

## SOP#116: Artificial Cover Object Swabbing for eDNA Recovery

**Purpose:** Sample eDNA off an artificial cover object (ACO) for later extraction and PCR analysis.


### Materials Required for ACO Swab Kit Preparation:

Latex or Nitrile gloves (non-powdered)	10% Bleach (verify expiry date)
Cotton finger cot (one per ACO)	Distilled water (dH <sub>2</sub> O)
Ziplocking plastic baggies: ~200 mL (snack size): 1 per finger cot	Paper coin bags (5.5 x 9 cm): 1 per finger cot
Permanent black marker	Laminar flow hood

### ACO Swab Kit Preparation (in Laminar Flow Hood):

1. Put on gloves. Turn on laminar flow hood. Clean working surfaces with 10% bleach, then rinse with water and wipe with 70% ethanol.
2. Insert one finger cot into a coin bag each and put each coin bag into a separate ziplocking baggie. If the site IDs to be sampled are known, pre-label the coin bags with site ID, sampling date, and sampler initials with the permanent black marker.

### Materials Required for Fieldwork:

70% Isopropyl (rubbing) alcohol in a spray bottle	Latex or Nitrile gloves (non-powdered)
Permanent black marker for labelling	ACO swabbing kit (Prepared as above)
Tablespoon-sized scoop for silica beads	
<p>Orange colour-indicating Silica Beads (1 tbsp/coin envelope up to three coin envelopes with 3 tbsp silica beads in a resealable plastic baggie). These beads can be purchased in 1 quart (2 lb) containers on Amazon from Dry &amp; Dry 1 quart orange premium dessicant indicating silica gel beads (industry standard 2-4 mm) reusable (Product #CRH-16036).</p> <div style="display: flex; align-items: flex-start;">  <ul style="list-style-type: none"> <li>• Beads will <b>turn from Orange (Active) to Dark Green (Water Saturated)</b> when 50-60% absorbed with moisture.</li> <li>• Free of Cobalt Chloride (there are blue ones that turn pink when saturated – don't get those as they are toxic).</li> <li>• Beads can be reactivated by placing in the oven for 0.5-2 hours at 200-250°F or microwave for about 10 min at DEFROST. Don't use over 250°F in the oven. Depending on beads' condition, they may take shorter than the recommended time.</li> </ul> <p>Check the beads' color periodically.</p> </div>	

### Field Methods:

1. Put on gloves, then turn artificial cover object (ACO) over to expose smooth underside.
2. Retrieve finger cot from coin bag with gloved hands, put the cot on an index finger.

- Spray the finger cot with 70% Isopropyl alcohol until damp.

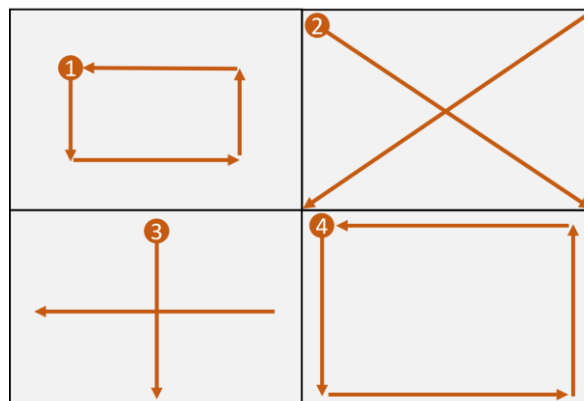


- Starting midway between the ACO's top left corner and its center, drag moistened finger cot in a small square while applying light pressure with fingers to maximize contact with the ACO. Depending on the heat of the ACO, it may be necessary to re-moisten the filter midway through the swabbing pattern.

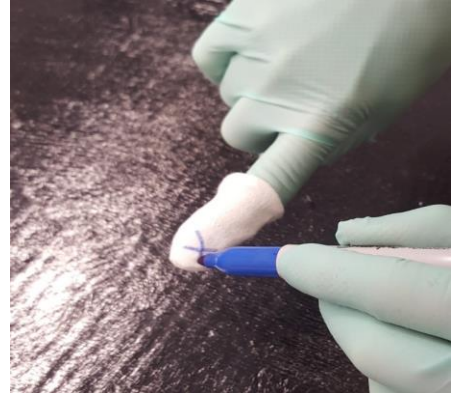


- Drag finger cot in an "X" connecting each corner followed by a "+" pattern.
- Finally, drag the finger cot along outer edges of ACO in the specific patterns indicated below.

**ACO Swabbing Pattern:**



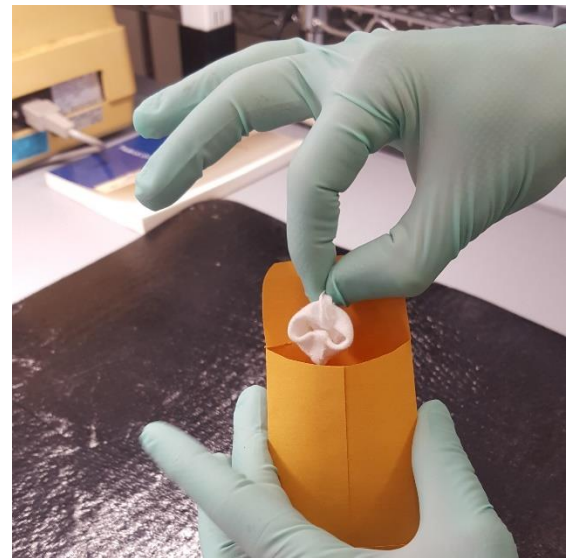
7. Mark the fingernail region with an “X” to identify the top of the finger cot.



8. Carefully slip off the finger cot so that the side which contacted the ACO is inside, taking care not to touch the inside.



9. Place the finger cot into its coin bag. If not pre-labelled, label the coin bag with the ACO site information, date, and sampler initials using a permanent black marker.



10. Put coin bag into its locking plastic baggie, add 1 tablespoon (~15 mL) of dry silica beads to the baggie and store baggies in a refrigerator or freezer as soon as possible. For storage longer than a few days, store in a freezer that is NOT frost-free.



### References:

1. Allison, M.J., Round, J.M., Bergman, L.C., Mirabzadeh, A., Allen, H., Weir, A., and Helbing, C.C. 2021. The effect of silica desiccation under different storage conditions on filter-immobilized environmental DNA. *BMC Research Notes*, 14: 106. DOI: 10.1186/s13104-021-05530-x.
2. Matthias, L., Allison, M.J., Maslovat, C.Y., Hobbs, J., and Helbing, C.C. 2021. Improving ecological surveys for the detection of cryptic, fossorial snakes using eDNA on and under artificial cover objects. *Ecological Indicators*, 131: 108187. DOI: 10.1016/j.ecolind.2021.108187

**Last Updated:** November 2021

**Update Author:** Caren Helbing