Methodist Health Services Corporation & UnityPoint Health MethodistlProctor Laboratory HISTOLOGY	Page # 1 of 3	Section: UPM HISTO	Procedure # 7.002
	Approved by: see signature block at end of document		Date: 12/8/16
	Date Revised: 9/3/12, 3/11/14, 9/10/15, 2/1/16, 4/22/16, 10/17/16 Date Reviewed:		
	Policy/Revision Submitted by: Terrance Howard/ Bobbie Deppolder/ Dr. Tracy Lundberg		
	CAP Standard: NA		
POLICY GUIDELINE ON: **Validation of Immunohistochemistry/In-situ Hybridization Assays**			

I. PRINCIPLE:

The performance characteristics of each assay in the immunohistochemistry/in-situ hybridization laboratory must be appropriately validated before being placed into clinical use.

II. CLINICAL SIGNIFICANCE:

Immunohistochemical/in-situ hybridization assays utilize antibodies or probes to target the cells for certain antigens or genetic material. Through the use of a combination of immunohistochemical stains, in-situ hybridization assays and routinely stained H&E sections, a diagnosis can be rendered on many poorly differentiated neoplasms. All assays used in patient diagnosis must be fully validated.

III. SPECIMEN:

10% Neutral-buffered formalin fixed tissues (4 um sections) are preferred.

IV. REAGENTS:

1. Primary Antibody or Probe
2. Protease 1-3 (as needed)
3. Hematoxylin Counterstain
4. Bluing Reagent – lithium carbonate solution of high pH.
5. Amplification Kit (Cat# 760-080) – AMPLIFIER A&B (as needed to amplify weakly staining antibodies)
6. Detection Chemistry (unless otherwise recommended, all new antibodies will be validated using ultraVIEW DAB or ultraVIEW Red Detection Kits as these kits are avidin/biotin free and will prevent the staining of endogenous biotin).
7. Bulk Fluids (see 7.1 Immunohistochemical Procedure – Benchmark XT and Benchmark ULTRA).
8. Dawn Detergent – to remove the liquid coverslip from the slide before coverslipping on the Sakura coverslipper.

V. PROCEDURE:

A. New assays:

1. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. If you are unsure where to start:
    1. Use the primary antibody manufacturers suggested protocol for Ventana instrumentation.
    2. Call Ventana Customer Support at (800) 227-2155 for additional assistance in optimizing the antibody. Ventana may suggest protocols or may ask for unstained slides to be sent to the Applications Lab. In the event that the Applications Lab is used, this will be facilitated by the Ventana Account Executive but is a free service Ventana offers.

2. Once optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of this validation will vary with antibody and is at the discretion of the Laboratory Director. Means of validation may include, but are not limited to: 1) correlating the results using the new antibody with the morphology and expected results; 2) comparing the results using the new antibody with the results of

prior testing of the same tissues with a previously validated assay; 3) comparing the results using the new antibody with the results of testing the same tissue in another laboratory with a validated assay; or 4) comparing the results using the new antibody with previously validated non-IHC tests or testing previously graded tissue challenges from a formal proficiency testing program. For initial validation, 90% overall concordance between the new test and the comparator test or expected results is required, unless otherwise noted.

3. Non-Predictive Markers: Whenever possible, a panel of 10 positive and 10 negative neoplasms will be used at a minimum. An exception to these requirements is that studies may not be feasible for antigens such as ALK that are only seen in rare tumors. Any exception determined by the Laboratory Director should be documented and rationale for the decision recorded.

4. Predictive Markers: Validation for ER/PR and HER-2/neu assays will follow current ASCO-CAP Guidelines.
    a. ER/PR validations require a minimum of 40 cases (20 positive and 20 negative) for FDA-approved/cleared tests. A minimum of 40 positive cases and 40 negative cases are required for laboratory developed tests. (LDT's)Validation should be performed by comparing the results with another assay that has been appropriately validated. Acceptable concordance levels are 90% for positive results and 95% for negative results. If significant changes are made to the testing methods (e.g. antibody clones, antigen retrieval protocol or detection system), revalidation is required.
    b. HER-2/neu assay validations must be performed on a minimum of 20 positive cases and 20 negative cases for FDA approved tests, or 40 positive cases and 40 negative samples for LDT's. Equivocal samples need not be used for validation studies. If initial validation of existing assays does not meet the current standard, it must be supplemented and brought into compliance. It is permissible to do this retroactively by review of performance on past proficiency testing challenges. Validation may be performed by comparing the results of testing with a validated alternative method (i.e. IHC vs. FISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs. Acceptable concordance levels are 95% for positive and negative results. All Methodist HER-2/neu testing will be done on specimens fixed ONLY in 10% neutral buffered formalin. If significant changes are made in testing methods (e.g. antibody clone, antigen retrieval protocol or detection system, FISH probe or pretreatment protocol), revalidation is required.
    c. Other Predictive Markers: Validation should include a minimum of 20 positive and 20 negative tissues. Any exception determined by the Laboratory Director should be documented and rationale for the decision recorded.
5. Decalcified Tissue: If decalcified tissues will be tested clinically, validation must also be done on decalcified tissue. The laboratory should test a sufficient number of such tissues to ensure that assays consistently achieve expected results. The laboratory director is responsible for determining the number of positive and negative cases to test.

