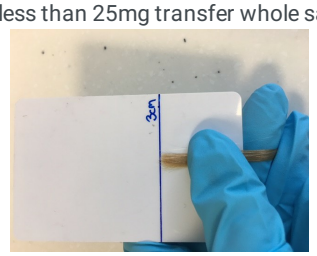
5 Place corresponding barcoded 4.5ml sample tube (ThermoFisher Scientific 342800-0045) in beaker and tare.

6 Hold scalp end of hair sample on line of sample length measure, pull hair taut and cut at very edge of measure using sharp scissors. Clean scissors with 70% IMS after use.

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6

7 Transfer hair sample to sample tube using clean tweezers.

8 Add/remove hair as necessary using tweezers to get as close as possible to 25mg. Record exact weight. For samples less than 25mg transfer whole sample and record weight. Cap sample tube.



9 Clean tweezers with 70% IMS and ensure balance is clean. Repeat from step 4 with remaining samples.

Hair sample washing

10 Add 2ml isopropanol (Propan-2-ol, >=99.8%, CAS:67-63-0) each tube in batch (batches of approximately 10 samples), replace cap.
(*Batch size depends on individual speed, ensure samples are not left soaking in isopropanol for extended length of time, aim for no longer than 5 minutes - 10 minutes maximum)*

11 Shake batch of samples on platform shaker for 3 minutes.

12 Remove isopropanol using pipette and long tips, this must be done carefully to ensure no hair is removed along with isopropanol.

13 Add another 2ml isopropanol to each tube in batch, replace cap.

14 Shake batch of samples on platform shaker for 3 minutes.

15 Remove isopropanol using pipette and 1250µl tip (Sarstedt 70.1186.100), this must be done carefully to ensure hair is not removed along with isopropanol.

16 Leave samples uncapped in fume cabinet at room temperature for a minimum of 48 hours to dry completely.

Sample grinding

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6

17 Add 3 x ceramic balls (MP Biomedicals 116959050) to each sample and replace cap securely.

(We had previously used 5 ceramic balls until testing established that 3 ceramic balls gave equivalent results)

18 Place samples into FastPrep-24 (MP Biomedicals), and grind 6 times at speed 6 m/s for 30 seconds each, allowing 5 minute cool down between grinds.

Extraction into methanol

19 Add 2ml methanol (Analytical reagent grade, CAS:67-56-1) to each sample tube. Replace caps tightly.

20 Rack samples and position horizontally on platform shaker.

21 Shake continuously for 24 hours at room temperature to extract cortisol.

Transfer of clear supernatant

22 After incubation, remove samples from platform shaker. Shake sample and tip contents of tube (including hair powder, methanol and ceramic balls) into the insert of corresponding pre-labelled Salivette tube (Sarstedt 51.1534).

23 Centrifuge Salivettes at 1500RCF for 5 minutes.

24 Dispose of Salivette inserts containing ceramic balls, replace caps.

25 Centrifuge Salivettes (now just containing methanol and hair powder) at 3000 RCF for 15 minutes.

26 Transfer 1.4ml clear methanol supernatant to corresponding pre-labelled 2ml tube (VWR 211-0093). Cap up samples as transferred.

Sample dry down

27 Uncap samples and load into Scanvac vacuum centrifuge.

28 Run at 1700RPM for 3 hours at 37° to completely dry down Methanol.

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6

29 Replace caps and store at -80°C until day of assay.

Reconstitution

30 On day of assay reconstitute samples by adding 150µl Salimetrics assay diluent. Replace caps.

31 Vortex samples for 10 seconds, then invert samples and vortex for a further 10 seconds.

32 Samples should be left to sit for a minimum of 20 minutes after being vortexed.

33 Soon before start of assay, again vortex samples for 10 seconds, then invert samples and vortex for a further 10 seconds.

34 Centrifuge samples at 1200RCF for 2 minutes.

35 The samples can then be assayed according to Salimetrics assay protocol.

Citation: Katy Parker, Matt Bristow (12/31/2020). Hair cortisol analysis protocol. https://dx.doi.org/10.17504/protocols.io.bqevmte6