Sampling tips for various types of ancillary necropsy testing (CSG Veterinary Workshop, 2016)

Test	General comments	Specific sampling notes
Histology	-Primary routine ancillary testing -Submit a standard broad range of tissues, even if they appear grossly normal -Also sample any lesions and describe them to the pathologist -Sample any margins of lesions with normal tissue	-Preserve tissues in formalin (10% phosphate buffered), at 1:10 tissue:formalin ratio. -Tissues may be mixed in one jar -Tissues should generally not exceed 1 cm diameter -For small crocs, entire "pluck" of tissues in body cavity can be removed as one piece and placed in formalin -Store formalin-fixed samples at room temperature
Cytology	-Not routinely performed when necropsy tissues for histological evaluation are available -Organ impressions may be useful in conjunction with histology to provide cellular detail or detect organisms -In some cases, cytology performed by the clinician may provide sufficient diagnostic information	-Slides are fixed in methanol and usually stained with Wright's type rapid stain -Additional diagnostic potential can be obtained by use of Gram's stain for bacteria and periodic acid-Schiff stain for fungi -Smears are destroyed by formalin fumes (keep separate)
Bacterial and fungal culture	-Minimise contamination as it will influence culture results and/or confound interpretation -Some bacteria (eg. *Mycoplasma* spp.) require specialized storage media and culture conditions (contact lab) -Accurate anaerobic culture requires use of specialised swabs or immediate transport of sample to lab -For fungal culture, tissue samples are generally better than swabs -If you have sampled a heavily contaminated site (mouth, intestine), advise the lab of the bacterial species of interest -If *Salmonella* spp. is suspected, request specific culture	-Aseptically sample filtering organs as soon as the body cavity is opened (liver, kidney, lung and spleen) -Sterilise instruments with flame prior to sampling -Sample 1 cm or larger pieces of tissue into individual sterile vials. -Moisten small (<5 mm) pieces of tissue with normal saline -Use swabs in bacterial transport medium for exudates -Store refrigerated (a few days) or frozen
Molecular testing	-Polymerase chain reaction and/or genetic sequencing -Commonly used for detecting reptile viruses -May also be used for fastidious bacteria and fungi -Tests are specialised and may only be available at research labs	-Generally, fresh tissues or plain swabs can be used (contact lab) -Moisten small (<5 mm) pieces of tissue and swabs with normal saline -Store refrigerated (a few days) or frozen -Testing of formalin-fixed, paraffin embedded tissues may be possible but is not ideal

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Virus isolation	-Specialised testing, usually requiring reptilian (probably crocodile) cell lines -May take weeks-months -Isolates require further testing for identification -Generally pursued only by researchers looking for novel viruses	-Generally, fresh tissues or plain swabs can be used -Moisten small (<5 mm) pieces of tissue and swabs with normal saline (or specialized viral transport medium if available) -Store refrigerated (a few days) or frozen
Serology	-Testing for antibodies to a specific disease -Not commonly used due to poor or unreliable reptilian humoral response -Limited availability for only a few diseases (contact lab/researcher)	-Useful serum or plasma may be obtained from carcase blood after death (as long as there is minimal decomposition) -Store refrigerated (a few days) or frozen
Parasitology	-Identification of parasites may be by morphological or molecular means -Availability of testing may be poor (contact lab/parasitologist)	-Basic preservation of helminths and arthropods in 70% ethanol -Optimal preservation (best chance at speciation) see CSG notes on parasite preservation by M. Tellez). -Gross examination and fecal flotation for eggs are generally more sensitive for identification of helminth infections than histology -Histology is more useful for detecting protozoal infections -Make a blood or lung impression smear if haemoparasitism is suspected
Toxicology	-Specialised testing by referral labs -Lab needs to be advised which toxin(s) to test for -Interpretation of significance of results may be difficult	-Sample type required depends on toxin suspected -Common samples are liver, kidney and stomach content -1-5 g of tissue/material may be required -Store frozen
Haematology and clinical chemistry	-May be useful under some necropsy circumstances to clarify gross or histological findings (eg. hypocalcaemia, anaemia, hepatic necrosis)	-Only samples taken prior to euthanasia or from carcass within minutes of death can be used -Lithium heparin anticoagulated blood sample can usually be used for both haematology and clinical chemistry -Store whole blood under refrigeration -Store serum and plasma refrigerated (few days) or frozen