PATHOLOGY AND LABORATORY MEDICINE
Mount Sinai Hospital
Toronto, Canada M5G 1X1

MOUNT SINAI HOSPITAL

ER/PR

Immunohistochemistry (IHC) Standard Operating Procedure

Special Histology Hard Tissue Procedure Manual

Project Specific Protocol

Version #2

Authorization by Study Director: _____ Date: _____
Dr. Brendan Mullen

Written by. _____ Date:_____

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Overview Flowchart

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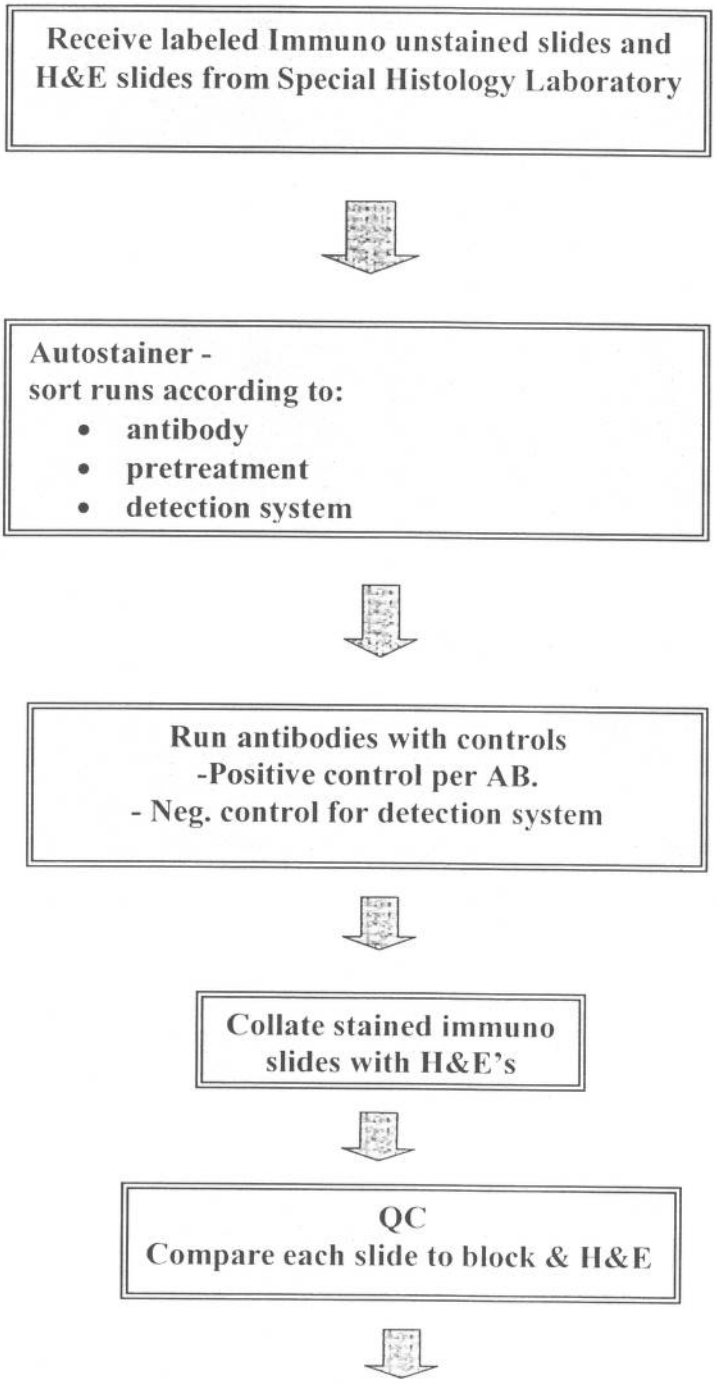

Give Slides to Pathologist

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Principles of Immunohistochemistry:

Refer to ***Histology/ Immunohistochemistry procedure manual SOP# 3: Principles of Immunohistochemistry***

Detection Systems:

Optimal antibody titer and detection system are predetermined for each of the antibodies to be run and and the information recorded .

Methodologies

First few runs (refer ***Attached Excel Sheet***) are run on DAKO Autostainer (refer ***Histology/Immunohistochemistry SOP***) . After validation and correction of Antibody dilution the project is continued on LabVision Autostainer 720.

