SPECTRUM HEALTH

SPECTRUM HEALTH – RESEARCH DEPARTMENT

Immunohistochemistry

CUSTOM IMMUNOHISTOCHEMISTY:
MICHIGAN STATE UNIVERSITY GRRC BUILDING

Jane A. Tol HT (ASCP)
7/1/2018

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General Information and Location

The Spectrum Health Immunohistochemistry Core at Michigan State University's Grand Rapids Research Center (GRRC) is a service core dedicated to supporting research at Spectrum Health. These services are available to MSU faculty at the same internal rates as Spectrum researchers. The Core will provide the principal investigator (PI) with reproducible test results. Conscientious handing of the tissue and care in producing the best possible slides can substantially affect and improve the accuracy of the project.

The IHC core will follow set procedures for methods used for frozen section(s), fixation, routine processing, and paraffin embedding, sectioning and staining of animal and human tissues. The laboratory occupies just under 500 square feet of space, including an alcove dedicated to processing samples and frozen section (FS), one workbench, a write up desk and a separate room dedicated to sectioning, routine H&E stain, Immunohistochemical stains (IHC) and special stains.

Location:
Michigan State University
Grand Rapids Research Center
400 Monroe Street, NW
Grand Rapids, MI 49503

Immunohistochemistry Core (IHC)
Histotechnologist
Jane A Tol, HT (ASCP)
Contact Information: 616.486.8667
jane.tol@spectrumhealth.org

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Workflow

Study Initiation:

1. Contact Jane Tol to schedule an appointment
2. Bring approved IACUC or IRB
3. Bring study design and sample information.

Sample Submission:

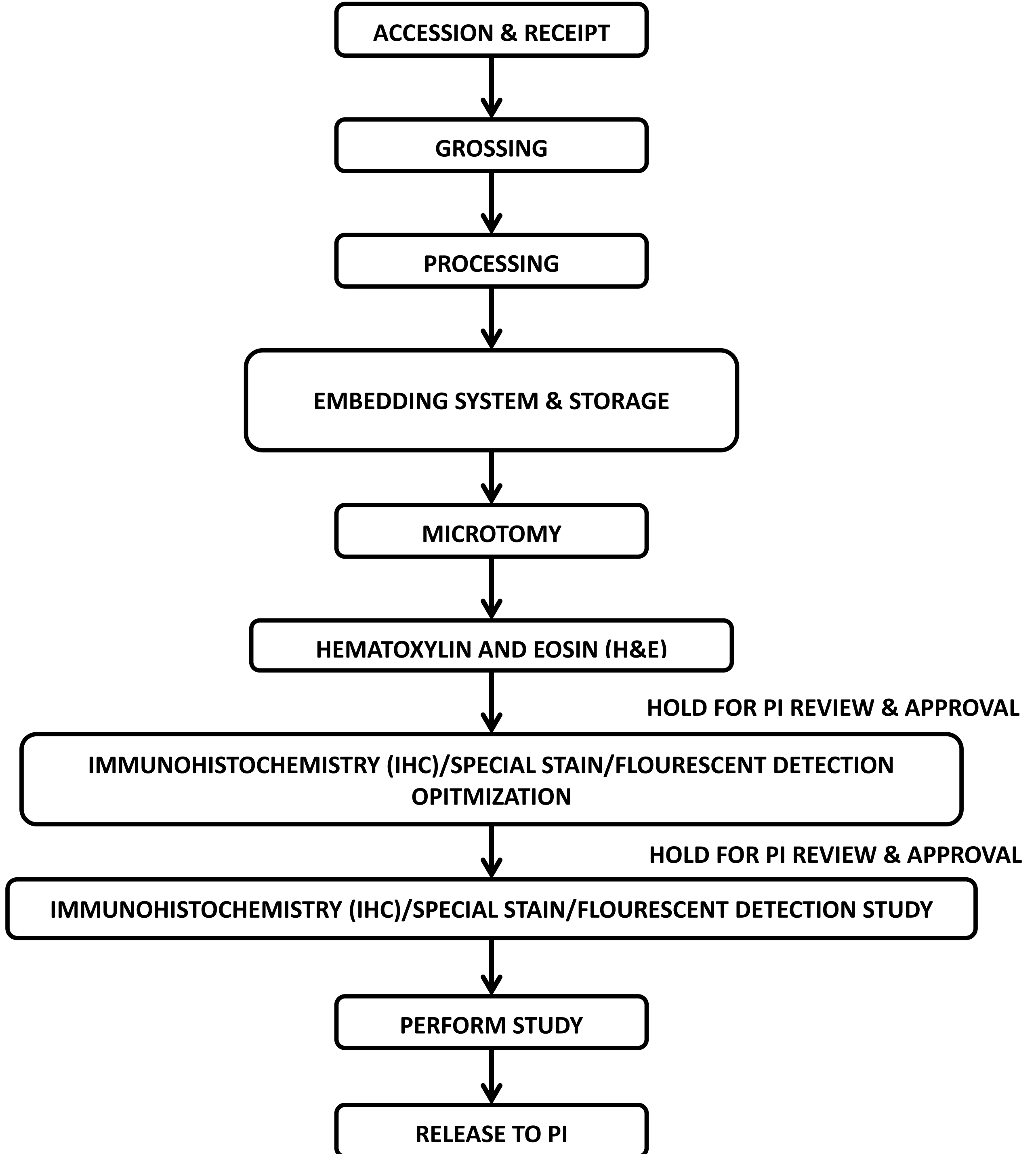
1. Please fill out the Digital Form and Email to jane.tol@spectrumhealth.org.
2. Please print a copy to send with all samples. (Note: Samples will not be processed without a copy this form).
3. PI Name, Contact Information and Request Date are required for all projects (Note: Samples without this information will not be processed).
4. IRB/IACUC are required when applicable
5. State on label container what reagent sample is in (95% alcohol/fresh/10% formalin/paraformaldehyde/saline?)
6. If sending frozen samples on **Dry Ice**, please notify the lab at 616.486.8667 **24 hours** in advance.
7. Requests for grossing samples must be discussed during initial request.