B. New Reagent Lot and shipment Verification: The performance of new lots and shipments of antibody and detection system reagents are compared with old lots on the same tissue block before (or concurrently with) being placed into service:
    1. When a new antibody lot or shipment is received the last lot stain for that antibody should be retrieved from the "Lot-to-Lot Validation" storage box along with a new control slide from the same block (block should be kept in the "Lot-to-Lot Validation" storage box to ensure availability). The new antibody should be used to stain the new control slide and then delivered to the pathologist with the previous lot stain for comparison. The pathologist will notate any changes in performance with the new lot or shipment (assessing both positive controls and internal negative controls) on the Ventana Run Report and approve the antibody for use. The new slide replaces the old slide in the "Lot-to-Lot Validation" storage box and the Run Report is

filed in the Lot-to-Lot Binder. Fill out the "Antibody Validation Log" form in the Lot-to-Lot Binder. The old slide should be filed.

2. When a new detection system lot is received the last detection lot-to-lot validation slide should be retrieved and delivered to the pathologist along with a newly stained control slide (from the same block as the last validation slide) with the test detection lot. The pathologist will notate any changes in performance with the new lot (assessing both positive controls and internal negative controls) on the Ventana Run Report and will approve the detection kit ready for use. The new slide replaces the old slide and the Run Report is filed in the Lot-to-Lot Binder. The old slide should be filed.

C. New Clone Verification: The performance of new clones of antibodies are verified using a limited antibody validation. A minimum of five cases should be chosen for testing (ideally five cases used in the original validation, if possible). In addition, the block from the "Lot-to-Lot Validation" should be stained and compared with the previous lot of the former clone.

D. Assay Performance Verification: The performance of an existing, validated assay will be verified when any of the following changes are made: antibody dilution, antibody vendor, fixative type, antigen retrieval method (e.g. change in pH, different buffer, different heat platform), antigen detection system, tissue processing or testing equipment, environmental conditions of testing (e.g. laboratory relocation), or laboratory water supply. A minimum of five cases should be chosen for testing (ideally five cases, including at least two known positive and two known negative cases, used in the original validation, if possible). In addition, the block from the "Lot-to-Lot Validation" should be stained and compared with the previous lot.

E. Instrument Performance Verification
The performance of all instruments is verified upon installation, and after major maintenance or service to ensure that they run according to expectations. After decontamination or service, a CD20 is performed to verify the instrument is staining properly. After installation of an instrument an array of antibodies: C-kit, p63. PAX-5, CK7, CD3, CMV DAB, Mart-1 Red, CMV Red, PIN-4, BRAF, Kappa ISH, Lambda ISH, is selected to represent each detection method performed on the instrument. Two known positive and two known negative cases will be chosen for each antibody, reviewed by the Pathologist section director for histology, and documented on the new instrument validation log.

VI. MAINTENANCE AND STORAGE

A. All policies and procedures are reviewed every two years, (except for Safety procedures which are yearly) by Laboratory Administration and or the Medical Director of the Laboratory or designee when there are changes in practice standards, or requirements.
B. All policies and procedures are reviewed every two years (except for Safety procedures which are yearly) by staff or at the time new or revised ones are put in effect.
C. All policies are retained 8 years after being discontinued or revised.
D. All procedures are retained 2 years after being discontinued or revised

UnityPoint Methodist Laboratory is a CAP accredited facility. As of 7/1/11 the responsibility of new and/or substantially revised policies and procedures will be restricted to the Laboratory Director whose name appears on the CLIA certificate, and signature appears below. The biennial review will be completed by the Administrative Director.

REVISION HISTORY (began using in 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	New Release of Procedure	D. Spears	9/3/12
2	Added verbiage to specify that new reagent lots must be tested on the same tissue block for comparison.	D. Spears	9/30/12
3	Added verbiage to specify that new antibody clones must be tested in a limited validation as well as on the lot-to-lot tissue block and compared to previous staining.	D. Spears	3/11/14
4	Added verbiage to explain different acceptable types of validation. Added validation requirements for predictive markers other than ER/PR/HER2. Included Assay Performance Verification to ensure any changes in procedure would qualify for re-validation.	D. Spears	9/10/15
5	Added verbiage to explain validation requirements for FDA approved tests and laboratory developed tests.	T. Howard	1/28/16
6	Add "and shipments" after new lot for validation.	B. Deppolder	10/17/16
7	Added "E. Instrument Performance Verification"	T. Howard	12/6/16

Reviewed by

Lead	Date	Coordinator/ Manager	Date	Medical Director	Date
		Dana Spears	9/4/12	Denise V. Tirpak	9/4/12
		Dana Spears	9/30/12	Elizabeth A. Bauerlein MD	10/1/12
		Dana Spears	3/11/14	Elizabeth A. Bauerlein MD	3/11/14
T. Howard	1/28/16	Bobbi Harris	1/28/16	Elizabeth A. Bauerlein MD	2/1/16
T. Howard	4/22/16	Bobbi Harris	4/22/16	Elizabeth A. Bauerlein MD	5/3/16
Terrance Howard	10/17/16	Bobbi L Deppolder	10/17/16	Elizabeth A. Bauerlein MD	10/17/16
Terrance Howard	12/6/16	Bobbi L Deppolder	12/7/16	Tyler Gross	12/8/16

Antibody Validation Log											
	Run Date	Antibody	Rec Date	Ship Date	Lot #	Run #	Instrument	Acc/Unacc	Comments	Tech	Path
1											
2											
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