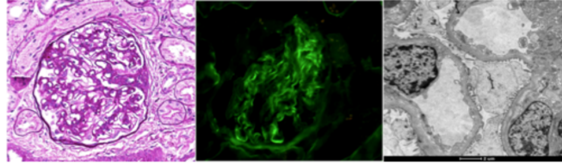
EUROPEAN VETERINARY RENAL PATHOLOGY SERVICE



Kidney Biopsy Instruction Form

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1. Contents of the kidney biopsy kit

Please check all contents upon receipt. If any contents are missing or damaged, please contact Dr. A.M van Dongen prior to obtaining specimens: email: A.M.vanDongen@uu.nl.

a. Tools
- Clean glass slides, 2 each (wrapped in tissue)
- Single-edged blades, 1 each
- Plastic transfer pipettes, 3 each

b. Fixatives
- Fixative for light microscopy (**LM**): 4% buffered formalin in glass vial with yellow label
- Fixative for electron microscopy (**EM**): Karnovsky in cryo vial with orange lid
- Fixative for immunofluorescence (**IF**): Michel's transport medium in plastic vial with white lid

Note: Please save all packing materials for return shipping.

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2. Tissue handling

Key aspects:
- **For storage of kits longer than 3-5 days prior to use, keep kits cool (not frozen).**
- Process biopsies as soon as possible (within 5 minutes) after collection/excision. Never allow the tissue to dry out. If there is any delay, keep the tissue moist using physiologic (normal, 0.9%) saline.
- During the process of partitioning and fixing the biopsies, do not risk cross-contaminating tissue samples with fixatives/transport medium. Replace the cap tightly and invert several times to be sure

the tissue floats freely in the liquid and is not stuck to the lid.
- Minimize handling of biopsies; do not use forceps (produces crush artefacts).

Steps: *

a. Remove the biopsy core from the needle:
- Use physiologic saline (in a 12 ml syringe fitted with a 25 G needle)
- GENTLY wash the tissue out of the needle onto a glass slide (i.e., held just under the needle)
- End up with the biopsy core in a small puddle of saline on the slide
- Use the saline syringe/needle to wash the needle (with vigour, if needed) before the next pass

Tip: To manipulate the tissue core on the slide, use the point of a 25 G needle and/or the corner of a single-edge razor blade to nudge it around in the saline puddle on the surface of the slide (like tug-boats moving a bigger ship around in a harbour).

b. Promptly assess tissue adequacy (that the cores are of cortex and contain glomeruli) and:
Obtain at least:
- 2 good cores that are ≥ 10 mm long or 3 good cores if they are < 10 mm long

Divide the total available material for EM, LM, and IF:
- Use a fresh, clean single-edge razor blade(s) to cut the core(s) transversely
- Separate the pieces for EM, LM, and IF.
Guidelines of how the tissues can be divided for LM, EM and IF are given in the publication '*Practice guidelines for the renal biopsy*' by Walker and co-authors and The Ad Hoc Committee on Renal Biopsy Guidelines of the Renal Pathology Society, Modern Pathology, 2004, 17, 1555–1563:

''In the absence of direct glomerular visualization (with a dissecting microscope), a standard protocol for dividing the tissue obtained at each 'pass' should be used to avoid inadequate glomerular sampling for LM, IF or EM (Figure). There are several acceptable approaches; … The standard approach is to first procure tissue for EM from each core by removing 1 mm cubes from the ends and placing them in formalin, cooled glutaraldehyde or other fixative suitable for EM. Some clinicians prefer that the pathology laboratory obtain tissue for EM from the ends of the formalin-fixed tissue. If the specimen is to be sent to a laboratory that uses IF, the first core can be cut in half by cross-sectioning and the larger piece placed in formalin or another fixative suitable for LM; the smaller portion is saved for immunofluorescence evaluation. If a second core is obtained, the ends should be taken for EM and the specimen again divided almost in half with the larger tissue core now kept for IF and the smaller for LM. Alternatively, if both cores contain cortex, one core can used for LM and one core for IF. Tissue from further passes should be divided to balance whatever was obtained initially''.