Functions and Process overview:

1. The core Histologist will:
   a. Log in new requests
   b. Log in and process animal or samples
   c. Collect human samples from SHS Biorepository: **Spectrum Health Policy #8789**[1]
   d. Collect human for positive controls from SHS Biorepository
   e. For all samples; process, embed, formalin fixed-paraffin samples or frozen tissues, or section/cut curls.
   f. Work up immunohistochemistry or special stain
   g. Perform immunohistochemistry or special stain
   i. Record all procedures/methods performed and samples analyzed
   NOTE: For all immunohistochemistry, all antibodies must be supplied by the PI
2. Documentation of time
   a. A daily log will be used to document the specific time spent on each project for charging purposes.
3. Prioritization of Requests
   a. Requests for smaller volumes of samples may be fit into larger projects
   b. These will be decided on a case-by-case basis, with consideration of grant deadlines, completion of grant aims, etc.

[1] Available by request. Contact Jane Tol: 616.486.8667; jane.tol@spectrumhealth.org

GENERIC IHC WORKFLOW



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Effective: July 2017

Item #	Item	$/Slide	$/Sample/Section	$/Box	$/Hour
1	Grossing		$3.00		
2	Processing		$0.85		
3	Embedding Paraffin Samples		$1.75		
4	Embedding Frozen Samples		$4.50		
5	Sectioning for Curls for Genomics		$5.00		
6	Sectioning, unstained, single section/slide	$1.70			
7	Sectioning, unstained, extra section/slide	$0.10			
8	Sectioning, unstained, extra slides	$0.70			
9	Sectioning Frozen Samples	$3.75			
10	Punch block		$5.00		
11	Slide : Untreated	$0.07			
12	Slide: Poly-l-lysine	$0.13			
13	Slide: Charged	$0.33			
14	Storage Box (100 slides ambient)*			$7.35	
15	Storage Box (100 slides -80° C)*			$13.00	
16	Storage Box Block (500 blocks)*			$11.20	
17	Slide Cardboard folder (20 slides)*			$17.00	
18	Staining: H&E	$3.43			
19	Staining: Immunohistochemistry**	$19.00			
20	Antibody Optimization **				$50.00
21	Special Stain***				$75.00
22	Staining IHC HER2, CD117, CD20, ER, PR***	$19.00			
23	Pathologist Charge (Human)				TBD
24					
25	Retrieval charge from Kent Records		$3.00/case & $25.00/each trip		
26	Cryostat use (no technical assistance)				$5/hr.

***Property of Spectrum Health – Free if returned**

****It takes up to 20 slides to optimize an antibody. Difficult antibodies may require additional optimization. At this point the Core lab will discuss findings with PI to determine how to proceed.**

*****PI's must cover the cost of antibodies and kits and provide datasheets. Pathologist charges may apply for scoring and/or verification of sample.**

Use of the Core and access to Spectrum Health prices are restricted to Spectrum Health System and affiliated investigators.

Requests for services for non-collaborative studies will be considered case-by-case. Requests for pricing for services related to non-collaborative studies should be directed to Jane Tol via email at jane.tol@spectrumhealth.org.

If your project requires services or supplies not listed, please discuss this at your project consultation and the core will be happy to research and define your project within the scope of the core abilities.

Note: Spectrum Health Immunohistochemistry Core reserves the right to change the prices at any time.

