Clinical Histology
Procedure
Histo03.01

Automated Immunohistochemical Staining
Utilizing the Ventana Benchmark Instrument

Final Approval: May 2010  Effective: May 2010  Next Review Date: May 2012

List all stakeholder(s) and dates of approval:
Stakeholder Name(s): Shelly Siegel, HT  Date: 9.21.10  Reviewed ☑  Revised ☐
Stakeholder Name(s): Mark Magilner, MD  Date: Reviewed ☐  Revised ☐
Stakeholder Name(s): Shelly Siegel Date: 5/3/11  Reviewed ☑  Revised ☐
Stakeholder Name(s): Date: Reviewed ☐  Revised ☐

Describe briefly the most recent revision made to this policy, procedure or protocol & why:
New Procedure

Purpose/Policy Statement:
The demonstration of antigens in tissue and cells by immunostaining is a process involving first, the binding of an antibody to an antigen of interest and second visualization of the bound antibody by an indirect biotin-avidin system coupled to an enzyme product. Ventana Medical Systems Detection Kits are indirect biotin-avidin systems for detecting specific mouse and rabbit monoclonal or polyclonal primary antibodies; The Detection Kit utilizes biotinylated secondary antibodies to locate the bound primary antibody and is followed by the binding of an avidin enzyme conjugate to the biotin. The complex is then visualized using a precipitating enzyme generated product.

Ventana Medical Systems Detection Kits are:
- The Ventana Medical Systems ISH iVIEW™ Blue Detection Kit
- The Ventana Medical Systems ultraView universal AP Red Detection Kit
- Ventana ISH- INFORM Kappa, Lambda, Negative control probe
- The Ventana Medical Systems ultraVIEW Detection Kit(s)

Slide barcode labels operate the instruments automated, sequential dispensing of appropriate reagent and antibody dilutions.

Definitions:
- n/a

**STEPS / KEY POINTS**

**SPECIMEN:** Paraffin sections of 3-micron or 4-micron thickness are collected from an 40-44°C flotation bath containing deionized water and mounted on 25 x 75 mm positively (+) charged slides or cytological samples prepared by smear or Cytospin® technique fixed in 95% alcohol.

**MATERIALS, REAGENTS:** VENTANA accessories:

VENTANA EBAR Slide Barcode Labels
3% Hydrogen Peroxide
Red Counterstain II - 780-2218
Negative Control IgMouse
Negative Control Rabbit Ig
EZ Prep (10X Concentrate) 950-102
Liquid Coverslip (LCS High Temp) 650-010
Cell Conditioning 1 (CC1) 950-124
SSC – Sodium Chloride Sodium Citrate 950-110
Reaction Buffer (10X Concentrate) 950-300

Hematoxylin II – 790-2208
Protease I – 250-2018
Ventana predilute antibody dispenser and/or
dispenser prepared by SHRL Histotechnologist
Hematoxylin – 760-2021
Bluing Reagent - 760-2037
Antibody Diluent (negative control) - 251-018

INSTRUMENTATION OR EQUIPMENT: VENTANA BENCHMARK XT instrument.

PROCEDURE:

1. From a tissue flotation bath of deionized H2O, retrieve the cut paraffin section of the patient case on the bottom of the microslide which contains the known positive tissue or multi-tissue controls on the top of the slide for the requested antibody. Only in circumstances where the patient tissue sample is larger than the provided slide space, use the slide as a control only and place the patient tissue sample on a 25 x 75 mm positively (+) charged microslide.

2. Using a permanent marking pen, label slide with:
   - antibody name
   - procedure date
   - accession number (at bottom edge of the extended paint).

3. Routinely dry slides at 60°C in convection oven for 30 min. EXCEPTION: HER-2/neu slides are dried overnight at room temperature per FDA approved protocol.

4. Remove from IHC refrigerator the Reagent Carousel Tray(s) containing any and all dispensers required for protocols being performed.
   Prepare for operation by uncapping, priming, and visually validating the satisfactory reagent volume of all dispensers.

5. Similarly install onto Reagent Carousel Tray(s) all primary antibody dispensers that will correspond with the prepared slides and validate the satisfactory reagent volume of all dispensers.

6. Install the Reagent Carousel Tray(s) onto the Benchmark instrument

7. Allow antibodies/reagents/stains to reach room temperature prior to staining.

8. Prepare slide barcode labels.

9. Turn ON VENTANA BENCHMARK XT instrument at the operational PC CPU and at also at the switch located on the front of the instrument, lower left side, behind the bottom left cabinet door.