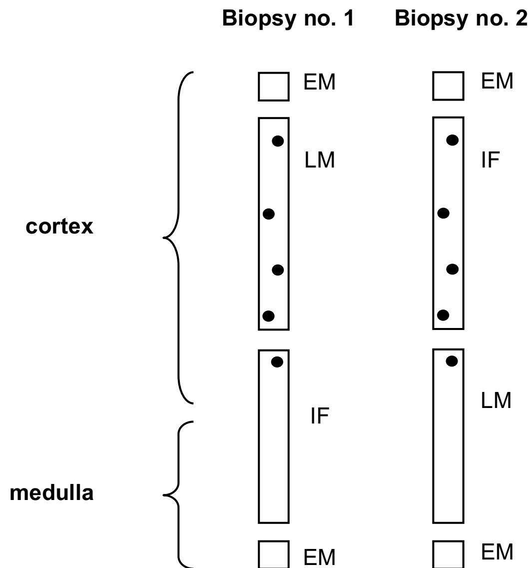

Diagram to illustrate division of renal biopsy cores in the absence of a dissecting microscope for clinicians using IF. The ends from all cores are taken for EM with the remainder divided for LM and IF (after Walker).

Tip: Presuming that each portion contains glomeruli, the ‘amount of specimen’ requirements are smallest for EM, medium for IF, and largest for LM; thus, divide the total amount of cortical tissue that is available accordingly.

c. Transfer the pieces into their respective fixative/preservative solutions
- Fix the EM piece(s) first (should be placed in the container within 5 minutes)
  - put it (them) in the 3% glutaraldehyde solution in small container with white lid
- Fix the LM piece(s) next
  - put it (them) in the 10% formalin solution in large container with yellow lid
- Fix the IF piece(s) last
  - put it (them) in the Michel’s transport medium in long vial with white lid

Tip: To transfer the pieces, we usually use a single-edge blade to ‘scoop’ up the piece, which typically clings (due to surface tension) to the ‘wet-with-saline’ edge of the blade, and we then use a pipette containing some fluid from the destination vial to wash the sample off the blade and into the bottle using a new blade and pipette for each tissue transfer task. The last piece(s) on each slide can be washed directly into the destination vial off a corner of the slide.

* protocol (with adaptations) from: Dr GE Lees, International Veterinary Renal Pathology Service

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3. Packing and Return Shipping Instructions

Key aspects:

- Use a commercial courier service to send the kit back to the laboratory.
- The cost of sending the kit back to the laboratory is the responsibility of the clinician/sender.

Please note:

- Please be sure the caps are tight on the bottles before placing them in the plastic bags.
- Please contact the service through the website (www.evrps.net) to confirm the expected arrival date of the specimen, name of courier and tracking number.
- Please note that the laboratory is closed Sundays and it is therefore best to send the kit back on Monday-Wednesday.

Submission of tissue to laboratory:
For return shipping, the final shipping box will contain 2 inner packagings.

a. Primary (innermost) package
- Place each of the 3 specimen containers/vial in its provided plastic bag with absorbent material in bottom of bag. Please ensure that all caps are tightly closed. Re-use provided absorbent material or replace with similar material if soiled.

b. Secondary package
- Place all 3 primary packages in second large ‘’safety bag bubble’’ (everything should be ‘’double-bagged’’)
- Place completed submission form or other relevant documents in the side compartment of the ‘’safety bag bubble’’ to keep them dry.

c. Outer package
- Put the secondary package (containing the specimens) in the cardboard mailbox with adequate cushioning material around it.
- Seal outer package with packing tape
- Affix the new address label over old address label.
- Affix the “UN 3373 Biological Substance Category B” label near address label