Questions should be referred to IHC Core:

Jane A Tol HT (ASCP) Phone: 616.486.8667 Jane.Tol@Spectrumhealth.org

Methods and Materials

The staining methods available to each PI include Hematoxylin and Eosin (H & E), special stains (Masson Trichrome and PASH, etc.), and Immunohistochemistry (IHC) antibody stains. As different procedures can lead to different staining patterns, it will be the responsibility of the PI to review the processing and staining options they feel best represents their desired end product.

Projects that will include biospecimens from the Biorepository at Spectrum Health will be handled under the policies set by the Biorepository Laboratory for Spectrum Health. These specimens may be used in Research projects or for positive and negative control(s). ***See Governance and Guardianship of Patient Bio-specimens for Biorepository at Spectrum Health (policy #8789).***

Upon arrival in the laboratory, the sample is given an identifying accession number. When previously given a number by the PI, that specific number sequence(s) will be maintained throughout the entire project. The PI's initials will be placed on all rodent number sequences maintaining the specific identify of each PI/project. For projects outside of IACUC and IRB, each sample will be given its own identification number in the IHC core system.

Routinely, the specimen is sectioned and representative tissue pieces (blocks) are placed in cassettes and immediately placed in a solution called a fixative that will prevent decomposition and preserve the tissue. After several hours, the tissue blocks are processed through a series of solutions that wash, dehydrate, and clear the tissue. The tissues are then infiltrated with a substance (usually melted paraffin) which will support the cells and make it possible to cut very thin sections of the tissue. Almost all laboratories accomplish this phase on a tissue processor. This process usually takes approximately 8 to 24 hours.

Once processed, the tissues are embedded (placed in a small mold containing melted paraffin) and allowed to solidify into blocks. Each block is placed into a machine called a microtome which is designed to cut sections of tissue-paper thinness. The sections are placed on glass slides, and allowed to air dry. Once sectioned, the tissue is sealed with a light coat of paraffin for long term storage. When the slides are for special staining purposes, IHC or Special Stains, they are stored at 4°C. The slides are dried in a drying oven. The paraffin is then removed from the tissue on the glass slide and the section is stained. After staining, the sections are sealed under a glass coverslip for preservation. The sections are then available for reading under a microscope.

Fresh Tissue for Frozen Section: Before any tissue can be cut into thin sections, it must be supported by some medium. Freezing the water in the tissue hardens it and is one way of providing necessary rigidity. This process is called Frozen Sectioning. Frozen sections will be snap-frozen, in a Sakura Tissue-Tek® Cryo³® Plus Microtome/Cryostat using Sakura Optimal Cutting Temperature (O.C.T.) Embedding Medium for frozen tissue specimen. Frozen sections are accomplished by placing a microtome into a machine called a cryostat. The frozen blocks will be sectioned at the thickness requested by the PI. Each frozen
section sample(s) will be placed on charged Superfrost Plus Microscope Slides (Cardinal Health,-Waukegan, IL) or Poly-Prep Slides (Sigma Life Sciences-St Louis, MO). Once sectioned, the slides can be immediately stained or placed in the -80° C freezer for storage.

Hematoxylin and Eosin Stain (H & E): Hematoxylin, a natural dye, is used to stain the nuclei of cells. Eosin is used to stain the cytoplasm of cells. Used alone, hematoxylin is a nonspecific stain, and is dependent on the metals with which it is combined. Hematoxylin combined with aluminum salts will stain blue and hematoxylin combined with ferric salts will stain blue-black. In the IHC lab, hematoxylin will be applied as a regressive stain, where the tissues are over stained and then decolorized (acid wash) to remove the excess color. Eosin, an acid synthetic dye, stains cytoplasm with different degrees of intensity.

Antibody-IHC and Special Staining: All slides will be air-dried overnight, and then dried in a Biocare Medical Desert Chamber Pro™ oven for 30 minutes at 60°C. Each slide will be routinely de-paraffinized to distilled water using Sakura's Tissue-Tek® DRS™ 2000 Automatic Slide Stainer – (SS to water program-Two changes of Xylene-removes paraffin, two changes of 100% alcohol, two changes of 95% alcohol, running water-two minutes, distilled water). Sections will be stained using the protocol(s) requested by the PI. When required, adjacent sections from each block will be staining following the protocol set by the PI, either IHC or special stains. Upon completion of each protocol, the sections are sealed under a glass coverslip for preservation. The sections are then available for reading under a microscope.

Each project will establish a known positive control or make a cell block of known positive cells, if available. Ten sections will be cut and placed on charged slides, air dried overnight and placed in the oven for 30 minutes at 60°C. Negative controls will be run on each project. Each negative control slide will be treated exactly as the experimental material without antibody or special stain. Ten sections will be cut and placed on charged slides, air dried overnight and placed in the oven for 30 minutes at 60°C. These slides will be used for validation purposes.

