The Lymph Node Locator 300: A Novel Technique for Lymph Node Retrieval in Colorectal Cancer Specimens

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Introduction

Colorectal cancer (CRC) is the most common gastrointestinal malignancy and the third most common cancer overall for both sexes in Canada with an estimated 26,900 new cases in 2020.[1,2] In the United States, CRC is the second most common cancer resulting in death with an estimated 52,580 deaths in 2022.[3,4] The American Joint Committee on Cancer (AJCC) stages colorectal cancer from 0 to IV depending on the extent of the tumor (T), number of lymph nodes with metastatic spread (N), and extent of distant metastasis (M).[5] Identification of lymph node metastasis increases a patient's stage to a minimum of stage III.[5] Five-year survival rates in patients without lymph node metastasis average 70-90%, whereas in patients with positive lymph node metastasis the survival rate is lower at 30-70%.[6,7] Adjuvant chemotherapy is recommended for patients with stage III CRC and, as such, the identification of lymph node metastasis has important clinical significance.[8] The Union for International Cancer Control (UICC), College of American Pathologists (CAP), and the AJCC state a minimum of 12 lymph nodes must be retrieved from CRC resection specimens to obtain an accurate diagnosis and avoid false negatives, however, the minimum of 12 lymph nodes is often not met.[5,9–12] Lymph nodes are typically retrieved from the adipose tissue of CRC specimens by means of manual palpation by the pathologists' assistant. This technique is labor intensive and time consuming, requiring identification of lymph nodes based on feel, consistency, and visual cues.

The size and number of lymph nodes within CRC resection specimens can be influenced by multiple factors, including advanced patient age,[12,13] tumor location,[11,12,14,15] extent of surgical resection,[15,16] and use of neoadjuvant therapy.[13,17–19] Fewer lymph nodes may be identified in older patients, however, due to an interplay of more conservative surgical approaches and age-related immunological degenerative changes.[12,13] Regarding tumor location, lymph node retrieval in colonic resection specimens is often greater compared to rectal specimens.[14] Furthermore, right-sided colon cancer specimens tend to contain significantly more and larger lymph nodes compared to left-sided specimens.[11,12,15] Increased dissection efforts may be required for left-sided colon and rectal cancer specimens to ensure at least 12 lymph nodes are retrieved for histological assessment. A moderate correlation has been demonstrated between the length of the specimen and the number of lymph nodes present within the adipose tissue.[16] Additionally, lymph node metastases have been identified in lymph nodes up to 5-10 cm from the tumor.[15] A focus on palpating and identifying large lymph nodes in close proximity to the tumor may be insufficient to capture the true status of lymph node metastasis in CRC specimens and pathologists' assistants must investigate the adipose tissue throughout the length of the specimen for distant lymph nodes. Neoadjuvant therapy is the use of radiation and/or chemotherapy prior to surgical resection and is recommended for patients with clinical T3 or T4 rectal adenocarcinoma.[20] While neoadjuvant therapy may lead to a reduction in size or stage of the tumor, it also leads to the shrinkage, fibrosis, and replacement of lymph nodes with adipocytes rendering manual palpation and visual identification of lymph nodes more challenging.[18,20]

A correlation between lymph node size and metastasis has not been clearly identified.[21] Some studies have demonstrated that large lymph nodes more often harbor metastases compared to smaller nodes.[15,22,23] In contrast, research has also identified that small lymph nodes (2-6 mm in diameter) contain nearly half of all lymph node metastases within neoplastic gastrointestinal specimens.[22–26] A growing body of research indicates as many lymph nodes as possible should be identified and submitted for histological analysis as there is a positive association between the number of lymph nodes assessed histologically and improved patient prognosis in CRC.[10,25,27,28] As such, identifying all lymph nodes regardless of size aids in obtaining the most accurate diagnosis for the patient.

