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| **Von Kossa Stain** |
| **Purpose** | This staining method detects anions combined with calcium, not the calcium itself. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | The black deposits in this reaction are due to a reduction of silver by organic matter in the sections. The black reaction product is an artifact that occurs only when the sections are exposed to strong light. If the reaction is carried out in subdued light, yellow to yellowish-brown silver phosphate is selectively demonstrated. Silver carbonate is soluble in sodium thiosulfate and does not stain. |
| **Materials** | **Supplies** | **Reagents** |
|  | * Coplin jars with lids
* PPE
* UV light
 | * Silver Nitrate
* Sodium Thiosulfate
* Nuclear Fast Red
 |
| **Sample** | FFPE tissue  |
| **Quality Control** | Tissue containing calcium All glassware must be clean and rinsed in distilled water before use. Do not use metal forceps. |
| **Special Safety Precautions** | Silver Nitrate may cause severe burns. Avoid contact with skin and eyes. Poison: avoid breathing in, corrosive. |
| **Stock Solutions** | Silver Nitrate, 5%Sodium Thiosulfate, 5%- Retic kitNuclear Fast Red- Retic kit |
| **Working Solutions** | **All reagents are purchased from Newcomer Supply ready to use**   |
| **Procedure** |  |
|  | **Step** | **Action** |
|  | 1 | Deparaffinize slides and hydrate to water |
|  | 2 | Place slides in coplin jar of Silver Nitrate, 5% in front of a UV light for.............**10-30** minutes  |
|  |  | Check slides periodically and remove from light source when control slide show black-brown deposits macroscopically |
|  | 4 | Rinse in several changes of Distilled water |
|  | 5 | Place slides in Sodium Thiosulfate, 5% for ………… **2** minutes |
|  | 6 | Wash in several changes of Distilled water |
|  | 7 | Counterstain in Nuclear Fast Red …………….. **5** minutes |
|  | 8 | Rinse well in Distilled water |
|  | 9 | Dehydrate, clear and coverslip |
| **Results** | Calcium salts……...….Black to brown/blackNuclei.........………....…RedCytoplasm.....................Light pink |
| **Notes** | Iodine solutions used for the removal of mercury pigment may also remove some of the calcium salts from the tissue. |
| **Result Reporting** | By Pathologist |
| **References** | Histotechnology A Self-Instructional Text, F.Carson 1990Sheehan & Hrapchak:Theory and Practice of Histotechnology, 2nd edition 1980 Battelle Press |

**Historical Record**

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| Version | Revised by | Effective Date | Summary of Revisions |
| 1 |  |  | Initial version. |
| 2 | A. Dubbelde | 6/27/19 | Update format, add version, and update to match current staining procedure used. |
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