All complete projects will include a written discussion of methods for use in a paper. Examples follow, and will include, but are not limited to:

Routine Hematoxylin and Eosin Stain:

Sections were dried overnight and placed in an oven at 60°C for 30 minutes. Using the Tissue-Tek ® DRS ™ 2000 automatic stainer (Sakura Finetek, USA, Inc., Torrance, CA); Sections were deparaffinized using 3 changes of Xylene, followed by 2 changes each of 100% and 95% alcohol. Sections were rinsed with running water and stained in Gill 2 Hematoxylin (72511, Richard-Allen Scientific, Kalamazoo, MI); for 2 minutes. Sections were rinsed in 5% acetic acid wash (5 ml acetic Acid/ 95 ml distilled water, Acetic Acid (Sigma-Aldrich, Co, St Louis, MO for 45 seconds, followed by rinsing in 95% alcohol for 45 seconds. Sections were stained in EOSIN-Y (7111 Richard-Allen Scientific, Kalamazoo, MI) for 2 minutes. Sections were rinsed in 98% reagent alcohol (98 ml reagent alcohol/2 ml distilled water), followed by 3 changes of 100% reagent alcohol, 1:1 Xylene/ alcohol, 3 changes of xylene and coverslipped with CYTOSEAL™ 60 (8310-4; Richard-Allen Scientific, Kalamazoo, MI).

Immunohistochemistry

Adjacent sections were retrieved for the human Mitochondrial marker,

SPARC, Ki-67 (HIER with Citrate buffer pH 6.0), PTEN (rodent Decloaker at 97°C for 25 minutes), and Factor VIII (0.4 % pepsin for 50 minutes at 37°C). Sections were then immunohistochemically stained for human Mitochondrial marker (1:100 for 60 minutes; E5204, Spring Bioscience), SPARC (1:20000 in 0.25 % BSA for 60 minutes; AON-5031, Hematologic Technologies, Inc), Ki-67 (1:100 for 30 minutes; M7240, Dako), PTEN (1:400 for 40 minutes; CM278, Biocare Medical), and Factor VIII (1:1000 for 40 minutes; A008a,Dako) on a intelliPATH FLX® stainer using Biocare reagents (Biocare Medical). Control sections were processed substituting the primary antibody with the appropriate immunoglobulin isotype. Detection was performed with streptavidin/biotin-HRP and DAB for 5 minutes and then counterstained with Hematoxylin for 5 minutes.

Special Stains

Masson Trichrome tissue staining. Tissue sections were snap frozen in a Sakura Tissue-Tek® Cryo³® Plus Microtome/Cryostat using Sakura Optimal Cutting Temperature (O.C.T.) Embedding Medium for frozen tissue Specimens, (4583,Sakura Fine-Tek, USA). Sections were stained using Trichrome Stain Kit, Gomori One-Step, Fast Green, (9175, Newcomer Supply-Middleton, WI). Frozen sections were placed in Gill 2 Hematoxylin (72511, Richard-Allen Scientific, Kalamazoo, MI); for 5 minutes, and then rinsed in running tap water for 10 minutes. The sections were stained in Gomori one-step solution for 20 minutes and then rinsed in distilled water until the water runs clear. Sections were differentiated in 0.5% acetic acid wash for 20 seconds. Sections were rinsed in distilled water for 2 minutes and dehydrated in two Changes of 95% and 100% reagent alcohol. Sections were cleared in 2 changes of Xylene and coverslipped with CYTOSEAL™ 60 (8310-4; Richard-Allen Scientific, Kalamazoo, MI).

Order: Please use the RESEARCH IHC CORE ORDER FORM and any appropriate appendices.

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Project Examples
IHC Research Core
(Not limited to)

1. Thick sections for Genomics:

- •Process wet tissue samples (6 hrs. 40 min) or receives previously processed & blocked samples
- •Embed paraffin infiltrated tissue into blocks(s) (l.5minutes embed; 10-15 minutes cool down/block)
- •Section thick sections at specific micron and number of curls requested (10 minutes/block)
- •Place sections in labeled ZIPPIT bag/transfer tube for transport to PI (1.0 minute/block)

2. Routine Hematoxylin & Eosin (H&E) stain –paraffin:

- •Process wet tissue samples (6hrs. 40 min)
- •Embed paraffin infiltrated study tissue into block(s) (1.5min embed; 10-15 minutes cool down/block)
- •Section each block at requested micron and place on un-charged slide(s) (2 min/slide/section)
- •Stain each slide with H & E; coverslip and label; to PI for review (stain: 24-34 minutes/up to 40 slides; coverslip: 30 second(s)/slide).