Efforts to increase lymph node yields have focused on improving visualization for the pathologists' assistant by means of fat clearing agents, such as GEWF (glacial acetic acid, ethanol, distilled water, and formaldehyde) or O-Fix (Leica Biosystems)[29], and injection of dyes, such as methylene blue.[26,29–32] Fat clearing agents aid in identifying lymph nodes by making their appearance more white, a process that improves yields of small (<5 mm) lymph nodes.[32] Despite the ability of fat clearing agents to increase lymph node yields, routine use can be costly and time consuming and the number of lymph nodes identified remains subjective between laboratory personnel.[11,17,33]

Unlike fat clearing agents, fat eluents such as acetone can dissolve adipose tissue. Acetone compression is a technique where adipose tissue is dissolved in acetone and compression is applied to displace the adipose.[34] Through displacing the adipose, the specimen reduces to 5-10% of its initial weight, allowing for all the tissue to be submitted for histological evaluation in a reasonable number of tissue cassettes. The compressed material consists of all possible lymph nodes in addition to nerves, vasculature, and the cell membranes and nuclei of lipocytes within the mesentery.[34,35] As all possible lymph nodes are submitted, combining a fat eluent such as acetone with compression standardizes the process of lymph node harvest and increases the likelihood of recovering and exceeding the minimum 12 lymph node guideline.[34–36] Acetone compression does not alter the morphology of lymph nodes, including parameters such as area, roundness, solidity, or histological quality when compared to traditional manual palpation.[34–36] Of note, acetone compression has been shown to retrieve an increased number of smaller lymph nodes (<2 mm in diameter) compared to manual palpation.[35,36] Furthermore, acetone compression outperforms manual palpation in lymph node retrieval from neoadjuvant therapy specimens.[35]

The earliest mention of acetone compression in the literature stems from a report by Brown et al.[16] After a lymph node search by manual palpation of a colonic adenocarcinoma resection specimen, the remaining mesentery was stripped and dehydrated

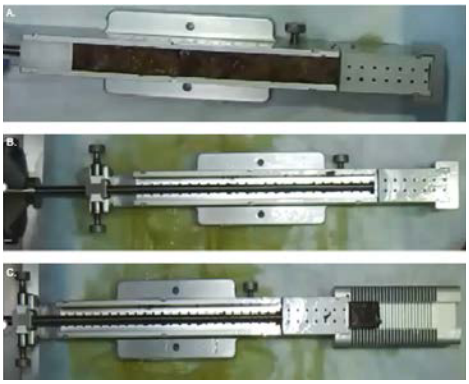

Fig. 1: Demonstration of compressing adipose tissue with the LNL300. Tissue is first placed in the loading chamber of the device (**A**). Following tissue compression, the lid of the device is removed demonstrating the tissue has been displaced (**B**). The cutting chamber is attached, and the tissue is pushed in to align with the knife-guidelines in preparation for sectioning (**C**).

in serial washes of alcohol and acetone.[16] The washings occurred over several days and the compression was achieved with a rolling pin.[16] Recent research on acetone compression demonstrates that acetone compression can be achieved in a much shorter period of time, with effective fat elution possible after four hours in heated acetone or after 12-24 hours in room temperature acetone.[34–36] Compression devices used in the literature have historically been created in-house. Recently the first commercially produced mesenteric fat compression device has become available for use in harvesting lymph nodes for pathology services.[37] The aim of this study was to validate the efficacy of the Lymph Node Locator 300 (LNL300) by Omnia Inventa Medical, Inc. for use in a high volume, acute care pathology laboratory service. To our knowledge, this is the first such study performed in Canada. The authors disclose that the device was provided, free of charge, from the manufacturer during a limited trial period for the study, after which the device was returned. The authors have no conflicts of interest to declare.

Methods

This study utilized adipose tissue from previously grossed CRC specimens with a final diagnostic report issued. The specimens were initially received at four adult acute care hospitals in Calgary, Alberta, Canada. The Calgary Metropolitan Region has a population of approximately 1.4 million residents, but the catchment area of the four adult hospitals in this region is larger and includes much of southern Alberta.[38] Specimens chosen were past the minimum eight-week retention period required by the local pathology service and were ready for discard. The use of patient data and specimens in this study was approved by the Health Research Ethics Board of the Alberta Cancer Committee (Study ID# HREBA.CC-21-0418).

