1.0 PURPOSE

The purpose of this document is to establish the procedure for a uniform and reproducible method for staining with Hematoxylin & Eosin (H&E) for the AIDS and Cancer Specimen Resource (ACSR). Tissues are collected from patients with informed consent.

2.0 SCOPE

This standard operating procedure (SOP) describes how sections of tissue should be stained using H&E. This SOP applies to all personnel from ACSR Regional Biospecimen Repositories (RBRs) and affiliates that are responsible for performing H&E staining specifically for the ACSR. The SOP does not cover detailed safety procedures for handling biohazardous material and it is recommended that personnel follow institutional biosafety guidelines.

3.0 REFERENCE TO OTHER ACSR SOPS OR POLICIES

ACSR SOP ID# Tech009 Specimen Handling

4.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel from ACSR RBRs and affiliate sites that are responsible for performing H&E staining.

<table>
<thead>
<tr>
<th>ACSR Personnel</th>
<th>Responsibility/Role</th>
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<tbody>
<tr>
<td>ACSR Staff Member</td>
<td>Conducting staining of tissue sections and/or sectioning/cutting of tissue blocks.</td>
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<tr>
<td>Pathology Personnel</td>
<td>Read H&amp;E slides for tumor content, necrosis and diagnosis. Evaluate staining</td>
</tr>
</tbody>
</table>
5.0 MATERIALS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

<table>
<thead>
<tr>
<th>Materials and Equipment</th>
<th>Materials and Equipment (Site Specific)</th>
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<tbody>
<tr>
<td>Statmark Pen or Ventana Medical System labels</td>
<td>Fisher #23-400-450</td>
</tr>
<tr>
<td></td>
<td>VMS #1358501</td>
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<tr>
<td>Tissue sections cut onto Superfrost charged slides</td>
<td>VWR# 48311-703</td>
</tr>
<tr>
<td>Xylene or Clear Rite 3</td>
<td>VWR # EM-XX0060-4</td>
</tr>
<tr>
<td></td>
<td>Richard Allan #6901</td>
</tr>
<tr>
<td>100% ethanol</td>
<td>Bulk from campus stores</td>
</tr>
<tr>
<td>95% ethanol</td>
<td>Bulk from campus stores</td>
</tr>
<tr>
<td>80% ethanol</td>
<td>80 ml of 100% ethanol and 20 ml of house distilled water</td>
</tr>
<tr>
<td>60°C Oven for deparaffinization</td>
<td>Fisher # S50172</td>
</tr>
<tr>
<td>Hematoxylin 1</td>
<td>Richard-Allan Scientific #7221</td>
</tr>
<tr>
<td>Ammonia Water</td>
<td>Bluing reagent - Richard-Allan Scientific #7301</td>
</tr>
<tr>
<td>Eosin</td>
<td>Richard-Allan Scientific #7111</td>
</tr>
<tr>
<td>Tap water</td>
<td></td>
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</tbody>
</table>

http://acsr.ucsf.edu
1% acid alcohol | 1% HCl in 95% ethanol
---|---
Coverslips (24mmx50mm #1 or #1.5) | Fisher 12-553-464
| Fisher 12-553-1-471
Staining dishes and slide carriers | Fisher #08-813D
Mounting Medium | Richard Allan Scientific #4112

### 6.0 DEFINITIONS

See ACSR Glossary.

### 7.0 PROCEDURES

This procedure is intended to ensure that tissue samples obtained from consented participants are processed in a safe and efficient manner.

#### 7.1 Special Safety Precautions

7.1.1 Comply with "Universal Precautions" when collecting and handling all specimens.

7.1.2 Use PPE (personal protective equipment) in accordance with collecting institution’s guidelines.

7.1.3 Standard best-practice working procedures include careful manipulation of the patient samples, disinfection of countertops and equipment used during testing, and disposal of biohazard waste into appropriate receptacles.
7.2 Verification of Identifying Information
   As applicable, verify the accuracy of patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on collection tubes. Ensure that all personnel are trained in the use of the electronic information system(s).

7.3 Hematoxylin and Eosin Staining
   7.3.1 Each slide should be labeled clearly and legibly with the identifier information.
   7.3.2 Deparaffinize slide by heating for at least 30 minutes in 60°C oven.
   7.3.3 Put slides in slide carry and submerge for 5-15 minutes in xylene or Clear Rite 3. Allow carrier to drain and then move slides as needed to each new bath for the time indicated
   7.3.4 5-10 minutes in xylene or Clear Rite 3
   7.3.5 5-10 minutes in xylene or Clear Rite 3
   7.3.6 25 dips in 100% ethanol
   7.3.7 25 dips in 100% ethanol
   7.3.8 25 dips in 95% ethanol
   7.3.9 25 dips in 95% ethanol
   7.3.10 25 dips in 80% ethanol
   7.3.11 Rinse in tap water for 30 seconds
   7.3.12 5 minutes in hematoxylin * Begin here if staining frozen sections slides after fixing in cold acetone for 15 minutes
   7.3.13 Rinse in tap water for 30 seconds
   7.3.14 Quickly dip in 1% acid alcohol
   7.3.15 45 seconds in ammonia water (do not dip)
   7.3.16 Rinse in tap water for 5 minutes. Do not shorten this step
   7.3.17 45 seconds in eosin
   7.3.18 5-6 dips in 95% ethanol until clear
   7.3.19 6 dips in 100% ethanol
7.3.20 6 dips in 100% ethanol
7.3.21 Clear in xylene or Clear Rite 3
7.3.22 Clear in xylene or Clear Rite 3
7.3.23 Coverslip – do not let slides dry while coverslipping
7.3.24 Record use of xylene and ethanol baths so replacements can be done as required – every 100 slides or every month

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 NCI Best Practices for Biospecimen Resources


8.4 US National Biospecimen Network Blueprint

http://bioethics.georgetown.edu/nbac/hbm.pdf

8.6 Declaration of Helsinki.
9.0 APPENDICES

10.0 REVISION HISTORY

<table>
<thead>
<tr>
<th>SOP Number</th>
<th>Date revised</th>
<th>Author</th>
<th>Summary of Revisions</th>
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