Supplementary Materials

Title: Recovering histological sections for ultrastructural diagnosis of glomerular diseases through the pop-off technique.

Sabrina Valente[1], Giorgia Comai[3], Deborah Malvi[4], Valeria Corradetti[3], Gaetano La Manna[3]*, Gianandrea Pasquinelli[1,2]*.

Affiliations: [1]Biotechnology and Methods in Laboratory Medicine, Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy; [2]Subcellular nephro-vascular diagnostic program, Pathology Unit, IRCCS, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [3]Nephrology, Dialysis and Kidney Transplantation Unit, IRCCS, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [4]Pathology Unit, IRCCS, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy.
*****Co-senior authorship.

Corresponding author: sabrina.valente2@unibo.it

Journal name: Journal of Nephrology

Part 1.

METHODS

The areas of interest were identified and marked at light microscopy (Fig. 1a). After area selection, histological paraffin sections stained with H&E or PAS or Masson's Trichrome as well as H&E frozen sections were dipped in xylene for 2-4 days to remove the coverslip. According to a previously described protocol [8], the glass slides were washed in fresh xylene followed by immersion in a 1:1 xylene - propylene oxide solution before putting them into pure propylene oxide; each passage was performed for 2 min at room temperature (rt). Then, the slides were sequentially immersed in a 2:1 mixture of propylene oxide - Araldite resin for 2 min at rt, followed by 1:1 propylene oxide - Araldite resin for 2 min at rt and by a 1:2 solution of propylene oxide - Araldite resin for 10 min at rt. A gelatin capsule was filled with pure Araldite resin and placed over the previously selected area (Fig. 1b). After resin polymerization at 60°C overnight, the gelatin capsule was removed using a hot plate for 15 seconds (Fig. 1c) obtaining a resin block with the stained section overlapped (Fig. 1d). The blocks were sectioned and ultrathin sections on grids (Fig.1e) were double-counterstained with uranyl acetate and lead citrate before ultrastructural examination.

Part 2.

RESULTS

The ultrastructural recovery of specific areas of renal tissues preliminary identified on histological slides was performed on 94 sections; the sections transferred from the glass slides (untreated and treated) to the resin block was successfully performed in 74.5%; a partial transfer of the area of interest previously identified or difficulties in the sectioning due to the presence of holes in the section were recorded in 11.7% of cases; the transfer failure was 13.8%. At low magnification, the ultrastructural composition of glomeruli was seen including the mesangium, the capillary basal membrane, endothelial cells, podocytes and their foot processes (Fig. 1f). GBM is seen at higher magnification in Fig. 1g. Additional recovery tests performed on H&E-stained frozen sections revealed significant cytological frozen damage to the renal tissue.

The influence of different microscope glass slides and tissue aging on the recovery of the sections

To assess the influence of different types of microscope glass slides on the section recovery, a quantitative analysis was performed in each group. After cutting, paraffin or frozen sections were recovered on untreated and treated microscope glass slides; the treated slides allow an increase in section adhesion that is useful to retain tissue when performing routine histological stains. When the sections have been collected on untreated glass, a complete and homogeneous transferring was achieved in 79.4% of the sections; a partial transfer has been found in 9.6% of the cases; a failure was recorded in 11% of the sections. The sections retrieved from treated slide glass showed that in 23.8% of sections the transfer failed, in 19% was partial and in 57.2% the transfer was successful. Comparing the average number of sections recovered from treated glass (2.6 ± 1.8) to those retrieved from untreated glass (2.6 ± 1.7), no statistical difference was detected. These results indicate that the transfer of stained section from the glass to the resin block is not affected by the characteristics of the glass slides used. Regarding the storage time, the pop-off was carried out on renal tissue sections processed in a wide period of time spanning from 2006 to 2018 without any evident influence on the recovery outcome.