3. **intelliPATH FLX – Immunohistochemical Staining: Stainer holds 1 to 50 slides**

- •Section previously blocked samples and place on charged slides – one each antibody requested plus negative control
- •Dry slides per protocol in Desert Chamber Pro (15-30 minutes*)
- •Program and create labels/protocol (20-40 minutes/per protocol/slides)
- •Deparaffinize and run down to water (7:34 minutes)
- •Load reagents and labeled slide(s) on intelliPATH (3 minutes/slide)
- •Start intelliPATH automatic IHC stainer (approx. 40 minutes/protocol up to overnight/run-depending on number of slide(s)/project
- •Remove slides; de-hydrate to Xylene; coverslip (30 minutes/20 slides)

4. Project using Fresh then Frozen Sectioned (FS) tissue for H&E/IHC/Fluorescence staining technique: 1block/8 slides total)

Samples are frozen and remain frozen during entire sectioning process

- •Using a Sakura Tissue-Tek® Cryo3® Microtome Plus® embed samples in Cryomold specimen mold(s) using OCT compound & allow sample to solidify. (2 minutes/orientation/sample)
- •Using Cryostat, section frozen tissue block(s) and place sections on charged slides. (3minutes/slide/1sample)
- •Stain slides 1 & 5 with H&E (17:34 minutes)
- •Stain slides 2 & 6 with Masson Trichrome (120 minutes)
- •Stain slides 3 & 7 with CD4 (130 minutes)
- •Store remaining slides #4 and #8 @ -20° for possible fluorescence staining

5. Small project including H&E, Special Stain and/or IHC stains- paraffin samples (FFPE)

1-10 blocks/10 slides per block Hand label slides/10 minutes/block	Section each block x 10 Levels; Place levels #1,4,7, 10 on **uncharged** slides, Dry overnight 10 minutes /block	Place slide levels #2,3,5,6,8, 9, on **charged** slides. Dry overnight *	Stain slides #1,4,7,10 with H&E (24:34minutes)*	Stain #3=IHC XXXX (70min) Stain # 6 = IHC XXXX (90 min) Stain # 8 = Special Stain (120 minutes)	Remainder of unstained slides saved for possible later studies. Store @ 2-8° C.

Large project including H&E, Special stain, or IHC Stains- Paraffin samples (FFPE)

1-100 blocks/50 sections Per block Hand label slides/40 minutes/block 60 minute/block/slide	Section 50 sections/block; place levels #!, 11,21,31,41 on **uncharged** slides. Stain with H& E. Place remaining levels on **charged** slides. Dry all slides overnight**.**	Stain slides 2, 12,22,32, 42, etc. with Masson Trichrome Stain. Batch up to 24 slides 120 minutes +	Stain slides 3, 13, 13, 33, 43, with IHC KI67 Batch run up to 50 Slides/overnight run	Stain slides 4,14,24,34, 44 with 2nd IHC stain Requested Batch run up to 50 Slides / overnight run	Stain slides #5,15,25 35,45, with 3rd IHC stain requested Save remainder of unstained slides for later studies-store @ 2-8°C.

7. Characterization of Cell Culture lines –

1 Known cell line – AB	2 Known Negatives – AB	3 #1 Unknown cell line - AB	4 #2 Unknown cell line - AB
5 Known cell line – NO AB	6 Known Negative – NO AB	7 #1 Unknown cell line – NO AB	8 #2 Unknown cell line NO AB

8. Validation New Antibodies and Special Stains *

For the initial validation, the IHC Core lab will test 5-10 each of known positive and known negatives samples. These IHC tests will include antigen retrieval times, primary dilutions. The special stain tests will performed on known positive and know negative control tissue, if possible, using the same fixative and processing methods as similar tissue previously tested tissue previously tested by the PI. These tests will follow procedures and protocols previously stated. (Protocol times and reagents similar to optimization) * Times for each protocol

Resection and Fixation of Tissue and Tumors

Tissue samples are harvested by the Principal Investigator (PI) and handed off to the research technician. Tissues for paraffin embedding should, ideally, not be more than 2 cm. square and not more than 4mm thick. The samples are placed in processing cassettes, labeled with the IRB number or PI specific accession number. At this point the samples should be placed in the preferred fixation chosen by the PI.

Fixation is the stabilization of protein and is the most important step in producing good histologic slides. The fixative prevents autolysis and decomposition, should not shrink or swell the tissue. It must not distort or dissolve tissue parts. It should modify tissue to retain its form when subjected to dehydrants, cleaning agents, embedding media, microtomy (sectioning), staining, or mounting reagents and not destroy or distort tissue from its original state. Usually 10% formalin is the preferred choice for fixative. The samples should not remain in formalin more than 24 hours and are then transferred to 70% ethanol for long term storage.

Fluorescence staining technique, the tissue is harvested and sectioned into 2 cm square and not more than 3mm thick pieces. It is placed into 4% paraformaldehyde for at least 7 days and transferred to 30% sucrose for long term storage at 4° C.

