

Sperm collection using cloacal massage on live birds (and sample preservation for microscopy)

Equipment:

- Capillary tubes (one per sample; for example, 10 μ l Vitrex end-to-end glass pipettes)
- 2 capillary holders with rubber bulb (to empty the capillary)
- Tubes (e.g. 2 ml Sarstedt vials with screw lids) prefilled with 500 μ l 12.5 % formalin (= 5 % formaldehyde). [Concentrated formalin = 40 % formaldehyde, so the working solution should be 1 part concentrated formalin mixed with 7 parts of water]
- A tube of stock PBS (phosphate-buffered saline)

Protocol:

1. Place a capillary tube in each of the two capillary holders (one for PBS, one for sperm).
2. Open a tube with formalin, put the lid down with the inside up, and use a capillary to add a drop of PBS (about 20-30 μ l) to the inside of the lid. Make sure there is no formalin inside the lid.
3. Take the bird and hold it so its underside is facing up and you can massage the cloacal protuberance (CP) with three fingers. For example, place the bird in the palm of the left hand (if you are right-handed) with the head/neck between the middle finger and the ring finger, and the legs between the index finger and the middle finger. Use the right hand middle finger to support the dorsal side under the cloaca.
4. Gently press the CP laterally at the base. For example, use the left thumb and index finger. Try to press down at the sides of the CP with support from the finger underneath, so the cloaca opens up.
5. Apply a gentle pressure with the right hand thumb on the posterior side of the CP, massage with all three fingers and some semen should appear in the cloacal opening (it is usually milky white,



- but the colour varies among species, from brownish to white).
6. Collect the semen with a capillary.
 7. Suspend the semen in the previously prepared drop of PBS in the inside of the lid of the tube with formalin.
 8. Mix the semen gently with the PBS, for example by filling and emptying the capillary a few times using the capillary bulb.
 9. Put the lid with the semen/PBS droplet carefully back on the tube and close it.
 10. Invert the tube gently so that the semen mixes completely with the formalin solution.
 11. Write sample information (sample number, species, ring number, [colour code], date, place) on the label. Also, put a mark on the tube lid, so that you see that the tube is used and avoid adding another sample to the same tube!
 12. Keep the sperm samples in a fridge or at ambient/room temperature. Avoid direct sun light and do NOT freeze!

Tips & Notes

- It is better if two persons can perform the procedure; one handling the bird, the other the capillary.
- It is important to dilute the sperm in PBS first. Otherwise, the sperm cells will glue together and it will be difficult to measure them individually.
- It may be helpful to modify the pressure and massage the CP in different directions.
- Feces or uric acid contamination is usually not a problem for the subsequent processing of the sample for microscopy, but the semen should ideally be as clean as possible. Uric acid has a compact white colour and does not dissolve as easily in PBS as does the semen.
- Practise the use of the capillary bulb before you start collecting samples. Do not block the hole in the bulb when sampling with the capillary; only block it with your finger when you want to push the sample out of the capillary.
- A video instruction is available at <https://youtu.be/f1KD3QnAegk>.

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Sperm samples can be sent by postal mail to:

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PO Box 1172 Blindern
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For shipment with courier, please contact us beforehand for details.