10. From the PC desktop menu, select the VENTANA icon.
    WINDOW Ventana Medical Systems – BENCHMARK
    SELECT Barcode Label icon
    SELECT Protocols
    WINDOW Select Slide Labels
    TAB Protocols
    BULLET BENCHMARK
    SELECT TEMPLATE Salem Hospital
    SELECT IHC PROTOCOLS
    HIGHLIGHT the desired protocol
    ENTER the number of copies
    SELECT Add
11. Apply each printed VENTANA bar-code label to its corresponding slide. Apply the label over the painted section of the slide with the label name at the top edge of the slide and exactly parallel to the top edge, without extension over any slide edge.

12. Open instrument Access Door to the Reaction Chamber and install an individual slide onto the Slide Carousel, positioning each with the bar-code label facing up and on the inner perimeter of the carousel.

13. Verify that each slide is secure and level.

14. Close instrument Access Door to the Reaction Chamber.

15. WINDOW Remaining Run Time will be displayed as instrument begins operation.

16. An audible alarm will sound at end of the staining program. In WINDOW Staining Module Messages, Staining Module Program Ran To Completion will appear.

17. Following completion of the instrument staining program:
   SELECT Sign Off icon
   SELECT Close

18. At the end of each workday, perform automated instrument-cleaning function.

19. Turn OFF BenchMark XT instrument.

CALIBRATION: Biannual calibration is performed on each micropipette and recorded on the Continuous Quality Improvement record sheet in the Immunological Staining Manual.

QUALITY CONTROL:
1. Positive result on negative control may be an indicator of endogenous biotin in certain types of tissue sample (brain, breast, kidney, or most frequently liver) in some patient cases. The resulting negative result on the negative control validates the prior staining reaction as endogenous biotin.

2. Daily prime and visually validate satisfactory reagent and antibody volume content of each dispenser (PROCEDURE Step 3 and Step 4).

3. Each time the procedure is performed a known positively reactive tissue for the specified antigen shall be simultaneously processed with the patient tissue.

4. For each patient block on which IHC is performed, a corresponding patient NEGATIVE control slide shall be simultaneously stained with the patient slide(s), excluding the application of the Primary Antibody. NEGATIVE control receives “most harsh” treatment of all protocols performed on the case.

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6. Ventana PATHWAY HER-2 4 in 1 Control Slide validates staining quality, per each protocol run.

7. The pH of Reaction Buffer is measured and documented each time a Working Solution is prepared.

8. Each time a five-gallon carboy of bulk ancillary reagent is emptied, it shall be decontaminated according to the Benchmark Instrument Quarterly maintenance protocol and then rinsed thoroughly with DH2O, wiped dry and/or drained and then air dried prior to refilling with newly prepared reagent.
9. Routine instrument maintenance is performed daily, monthly, and quarterly by a staff Histologist and documented in the instrument software program.

10. New lots of antibody and detection system reagents are compared to the previous lot using an appropriate panel of control tissue. Documentation of validated new antibodies and detection system reagents are maintained on the **Antibody Quality Control Sheet**. Control tissue blocks are validated prior to patient testing. It is documented and maintained on the **Tissue Block Quality Control Sheet**. The director of Pathology validates all antibodies and tissue control blocks before patient use.

11. P/M is performed annually by a Ventana representative under a purchased manufacturer service/maintenance contract.

**PROFICIENCY TESTING:** CAP Proficiency Survey / Immunohistochemistry, CAP HistoQip.

**RESULTS:**
1. Positive results will be demonstrated with variations of colored staining based upon use of specific detection kit systems; negative results will be demonstrated by the presence of counterstain only.

2. Positive result on negative control may be an indicator of endogenous biotin in certain types of tissue sample (brain, breast, kidney, or most frequently liver) in some patient cases. See QUALITY CONTROL.

**PROCEDURE NOTES:**
1. Dispensers may be placed randomly on the Reagent Tray with the following exceptions: DAB and DAB H₂O₂ must be adjacent.

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**Equipment/Supplies** (If Applicable):
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**Form Name & Number or Attachment Name** (If Applicable):
Histo03

**Author Position:**
Lead Histologist

**Review/Revision Authority** (Position Not Individual Name):
Lead Histologist

**Expert Consultant Position/s** (Not Individual Name/s):

**References** (Required for Clinical Documents):
2. NexEX Sofstware Manual, Version 8.0, Ventana Medical Systems, Part Number 1390400 Revision C.
3. Ebar SLS Printer Manual, Ventana Medical Systems, Part Number 1722500 Revision -.

**MANUFACTURER’S PACKAGING BROCHURE/INSERT:** N/A

**Is there a Regulatory Requirement?** Yes ☐ No ☐
If yes, insert requirement information here:

**Review History** (No Changes):

**Revision History** (Note changes in area under header):

**Computer Search Words:**