Marking Excision Margins of Surgical Specimens by Silver Impregnation

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Summary
Marking excision margins of surgical specimens by silver impregnation has several advantages over commonly used Indian ink: during the slicing the tissue preserves its natural color, the staining is permanent, and the pigment does not smudge over cutting surfaces. The pigment is clearly visible in tissue sections.
The tissue specimen is shortly dipped into a 10% water solution of argent nitrate ($AgNO_3$ with HNO_3). After slicing, the tissue specimens are developed in common black & white developer for several seconds and paraffin processed as usual. The method is suitable for formaldehyde fixed as well as fresh tissue specimens.
Key words: **surgical margin – surgical specimen – biopsy**

Souhrn
Značení resekčních okrajů tkáně stříbřením

Značení resekčních okrajů vzorků tkáně stříbřením přináší oproti běžnému značení tkáně tuší některé výhody: tkáň při přikrajování zachovává původní barvu, zabarvení je stálé, nedochází ke znečištění řezné plochy ani tekutin při sycení tkání parafinem, značení je dobře viditelné na tkáňových řezech.
Pro značení používáme krátké ponoření tkáně do roztoku 10% dusičnanu stříbrného $AgNO_3$ s přídavkem kyseliny dusičné; po nakrájení jsou tkáňové řezy vyvolány běžnou černobílou fotografickou vývojkou.
Klíčová slova: **resekční okraj – bioptický vzorek – biopsie**

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When preparing tissue blocks from surgical specimen of tumorous lesions it is usually necessary to mark surgical margins of the tissue in order to evaluate the adequacy of lesion removal. Common practice is to coat the tissue with India ink by immersion, spraying or painting by a small brush. The India ink can be coagulated by Bouin's solution. However, even the coagulation does not prevent smearing the ink onto the sliced surface. This may cause problems, because polluted cutting surface may simulate resection margin in askew embedded tissue blocks.

Another problem is the dark staining of the specimen before the slicing, which may cause problems with orientation of the tissue and evaluating fine anatomical details.

Therefore we sought a method which would fulfill following requirements:

- the method should work on wet fixed as well as fresh tissues
- the pigment must survive formol-paraffin process
- the tissue specimens should not be significantly stained before the slicing
- the pigment should not smear onto the sliced surface
- the pigment must be visible in the tissues stained by common methods and the process must not interfere with histological and immunohistological methods
- the staining of the tissue must be fast and the process easy
- the reagents should be easily available without strict demands as far as the purity and exact dosing

Materials and methods

The method is based on reaction of the argent nitrate ($AgNO_3$) with natrium chloride (NaCl) within the tissue. Newly formed argent chloride (AgCl) is developed by common developer for black and white photographic process. Thus the surface of the tissue is stained black by the mixture of silver and silver oxide.

We have found no literary reference to this

simple method. Because it's fast, easy and may be useful, we give the more detailed description:

- excess fixation fluid is wiped by the paper wool
- the specimen is shortly dipped (1 second) into the water solution of argent nitrate (*bath 1*); the specimen may change its color slightly; common light is enough for the initial reduction of argent chloride
- the specimen is dried again with paper wool and sliced in a usual way for paraffin blocks
- before or after putting the tissue into the plastic boxes the slices are developed in common developer for black and white films or papers in recommended concentration (we use Fomatol LQN 1:5, but any rapid b & w developer will do); the surface of the tissue gets almost immediately black
- the tissue is then rinsed in water quickly and processed as usual.

The process can be modified: the developer can be applied even before the slicing (but the advantage of working with unstained specimen is lost). The whole baskets of boxes with specimens can be developed simultaneously in large amount of the developer.

Results

After pre-treatment with argent nitrate the tissue preserves its color and details are preserved for the slicing. After developing the surgical margins stain black while the slicing surfaces remain unstained.

Discussion

This method cannot be used for certain fixation fluids (eg. Methacarn) or for acid baths used for decalcifying. The tissues stored in formalin for a long time may react less. In such situations it is necessary to use the India ink or (if possible) to stain the tissue before fixation with Methacarn.

The pigmentation may interfere with some staining methods. We use the method especially to stain surgical margins of excisions of skin tumors. We do not use this approach for small probatory excisions of inflammatory skin lesions. The cornified layer does not stain much, but even though it may interfere with silver impregnation methods like Grocott or Warthin-Starry. In hemangiomas and blood vessel malformations the pigment may get deep into the vascular spaces.

On the other hand we have not observed any interference with common stainings and immunohistochemical or FISH methods.

This process is suitable for other tissues where surgical margins are important, like eg. the cervical cone. Clean grayish-white cut surface contrasts with dark margins, which helps when orienting the tissue in the block. The baths remain clear, not polluted by the India ink.

The process can be used for staining fresh tissues in preparing frozen specimens. For this purpose it is better to use higher (10 %) concentration of the argent nitrate.

The method is slower, more laborious and more expensive, but the amount of the solution is only several ml/day and the demands on purity of the chemicals are low.

The reduced silver can be removed from the tissue if necessary. The method for rehalogenizing negative films can be used (1) and the silver chloride can be washed out by the common fixative used in black and white photography:

The tissue sections stretched on a slide, deparaffinized and hydrated are put into the rehalogenizing solution (*bath 2*) for about 15 s, rinsed in water and fixed for about 30 s in any common black and white fixative. Then the slides are washed and stained in a usual way. Similar method can be used to remove the silver from the surface of the whole tissue specimens (before paraffin saturation). Rehalogenizing may interfere with some staining or immuno-histochemical methods (not tested).

Bath 1: 5–10 % solution of argent nitrate with nitric acid

distilled water	80 ml
argent nitrate $AgNO_3$ cryst.	5–10 g
nitric acid HNO_3 conc.	5 ml
distilled water up to	100 ml

Store in dark bottle; use appropriate amount of fresh solution.

Bath 2: Re-halogenizing solution ORWO 710

distilled water	400 ml
copper sulphate $CuSO_4$ cryst.	50 g
sulphuric acid H_2SO_4 conc. 96 %	
12.5 ml	
natrium chloride NaCl	50 g
distilled water up to	500 ml

The solution is stable and can be stored indefinetly.

Conclusion

The method described is suitable for marking surgical margins of smaller tissue specimens, either fixed in formaldehyde or fresh. The pigment is developed after the specimens are cut, therefore the tissue color is not changed and the cutting is easy. Pale, unstained cutting planes and dark tissue margins help when embedding the tissue into the blocks.

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J. Lukáš a kolektiv

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