Flash Freezing Method - Sample Preparation Immunohistochemistry Core

In addition to standard immunohistochemistry, this technique can be used for metabolic assays or enzyme studies using histochemical methods.

1. The sample should be blotted with an absorbent towel to remove excess moisture before flash-freezing.
2. Do not leave forceps impressions in the sample.
3. Do not use pin/needles, tongue depressors.
4. Do not wrap specimen in tin foil.
5. A well frozen sample should have a chalky color.
6. Never place the frozen sample in an un-chilled container as the sample will thaw before it freezes again.
7. Puncture the lid of a screw cap specimen container to allow access iospentane to evaporate.
8. Place screw-cap specimen container in -20° C freezer or on dry ice to pre-chill.
9. Add 50-100 ml of iospentane (2-methylbutane) to a Nalgene® or metal beaker. Suspend the beaker in a bath of liquid nitrogen and stir continually with a -200°C thermometer until the iospentane freezes to -125° C and is thickened.
10. With a long forceps' and continuously stirring, place the sample in the iospentane for 15 seconds. (It is important to swirl the sample around to insure that the sample stays in contact with the colder, frozen iospentane).
11. After removing the sample from the iospentane, immediately place the frozen sample in the pre-chilled on dry ice. Do NOT wrap in foil.
12. Place cover on container.
13. Keep sample frozen at -80° or on dry ice from this point on.

IACUC Mouse/Rat Brain Harvesting Schedule

This is a template that is project specific and, if requested, will be created for each project.

In the IHC core, rodent brains are sectioned and representative tissue samples pieces (blocks) are placed in cassettes and immediately placed in a solution called a fixative that will prevent decomposition and preserve the tissue. Usually the fixative is 10 % formalin. The brains are sectioned using a mouse brain matrix (Braintree Scientific, Inc., www.braintreesci.com) Adult Mouse 30 gm Coronal or rat brain matrix (Braintree Scientific, Inc., www.braintreesci.com) Adult Rat 200-400 gm Coronal. After several hours, the tissue block(s) are processed through a series of solutions that wash, dehydrate, and clear the tissue. The tissues are then infiltrated with a substance (usually melted paraffin) which will support the cells and make it possible to cut very thin sections of the tissue. Almost all laboratories accomplish this phase on a tissue processor. This process usually takes approximately 12 to 24 hours.

When possible, the core will use samples that have been processed using the same fixative and processing methods to provide reproducible test results. When using samples from different institutions, the core cannot control the differences in sample handling and processing. The following schedule has been set up for consistent processing in the IHC Core.

Sac/Perfusion Day	2 mm Blocks Made	Tissue Processed
Monday	Tuesday	Tuesday Eve- Wed morning
Tuesday	Wednesday	Wednesday Eve- Thurs Morning
Wednesday	Thursday	Thursday Eve – Friday Morning
Thursday	Friday	Friday Night – Saturday Morning
Friday	Monday (heads stored in 50% Alcohol)	Mon Morn - Mon Afternoon
Saturday	Monday (heads stored in 50% Alcohol)	Mon Morn – Mon Afternoon
Sunday	Monday	Monday Eve – Tues Morning

Validation of New Immunohistochemical Antibodies

The Immunohistochemistry Core at Michigan State University's GRRC, is a service core dedicated to supporting research at Spectrum Health. The Core will provide the principal investigator with reproducible test results. This will require prior validation of antibodies, some standard and some novel to a particular study. When validating a new antibody, the IHC core will follow a set procedure, documenting the methods and antibodies used in the testing conditions, and personnel performing the test. Because the work is research based, there may not be a set standard for a particular antibody on a given tissue type. So workup will include control samples that are positive and negative for staining (if available) to determine sensitivity and specificity.

As different procedures can lead to different staining patterns, it will be the responsibility of the PI to review the staining options and select the procedure/antibody that they feel best represents protein expression, based on their review of the literature and/or manufacturer's antibody data sheets. They may wish to include pathology review of slides to help select best staining conditions. Upon completion of the validation process, the PI will select and approve that performance expectations have been met before the research project proceeds.