DISCUSSION

An aspect worthy of consideration is the possible interference that the type of slide glass used for collecting the sections could have on the recovery procedure. Here, the impact of two most commonly used types of glass slides was

evaluated; untreated glass was used for H&E and PAS stains while treated glass for TRICMAS. Considering that the section transfer from the slides glass to the resin block did not always happen successfully (11.7% partial transfer and 13.8% no transfer), we hypothesized that adhesive glass surface treatment could prevent the detachment of the sections from the slide at the end of the resin inclusion process. By comparing failure rates of the recovery technique from untreated and treated glasses, we conclude that the glass slides do not influence the pop-off technique.

The time elapsed between the date on which the biopsy was originally performed and the date on which the sections were used for this study, did not influence the efficacy of recovery. In fact, we recovered sections dating a wide time span, 2006 to 2018, with no evident differences.

SUPPLEMENTARY REFERENCES

S1. Amann K, Haas CS (2006) What you should know about the work-up of a renal biopsy. Nephrol Dial Transplant 21(5):1157-61. https://doi.org/10.1093/ndt/gfk037

S2. Walker PD (2009) The renal biopsy. Arch Pathol Lab Med 133(2):181-8. https//doi.org/10.1043/1543-2165-133.2.181

S3. Sementilli A, Moura LA, Franco MF (2004) The role of electron microscopy for the diagnosis of glomerulopathies. Sao Paulo Med J 122(3):104-9. https//doi.org/S1516-31802004000300006

S4. Haas M (1997) A reevaluation of routine electron microscopy in the examination of native renal biopsies. J Am Soc Nephrol. 8(1):70-6.

S5. Rivera A, Magliato S, Meleg-Smith S (2001) Value of electron microscopy in the diagnosis of childhood nephrotic syndrome. Ultrastruct Pathol 25(4):313-20. https//doi.org/10.1080/019131201753136340

S6. Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, Nadasdy T, D'Agati VD (2009) Proliferative glomerulonephritis with monoclonal IgG deposits. J Am Soc Nephrol. 20(9):2055-64. https//doi.org/10.1681/ASN.2009010110

S7. Kfoury H (2014) Epithelial cell foot process effacement in podocytes in focal and segmental glomerulosclerosis: a quantitative analysis. Ultrastructural Pathology 38(5): 303–308. https//doi.org/10.3109/01913123.2014.927405

S8. Lighezan R, Baderca F, Alexa A, Iacovliev M, Bonţe D, Murărescu ED, Nebunu A (2009) The value of the reprocessing method of paraffin-embedded biopsies for transmission electron microscopy. Rom J Morphol Embryol 50(4):613-7.

S9. Gonzalez-Angulo A, Ruiz de Chavez I, Castaneda M (1978) A reliable method for electron microscopic examination of specific areas from paraffin-embedded tissue mounted on glass slides. Am J Clin Pathol 70(4):697-9. https//doi.org/10.1093/ajcp/70.4.697

S10. Scimeca M, Pietroiusti A, Milano F, Anemona L, Orlandi A, Marsella LT, Bonanno E (2016) Elemental analysis of histological specimens: a method to unmask nano asbestos fibers. Eur J Histochem. 60(1):2573. https//doi.org/10.4081/ejh.2016.2573

S11. Lehmbecker A, Rittinghausen S, Rohn K, Baumgärtner W, Schaudien D (2014) Nanoparticles and pop-off technique for electron microscopy: a known technique for a new purpose. Toxicol Pathol 42(6):1041-6. https//doi.org/10.1177/0192623313509906

S12. Monga G, Mazzucco G, Coppo R, Piccoli G, Coda R (1976) Glomerular Findings in Mixed IgG-IgM Cryoglobulinemia. Light, Electron Microscopic, Immunofluorescence and Histochemical Correlations. Virchows Arch B Cell Pathol 20(3):185-96. https//doi.org/10.1007/bf02890338

S13. Foster K, Markowitz GS, D'Agati VD (2005) Pathology of thin basement membrane nephropathy. Semin Nephrol 25(3):149-58. https//doi.org/10.1016/j.semnephrol.2005.01.006