Änderungen gegenüber der letzten Fassung:

1. Aim

Measurement of Corticosterone, Aldosterone, DHEAS, ACTH, Catecholamines

2. Materials

Litium heparin coated tubes (orange cap) Fa. Sarstedt

200047 41.1393.005	Plasma Lithium-Heparin	1,3 ml	10,8 mm/ 45 mm	orange	1.000 Stück / 100 Stück X 11550

100 µl Pipette
100 µl Tips
Labeled 1,5 ml Eppendorf tubes
Ice (cool pack)
Cool box (syropor box)
Cling wrap (Frischhaltefolie)
Insulin syringe
Empty clean cage
Glass slap

3. Procedure

Methods of urine collection:

- spontaneous urination
- terminal urine collection after killing

3.1.	spontaneous urination	The mouse is allowed to roam around on cling wrap layered around a glass slap, put into an empty clean cage. The mouse is removed as soon as it urinated and the voided urine is then aspirated with a pipette tip. Avoid contaminating urine with faeces.
3.2.	terminal urine collection after killing	Direct puncture of bladder after killing and open of the body

Methods of blood collection:

- Cardiac puncture
- Decapitation (for steroids only)

3.3.	Cardiac puncture	Anaesthesia or cervical dislocation required for all exsanguination sites/methods. Cardiac Puncture: Using a 23 g needle and 3 ml syringe, approach the heart either from under the sternum (as illustrated) or from the lateral left thoracic wall near the point of the flexed elbow. Once the needle is in the heart (preferably a ventricle), stabilize the hub and gently apply negative pressure to the plunger (the heart chamber will collapse if the negative pressure is too high). Do not repeatedly probe

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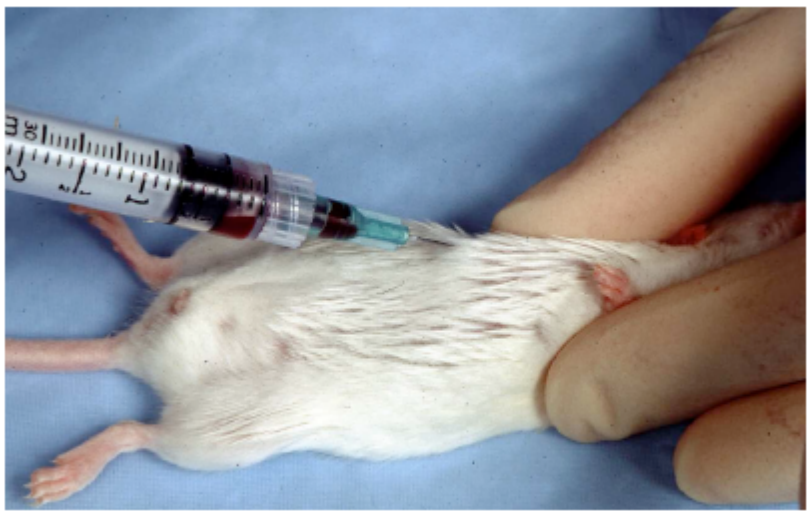
		with the needle. Be prepared to euthanize the mouse once the maximum blood volume has been collected. Cardiac punctures often produce samples that are haemolysed and/or clotted. These problems can be difficult to overcome, but with experience it is possible to obtain large volumes of blood relatively quickly.  Cardiac Puncture
3.4.	Decapitation	This method provides only momentary distress to the animal but is for steroids only.

4. After sample collection

- Store all samples at 4°C until freezing.
- Urine samples should be centrifuged to remove precipitates and only aliquots of the clear supernatant fraction should be used for analysis or frozen for storage. Divide the sample into two aliquots.
- Blood samples from the syringe should quickly transfered into the litium heparin coated tube and should be well mixed by inversion.
  It is important to centrifuge bloods within 2 hours of collection (but the sooner the better) at 5000 x g, for 10 minutes in a centrifuge at 8°C. If plasma/serum samples cannot be analysed fresh, they should be frozen as soon as possible at -20ºC for short term storage or -70ºC for long term storage.

5. Manifest (Excel table)

Number	DOB	gender	genotype	e.g. Cortisol level