The IHC core laboratory will use these guidelines to establish protocols before putting into use (this includes but is not limited to):

1. Correlation to similar morphology and expected results (if available in control specimens). Comparison results of previous tests in the same laboratory (if available). Comparison of SH test to results of to those from another laboratory using a validated protocol (if available through PI). Comparison of new test results to previously validated non-Immunohistochemical tests (if done by PI).
2. For the initial validation, the IHC core lab will test 10 positive and 10 negative samples if available in SH pathology). If available, but PI requests less than 20, the PI will document the rationale for less than 20 (validation will include high and low reactivity for positive cases, when appropriate, and if known.
3. When it is difficult to obtain 10 positive and 10 negative samples for validation purposes, the PI may use validation sets for comparison from other laboratories (if available).
4. When possible, the IHC core will use samples that have been processed using the same fixative and processing methods as cases that have been tested clinically. IHC core cannot control differences in sample handling and processing. This should be taken under consideration when interpreting results.
5. When using a new reagent lot, the IHC core will confirm an assay's performance by testing 1 known positive and 1 known negative.
6. The IHC core will confirm antibody performance with 2 positive and 2 negative cases if there is any change in antibody, dilution, antibody vendor (same clone), incubation or retrieval time (same method).
7. The IHC core will confirm antibody performance by testing 2 positive and 2 negative if there are any changes to fixative type, antigen retrieval (change in pH, different buffer, and different heat platform).
8. The IHC core will document all validation and verifications in compliance with research regulations and requirements.

References: Fitzgibbons, P., Bradley, L., Fatheree, L., Alsabeh, R., Fulton, R., Goldsmith, J., Haas, T., Karabakhtsian, R., Loykasek, P., Marolt, M., Shen, s., Smith, A., Swanson, P. *Principles of Analytic Validation of Immunohistochemical Assays. Arch Pathol Lab Med. 2014;138:1432-1443; doi:105858/arpa.2013-0610-CP*

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HISTOLOGY/IHC WORK FLOW – (SPECIFIC)

ACCESSION & RECEIPT

Numbering, labeling & record keeping*

GROSSING

Examination, dissection, and representative sample(s) taken
under hood (10% formalin)*

PROCESSING

Fixation (formalin), dehydration (alcohol), clearing (solvent) and infiltration (paraffin)
Maintenance* & Processing **

EMBEDDING SYSTEM & STORAGE*

Sakura Tissue-Tek® TEC™5
Samples are placed in small metal molds containing melted paraffin, & allowed to solidify into blocks.

PARAFFIN SECTIONING – Dirty Area

↙ ↘

Microtomy

↙

Each block is placed into a microtome,
sectioned from 4 (thin)-50 (thick) microns
& placed on glass slide(s).
Slides are allowed to air dry overnight &
stored at 2-8°C until needed.**
When ready to stain,
oven dry for 20 minutes at 70 °C.**

Thick sections for Genomics

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Each block is placed into a microtome
section tissue at specific micron requested.
# of blocks for curls
# of curls needed per block
Micron thickness for each block*

HEMATOXYLIN AND EOSIN STAIN (H&E) **

The sections are deparaffinized, re-hydrated through graduated alcohols. Different tissue elements are stained with dye solutions (hematoxylin = nuclei/eosin = cytoplasm) to identify normal and abnormal cell morphology.

The slides are given to the PI to identify area of interest.

↙ ↘

SPECIAL STAIN*

↓

New Protocol

↓

Control blocks chosen for optimization;
reagents purchased; blocks sectioned & dried;
protocol performed; reviewed by PI. If not approved,
repeat with agreed upon changes; if approved,
study group sectioned; dried; stained; sent to PI.

Optimized Protocol*

Section samples
Prep slides for staining Special Stains
Stain slides; Coverslip, Review, Send to PI

Masson's Trichrome*/**

Deparaffinize, run to water;
Mordant in Bouins fluid for 1 hour
Rinse in running water 10 minutes
Stain with Weigert Iron Hematoxylin 10 minutes
Rinse in running water 10 minutes
Stain in Gomori 1-step Aniline Blue for 20 minutes
Differentiate 10 seconds; rinse quickly in DI
Dehydrate through solvents to Xylene; coverslip
Send to PI

Other Special Stain(s)

Protocol times and reagents are unique to each stain.
Each is performed by manual technique

IMMUNOHISTOCHEMISTRIES & FLUORESCENC DETECTION*

↓

New Protocol

↓

Control blocks chosen for optimization;
reagents purchased; blocks sectioned & dried;
Protocol performed; Reviewed by PI. If not approved,
repeat with agreed upon changes; if approved,
study group sectioned; dried; stained; Sent to PI.

Optimized Protocol*

Section Samples
Prep slides for staining IHC/Florescence
Stain Slides, Coverslip, Review, Send to PI

Characterization of Cell Culture Lines*/**

receive 1-10, 8-welled cell culture slides hand label experiment ID info;
Perform manual staining applications for fixation,
blocking, primary antibody & negative control.
Remove well from slides, apply staining label,
load slides onto intelliPATH FLX IHC stainer.
Perform remainder of staining protocol on stainer until complete.
Remove slides, de-hydrate through solvents to Xylene; coverslip
Photograph slides using Nikon Eclipse Ni microscope/camera;
transfer photos to Lab's M drive

Immunohistochemistry Stains (IHC)*/**

Deparaffinize, hydrate slides using Sakura DRS 2000 –SS→H2O
Perform Antigen Retrieval per protocol (offline).
Program intelliPATH: prepare case(s), select protocol(s); Preview,
print labels, load & map slides on intelliPATH FLX, complete
prestart checklist and start machine. Upon completion, remove
slides, de-hydrate through solvents to Xylene. Coverslip.

