**UW Medicine - Pathology**

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Von Kossa for Calcium (Pigment and Mineral) Procedure

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| Adopted Date: 06/22/05  Revision Date: 09/07/12 |

PURPOSE

To identify the method for performing the special stain of Von Kossa's for Calcium.

PROCEDURE

**Fixation:**

10% buffered neutral formalin.

**Sectioning:**

Paraffin sections cut at 4 microns.

**Solutions:**

Use Type II de-ionized water for all solution preparation.

**5% Silver Nitrate**

Silver nitrate 5.0 gm

Distilled water 100.0 ml

Use bleach cleaned glassware. Discard after use.

**5% Sodium Thiosulfate**

Sodium thiosulfate 25gms

Distilled water 500mls

Stable for six months. Discard after use.

**Nuclear Fast Red**

Richard Allan Scientific. Ready made.

**Procedure:**

Use control slide.

1. De-paraffinize and hydrate to distilled water.
2. Place slides in 5% Silver nitrate solution exposed to direct sunlight for 60 minutes. A 100-watt lamp light may be used if necessary.
3. Rinse in distilled water.
4. Place slides in 5.0% Sodium thiosulfate solution for three minutes.
5. Rinse in distilled water.
6. Counter stain in nuclear fast red for five minutes.
7. Rinse well in distilled water.
8. Dehydrate in 95% and absolute isopropyl alcohol.
9. Clear in xylene and mount.

**Results:**

Calcium salts black

Nuclei red

Cytoplasm light pink

**Comments:**

This is a metal substitution technic for demonstration of calcium and depends on the anionic part of the calcium salt and hence is not specific for the calcium ion itself. Phosphate and carbonate are the most common anions in calcium deposits, and treatment with a metal results in the transformation of the calcium salt into the corresponding metal salt which is then visualized in different ways. The transformation depends in part on the relative solubility’s of the calcium and metal salts, and quantitative transformation occurs only if the metal salt is considerably less soluble than the original salt. In this technic, sections are treated with a silver nitrate solution and the silver is deposited, presumably by replacing the calcium, reduced by the action of strong light, and finally visualized as metallic silver.

REFERENCES

Luna, Lee G.: *Manual of Histologic Staining Methods of the AFIP,* Mcgraw-Hill Book Co., 1968, pg. 176-177.

Sheehan, D.C. and Hrapchak, B.B.: *Theory and Practice of Histotechnology ,* the C.V. Mosby co., 1980, pg. 227.

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