Preparation of Lymph Nodes from a Colorectal Specimen

Purpose: This document provides standardized direction for the collection and submission of lymph nodes from a colorectal specimen. Use of this procedure will support the Canadian Partners Against Cancer (CPAC) goal of having 90% of colorectal specimens removed for cancer have a minimum of twelve lymph nodes be examined.

Sample: Colorectal excision specimens only; exclude specimens for rectal cancer where neoadjuvant therapy may have been completed.

Procedure:

Fixation:

- There is no set time required for fixation of the colonic adipose tissue that contains the lymph nodes. However, complete fixation of the lymph nodes is required prior to processing to maintain the morphologic structures and avoid nuclear bubbling.
- Place specimens in 10% neutral buffered formalin for transport or immediately upon arrival in the pathology Laboratory if transferred in a fresh state.
- Secondary fixation to assist with lymph node identification can be completed using in house developed alcoholic formalin or a commercial fixative such as Hartman's (modified Davidson's) or Dissect Aid.

Lymph Node Dissection:

- Examination of the colonic adipose tissue for lymph nodes can be completed many ways dependant on the experience of the prosector.
    - Examine the colonic adipose tissue prior to complete fixation, as visual identification of lymph nodes is often possible due to their colour and texture being different from blood vessels and fibrous tissue.
    - Examine the adipose tissue where it attaches to the colon prior to removal, as this area can yield a high number of lymph nodes.
    - Remove adipose tissue and complete fixation in 10% neutral buffered formalin. Upon completion of second examination, if twelve lymph nodes cannot be identified secondary treatment with alcohol based fixative is an option.

Block Preparation:

- Multiple (up to 5) small lymph nodes less than 4 mm in diameter can be placed into the same cassette. Record the number of lymph nodes as part of the gross description.

- Lymph nodes larger than 4 mm should be bisected or serial sectioned into 2-3 mm slices. Submit all slices into cassettes. Record information about each lymph node dissected and cassette summary as part of the gross description.

Slide Preparation:

- Position block in microtome block holder following default position i.e. label to the right.
- Trim gently into the block surface until a full cross section of all the tissue pieces is visible. If required before cutting a ribbon of sections, cool the block surface by removing the block from the microtome and placing on ice or a cold plate. Limit exposure time on the wet ice surface or place uncut block surface on the ice to avoid absorbing water into the cut surface of the tissue pieces. Absorption of water may cause the tissue swell and create an uneven cutting surface resulting in incomplete sections (holes) when ribbon created.
- Cut a single (3 micron) section, place on one slide and stain with H&E.
- Discontinue default preparation of multiple slides, levels and IHC stains. This includes cutting and holding slides for IHC just in case ancillary testing is required.

Procedure Notes and Limitations:

- When using an alcohol-based fixative (in house or commercial) to identify lymph nodes, users must be aware that primary alcohol fixation effects tissue morphology (cell shrinkage) and hardens tissue that can create microtomy problems. Avoid these artifacts by completing the initial fixation with 10 % neutral buffered formalin prior to transfer to an alcohol-based fixative.
- Do not over crowd the cassettes with multiple lymph node sections, sufficient space is required to allow fluid circulation during processing.
- If less than 12 lymph nodes are located after an extensive examination, this may be a reflection of regional anatomic variation or the result of an inadequate resection, rather than technical inefficiency of the prosector.
- Additional levels, slides and stains (histochemical or immunohistochemical) can be requested as part of the initial examination, based on clinical need and pathologist discretion. However, multiple levels, additional H&Es and stains should not be the default process.

Statement of Use: Best Practice Recommendation; approved by the Provincial Anatomical Pathology Advisory Group. This may be included in Health Authority / facility specific procedures.