Sample Collection Techniques for Histology and PCR

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Why is histological examination needed?

- “A person not specially trained in veterinary pathology should not approach a necropsy with the idea that a diagnosis will be made during the post-mortem examination” (Terrell and Stacy 2007).
- The pathologist uses the data and samples collected to make the diagnoses.
- In most cases the cause of death or disease will not be determined until further analysis of tissues is done.
**Why is histological examination needed?**

- Many diseases are only seen at the microscopic level
- There are often multiple disease processes, revealed using routine and special stains
- Increases understanding of these species

**Taking samples**

- Sample all tissues – there is no such thing as having “too much” material
- This includes organs with a normal appearance
- The brain should always be collected, whole.
- Where the organ appears normal take a representative specimen
- Lesions – sample normal and abnormal; margins
- Active processes, causative agents often at margins
Tissue sampling

- Trachea – a complete ring
- Lung – sample both lungs in multiple areas, include large airways
- Glands – whole (incise to allow penetration of fixative).

Tissue sampling

- Stomach and intestine – avoid touching/handling the mucosa
- Stomach – near esophagus, body and pylorus.
Tissue sampling

- Intestine – tubular (un-opened). Proximal, middle, distal
- Liver – lobes, gall bladder
- Spleen – cross-section

Tissue sampling

- Kidneys – cranial and caudal poles, left and right, include cortex and medulla.
- Urinary bladder – avoid handing mucosa.
Labelling and submitting

- Put all relevant information on container label
- Provide a full post-mortem report with photographs – pathologists are trained to interpret them

Fixation

- 10% neutral buffered formalin
- Formaldehyde solution (40%)
- 1 part to 9 parts distilled water
- 4 grams monobasic sodium phosphate
- 6.5 grams dibasic sodium phosphate
- Colour indicator e.g. methylene blue (1-2 ml)

If at all possible, avoid use of formalin if DNA is to be extracted from samples later.
Fixation

- Fix for at least 24-48 hours
- Formalin can only penetrate 0.5 cm in 24h
- Use a sharp knife or scalpel and a cutting board
- 10x as much liquid as tissue in the container
- Very small specimens or from particular sites in plastic cassettes

Brain

- Always take it
- Fix whole
- Fix for at least a week, have the pathologist section it.
- Can fix in 40% “formaldehyde” just covering brain, then add 10% until buoyant. Brain sinks when fixed.
Fixation

- Fix eyes whole following removal of attached structures including extraocular muscles
- Larger eyes will require decalcification due to scleral ossicles

Long-term storage

- Various recommendations
- Long-term in formalin cross-links proteins and degrades DNA
- Long-term effects of alcohol not known
- Re-fill and re-bag/pot samples every few years
- Paraffin wax blocks require climate-controlled storage, may be discarded after a certain period
Sampling for PCR

- Polymerase chain reaction
- Rapid, inexpensive, simple method of producing large numbers of copies of DNA molecules from minute quantities of source material.
- Even when the source DNA is relatively low quality
- Pathogens, species identification for cooked meats, genetic analysis e.g. diversity in certain populations

It is best to store the sample properly from the outset
- 100% ethanol 10x the tissue volume
- Saturated salt/DMSO (20% DMSO, 0.25 M EDTA, pH 7.5, NaCl saturated).
- Salt preserves, DMSO helps salt penetrate. Long-term??

**Sampling for PCR**

- No more than 5 mm cubed
- Blood – allow drops to dry on absorbent paper (commercial product is Whatman FTA cards)
- Swabs of cheek cells are often used for humans (various commercial products) and can be air-dried

**Transportation of samples**

- 100% ethanol is classified as “dangerous”.
- Preserve for at least 3d in 100%, then transport in 24% ethanol
- Minimize time in 24%
- Ship in RNALater or DMSO salt-saturated solution, then transfer to 100% ethanol

Sampling for RT-PCR

- Reverse transcription PCR
- RNA reverse transcribed into complementary DNA
- Gene expression, pathogens (RNA viruses).
- RNA later

Sampling for RT-PCR

- RNA later rapidly penetrates tissues to stabilise and protect RNA
- Sample as for PCR
- Don't freeze first!
- Room temp 1d at 37°C, 1 week at 25°C
- Store in refrigerator overnight, then remove supernatant and move to -20 or -80°C for long-term storage
Any questions?