*Active time / **Non Active time Worked

SPECTRUM HEALTH

Research IHC Core Order Form Instructions

Samples received in IHC Core
Monday – Thursday; 8 AM – 4:30 PM

Questions: Call Jane Tol @ 616.486.8667

1. Please fill out the Digital Form and Email to jane.tol@spectrumhealth.org.

2. Please **print** a copy to send with all samples. **(Note: Samples will not be processed without a copy this form).**

3. PI Name, Contact Information and Request Date are required for all projects **(Note: Samples without this information will not be processed).**

4. IRB/IACUC are required when applicable

5. State on label container what reagent sample is in (95% alcohol/fresh/10% formalin/paraformaldehyde/saline?)

6. If sending frozen samples on Dry Ice, please notify the lab at 616.486.8667 24 hours in advance.

7. Requests for grossing samples must be discussed during initial request.

SPECTRUM HEALTH

Research IHC Core Order Form

Please Fill Out as a Digital Word Fillable Form and Email to:
jane.tol@spectrumhealth.org

Required for all projects

PI: ______________ Phone Number: ________

PI Email: ______________

University/Organization: ______________

Department: ______________ Req Date: ________

IRB #: ________ IACUC #: ________ Grant #: ________

Study Design / Sample Numbers

Fresh, Fixed, FFPE or Paraffin-embedded or Frozen Blocks	Click here to add sample type.	
Sample IDs	Click here to enter number.	
# of tissue blocks	Click here to enter number.	
Sample Species	Click here to enter species.	
Requested # of sections cut for each block	Click here to enter number.	
Section thickness (microns)	Click here to enter number.	
# of antibodies per block *(submit datasheets*); see Additional Antibodies/Stains Form for more antibodies	Click here to enter number.	
Antibody # 1	Click here to add antibody.	
Samples and # of sections per sample	Click here to enter Sample IDs.	Click here to enter # of sections.
Antibody # 2	Click here to add antibody.	
Samples and # of sections per sample	Click here to enter Sample IDs.	Click here to enter # of sections.
# of H & E stains / block	Click here to enter Sample IDs.	Click here to enter # of sections.
# of Special Stains / block; see Additional Antibodies/Stains Form for more stains	Click here to enter number.	
Special Stain # 1	Click here to add stain.	
Samples and # of sections per sample	Click here to enter Sample IDs.	Click here to enter # of sections.
Special Stain # 2	Click here to add stain.	
Samples and # of sections per sample	Click here to enter Sample IDs.	Click here to enter # of sections.

Known Staining Protocols

Yes ☐

No ☐

Work-up Required

Yes ☐

No ☐

Any questions should be directed to IHC Core

Jane A Tol HT (ASCP) Phone: 616.486.8667 jane.tol@spectrumhealth.org

Control Tissue

Antibody/Stain Tissue Controls	Click here to add antibody/stain.		
Known Positive Tissue	Click here to add tissue type.		
Provided?	Yes ☐	No	☐
No Antibody/Stain Control	Click here to enter number.		
Known Negative Tissue	Click here to add tissue type.		
Provided?	Yes ☐	No	☐
No Antibody/Stain Control	Click here to enter number.		

Special Comments/Requests:
Please add any special comments and/or requests here. This box will expand as needed.

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SPECTRUM HEALTH

Additional Antibodies/Stains Form

Please Fill Out as a Digital Word Fillable Form and Email to: jane.tol@spectrumhealth.org

Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
Antibody/stain	Click here to add antibody or stain.	
# Stained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.
# Unstained Sections	Click here to enter Sample ID.	Click here to enter # of sections per Block.

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SPECTRUM HEALTH

Control Tissues Form

Please Fill Out as a Digital Word Fillable Form and Email to:

jane.tol@spectrumhealth.org

Antibody/Stain Tissue Controls	Click here to add antibody/stain.
Known Positive Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Known Negative Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Antibody/Stain Tissue Controls	Click here to add antibody/stain.
Known Positive Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Known Negative Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Antibody/Stain Tissue Controls	Click here to add antibody/stain.
Known Positive Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Known Negative Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Antibody/Stain Tissue Controls	Click here to add antibody/stain.
Known Positive Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Known Negative Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Antibody/Stain Tissue Controls	Click here to add antibody/stain.
Known Positive Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.
Known Negative Tissue	Click here to add tissue type.
Provided?	Yes ☐ No ☐
No Antibody/Stain Control	Click here to enter number.

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