Automated IHC Method:

Programming the Autostainer - LabVision 720:

All information regarding antibody titer and detection system used is preprogrammed into the computer in the protocol template. (refer to ***Labvision manual, section 13.6.1 Creating a New Staining Program)***.

Organization of Slides:

Group the slides in accordance with the antibody, stated on the QC Antibody chart. Both antibodies (ER and PR) using Horse anti-mouse (HAM)/Elite-ABC detection system and grouped together on the same run. Positive controls for each antibody are included. The decision to use negative controls for every block is left up to the discretion of the pathologist. (refer to ***Attached Excel sheet)***.

Pretreatment:

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Blocking endogenous pigments:

Interstitial peroxidase activity may be present in the tissue sections . In order to suppress endogenous peroxidase activity in formalin fixed tissue, perform the following:

Deparaffinize the slides and take down to water using descending grades of alcohol. Block the endogenous peroxidase activity by using a 3% aqueous solution of hydrogen peroxide for a 15 minutes. Wash well in 2 changes of tap water.

Antigen Retrieval:

In order to unmask the antigen sites the Microwave Antigen Retrieval method is employed using the Micromed TT Mega with Tris Buffer pH 9.0 for both the antibodies . The method can be found in the ***Histolgy/Immunohistochemistry procedure manual SOP#4.***

Blocking of Non-Specific binding of Endogenous Avidin/Biotin Activity

Biotin is distributed in a wide variety of tissues. These tissues may bind avidin, biotinylated horseradish peroxidase or other Biotin/Avidin system components which cause non-specific binding. This occurrence can be suppressed by adding Avidin to the normal blocking serum and biotin to the primary.

Setting up the grid and map on the Autostainer:

The autostainer capacity is 84 slides depending on the number of antibodies and the number of detection systems used. Program the autostainer with the number of slides for the antibodies being run. The detection system information is pre-programmed into the computer. The software devises a map and a grid of the antibodies to be tested. The map and the grid for each run are saved, printed out, and logged.

Preparing reagents for the Run

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Antibody Preparation:

The optimal dilutions (*refer* ***Routine Antibody Data Sheet***) of the primary antibodies are made up according to the volumes stated on the Reagent map. The primary antibodies are diluted in Antibody diluent with the addition of Biotin. The quantities are prepared fresh for each run.
Primary Antibody used :

- ER – clone 6F11 (Vector ;Cat # VPE613)
- PR – clone PgR1294 (DakoCytomation ; Cat # M3568)

The blocking serum plus Avidin, the secondary and the tertiary antibodies are made up in Tris buffered saline and measured into the reagent vials according to the volumes stated on the reagent map.

Loading the Autostainer

The slides are loaded in the autostainer in accordance with the map. The positions of the reagent vials in the rack are checked against the map.

Completing the Staining Run

At the end of the staining run, save the Run Log. Remove the slides from the machine. Click on the Clean button to run the clean cycle.

Counterstaining the slides

Wash the slides in running water for a few seconds. Counterstain in Mayer's Hematoxylin, followed by washing in warm running water. Differentiate 10 -20 seconds in Scott's Tap Water followed by washing in warm running water. Dehydrate clear and mount. The slides are then collated with their corresponding Hematoxylin and Eosin stained slide.

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Quality Control

The quality control performed for the project is in compliance with standard laboratory practices (refer to ***Histology/Immunohistochemistry procedure manual SOP#10 Quality Control Program)***.

Each antibody tested is run with a positive control. Negative controls were run on each patient block at the beginning of the project .After Pathologist review it was decided that no Negative control per block would be required .Instead there was one Negative control per run (refer to ***Attached Excel Chart***). The control slides are dated along with the test slides that are run in the same batch and the positive control slides are filed in the laboratory archive. The Negative control slides / per block/ are filed with the test project slides .The Negative control slide /per run/ will be kept at our location.

Upon completion of staining, each IHC stained slide is compared to the block it was cut from and its corresponding H&E slide.

Each slide stained is checked microscopically for stain and section quality this is recorded on the **ER/PR *QC sheet.***

All discrepancies that arise during processing, cutting, and staining are recorded on a "Quality Assurance Problem Sheet." The description of the problem and the follow-up and/or corrective action are included on this document. These sheets are kept in the "QC Documentation 2005" project binder .