

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 1 of 6
Approved by ACSR Executive Committee	July 2, 2015

## 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.

ACSR Personnel	Responsibility/Role
ACSR Staff Member	Conducting staining of tissue sections and/or sectioning/cutting of tissue blocks.
Pathology Personnel	Read H&E slides for tumor content, necrosis and diagnosis. Evaluate staining

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 2 of 6
Approved by ACSR Executive Committee	July 2, 2015

## 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.

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

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 3 of 6
Approved by ACSR Executive Committee	July 2, 2015

1% acid alcohol	1% HC1 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.

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 4 of 6
Approved by ACSR Executive Committee	July 2, 2015

## 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

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 5 of 6
Approved by ACSR Executive Committee	July 2, 2015

- 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  
<http://biospecimens.cancer.gov/bestpractices/2011-NCIbestpractices.pdf>
- 8.2 Preece, Ann., H.T. (ASCP), 1972. A Manual for Histologic Technicians, 3<sup>rd</sup> ed., Little Brown and Company, Boston.
- 8.3 Best Practices for Repositories: Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).  
[http://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER\\_Best\\_Practices\\_3rd\\_Edi.pdf](http://c.ymcdn.com/sites/www.isber.org/resource/resmgr/Files/ISBER_Best_Practices_3rd_Edi.pdf)
- 8.4 US National Biospecimen Network Blueprint  
<http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp>
- 8.5 National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.  
<http://bioethics.georgetown.edu/nbac/hbm.pdf>
- 8.6 Declaration of Helsinki.  
<http://www.wma.net/en/30publications/10policies/b3/index.html>

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: July 2, 2015
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 1.0
ACSR SOP ID# Tech008	Page 6 of 6
Approved by ACSR Executive Committee	July 2, 2015

## 9.0 APPENDICES

## 10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions