



AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 1 of 7
Approved by ACSR Executive Committee	July 2018

## 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).

## 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. The AIDS Cancer Specimen Resource Regional Biorepositories and affiliates process and bank biospecimens under site specific approved Human Subjects Protocols. Biospecimens, Samples, Aliquots and Derivatives are entered into the ACSR database when consent for banking and research use have been verified by the Protocol PI or ACSR designee. Each RBR and affiliate site is responsible for Human Subjects compliance as per their institutional guidelines and their local approved Human Subjects Protocol. 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 Tech009 Biospecimen Handling

ACSR SOP Tech010 Biospecimen Labeling

## 4.0 DEFINITIONS

Term/Acronym	Definition
ACSR	AIDS and Cancer Specimen Resource
Aliquot	The sample has the original characteristics of the original or parent specimen but in smaller quantities (FFPE block vs unstained sections from the FFPE block)
Biospecimen	Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen.

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 2 of 7
Approved by ACSR Executive Committee	July 2018

Derivative	The original characteristics of the specimen are changed (FFPE vs DNA derived from FFPE).
H&E	Hematoxylin & Eosin
PPE	Personal Protective Equipment such as gloves, lab coat, face shield.
RBR	Regional Biorepository
Sample	This is an aliquot, derivative or if the Parent Specimen received is stored as a whole specimen, it is referred to as a sample, as per ACSR database definition.
SOP	Standard Operating Procedure. A set of written instructions that document a routine or repetitive activity followed by an organization.
Specimen	The same as Biospecimen. Human material such as urine, blood, tissue stored in a biorepository for use in laboratory research. For the ACSR this is considered the original or parent biospecimen/ specimen.
Universal Precautions	This is an approach to infection control to treat all human blood and certain human body fluids as if they were known to be infectious for HIV, HBV and other bloodborne pathogens,

## 5.0 ROLES AND RESPONSIBILITIES

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

<b>ACSR Personnel</b>	<b>Responsibility/Role</b>
ACSR Staff Member	Conduct 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: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 3 of 7
Approved by ACSR Executive Committee	July 2018

## 6.0 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.

<b>Materials and Equipment</b>	<b>Materials and Equipment (Site Specific or equivalent)</b>
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	
95% ethanol	95 ml of 100% ethanol and 5 ml of house distilled water
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 #7221
Ammonia Water	Bluing reagent - Richard-Allan Scientific 7301
Eosin	Richard Allan #7111
Tap water	
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 #4112

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 4 of 7
Approved by ACSR Executive Committee	July 2018

## 7.0 PROCEDURES

This procedure is intended to ensure that tissue biospecimens 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 handling all biospecimens.

**7.1.2** Use PPE (personal protective equipment) in accordance with the institution's guidelines.

**7.1.3** Standard best-practice working procedures include careful manipulation of the patient biospecimens, disinfection of countertops and equipment used during testing, and disposal of biohazard waste into appropriate receptacles.

### 7.2 Verification of Identification Information on Biospecimen vessel

As applicable, verify the accuracy of coded patient information (in keeping with privacy and ethical policies) and ensure that it corresponds with the information on labels on biospecimen and biospecimen vessel or tubes. Ensure that all personnel are trained in the use of the ACSR database and local 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 / remove paraffin from slide by heating for at least 30 minutes in 60°C oven.

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 5 of 7
Approved by ACSR Executive Committee	July 2018

- 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: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 6 of 7
Approved by ACSR Executive Committee	July 2018

**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 slides.

**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/practices/default.asp>
- 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 IV. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). Feb 2018 <http://www.isber.org/?page=BPR>
- 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>

## 9.0 APPENDICES

Not applicable.

AIDS and Cancer Specimen Resource (ACSR)	Effective Date: August 2018
Technical: Hematoxylin & Eosin (H&E) Staining Protocol	Version 2.0
ACSR SOP ID# Tech008	Page 7 of 7
Approved by ACSR Executive Committee	July 2018

## 10.0 REVISION HISTORY

SOP Number	Date revised	Author	Summary of Revisions
Tech008	3-1-2018	BGG/TY	Formatting, definitions and replace sample with biospecimen