Filter Paper (FP) Blood Sampling from Caribou

– Brief Notes (full SOP available from Kutz Lab) –

FIELD COLLECTION:

1) Sever a large vein (femoral vein is good; jugular is excellent but be sure to avoid contamination from esophagus).

   - when skinning the neck to expose the jugular, create a 'trough' just below the level of the jugular that will catch the blood and form a pool in which to dip FPs

2) Avoid fingers/dirt contacting the white filter papers (Fops). Dip ***full length*** of all FP strips in clean pool of blood. Shake off excess, put strips back in envelope, put envelope back in Ziploc.

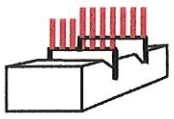
3) Freeze immediately and *keep frozen solid* until time of analysis
   OR dry and store dry (see protocol below) at room T.

****NOTE**: Samples must be dried before they are processed for diagnostic testing. Drying FPs before shipping helps ensure sample integrity en route (i.e., lighter/easier handling and no risk of thaw during transport) and after arrival at the lab.
Do NOT ship dried FPs in coolers with frozen or cooled samples.

DRYING PROTOCOL:

1) End of day in field (or immediately after defrosting frozen FPs):

   Tape racks down to a table/surface. Place FP sets upright in racks.
   - keep away from direct heat and sunlight
   - air dry overnight
   - allow envelopes to dry also



2) Next morning:
   Return FP sets to their dry envelopes.

3) Place 10-15 envelopes together in a clean large Ziploc and add approx. 10 desiccant packs. Squeeze out air and seal tightly.

You're ready to ship or store!

****NOTE**: Check desiccant weekly throughout the first month and refresh the pouches as needed based on indicator colour. Check/refresh every 3 months thereafter.

Filter paper elution

1. Blood collected on Filter Paper (FP) strips should be air dried.
2. Dried FP strips eluted as per previous protocol (Curry *et al.*, 2011). Eluates prepared from two strips of each animal's FP.
3. A stock solution of elution buffer made consisting of Dulbecco's phosphate-buffered saline with CaCl and MgCl (D-PBS 13, Gibco® Invitrogen™, Burlington, ON, Canada) and an antibiotic mixture (penicillin–streptomycin liquid, Invitrogen). The final penicillin and streptomycin concentrations in the stock solution are 100 U/ml and 100µg/ml, respectively.
4. For each eluate, clean (flamed and cooled) small scissors should be used to cut the absorbent portions of two FP strips into five or six pieces directly into a 1.5-ml microcentrifuge tube.
5. Eight hundred microliters (800µl) of stock solution of elution buffer were added and the tube was finger-flicked to ensure all fragments were in full contact with the fluid.
6. Tubes stored at 4 °C for 16 hrs, after which the 'eluate' (fluid) pipetted into a new, 1.5-ml microcentrifuge tube. Eluates spun briefly (15 sec) to draw all fluid to the tube bottom, and then stored at -20°C until testing. Each resultant two-strip eluate would be 400–440µl and estimated to be 1:10 serum concentration, according to the FP manufacturer's specifications.

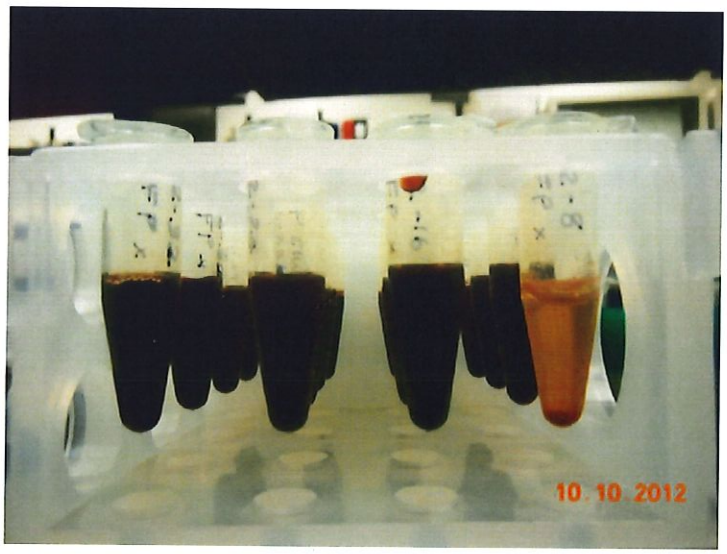


Image of normal eluates (generally dark red in colour).