Specimens were selected following a data pull of the final pathology report and patient demographics of all complex gastrointestinal specimens received in 2022. Each case was screened for exclusion criteria: benign specimen, re-excision negative for residual adenocarcinoma, lymphoma, gastrointestinal stromal tumor, and neuroendocrine tumor. The laboratory accession numbers of the remaining eligible cases were used to locate the physical specimens stored in 10% neutral buffered formalin (NBF) since the time of initial gross examination. Of the 34 eligible colon and rectal adenocarcinoma specimens identified, 25 specimens were included in this study due to time and resource limitations.

Age at time of surgery	40-99 (66.8)
Sex	
Male	13 (52%)
Female	12 (48%)
Type of procedure	
Right hemicolectomy	14 (56%)
Left hemicolectomy	2 (8%)
Sigmoidectomy	3 (12%)
Low anterior resection	5 (20%)
Abdominoperineal resection	1 (4%)
Post-neoadjuvant therapy (%)	
Yes	4 (16%)
No	21 (84%)
pT stage (%)	
T1	2 (8%)
T2	2 (8%)
T3	13 (52%)
T4	8 (32%)
Initial pN stage (%)	
N0	17 (68%)
N1a	3 (12%)
N1b	2 (8%)
N1c	0
N2a	3 (12%)
N2b	0
Number of lymph nodes	
Initially grossly identified	6-62 (25)
Initially histologically identified	8-45 (23)
Fat clearing agents used during initial gross (%)	
Yes	3 (12%)
No	22 (88%)
Weight of adipose tissue (g)	27.9-481.6 (204.7)
Number of cassettes used at initial gross	4-25 (14)

Table 1: Patient sample characteristics (n=25) presented as a range (mean) or frequency (%).

The following variables were extracted from the patient's final pathology report for all specimens included in the study: age at time of surgery, sex, type of surgery performed, previous treatment, pT stage, pN stage, number of lymph nodes grossly identified, number of lymph nodes histologically identified, number of metastatic lymph nodes, use of fat clearing agents at gross, and number of cassettes used to submit lymph nodes at gross.

The adipose tissue was stripped from the specimen, measured and weighed in aggregate, sectioned into 0.5-1.0 cm strips, and placed in room-temperature acetone at a 1:10 (tissue volume: reagent volume) ratio overnight.

Operation of the LNL300 device was performed as per the manufacturer's instructions.[39] Following immersion in acetone overnight, the adipose tissue was placed in the loading chamber (**Fig. 1A**), the drive shaft was loaded, and the lid was placed on top. The drive shaft handle was rotated which applied pressure to the specimen, squeezing the eluted adipose in the acetone out of the device through small holes on the exterior. After maximum compression was achieved (**Fig. 1B**), the resulting compressed tissue product was transferred to the cutting chamber (**Fig. 1C**). A specially designed blade handle provided with the device was used to cut the compressed product in pre-set intervals to produce cassette-sized pieces of tissue. The pieces of tissue were transferred into cassettes, ensuring the same face was embedded down, and cassettes were placed in 10% NBF until tissue processing. The tissue was processed on a 12- or 16-hour processing cycle and stained with hematoxylin & eosin (H&E) per standard laboratory techniques.

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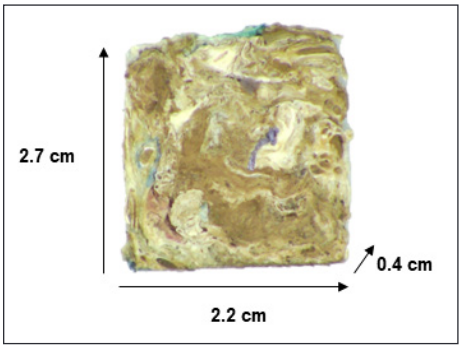

Fig. 2: Representative cassette-sized piece of tissue produced by the LNL300, and average dimensions (height, width, and thickness) based on all pieces generated.

A gastrointestinal subspecialty pathologist recorded the number of lymph nodes identified, the number of lymph nodes containing tumor metastases, the diameter of the smallest and largest lymph nodes per case, and the histological quality of the lymph nodes identified. The pathologist was blind to all case information and determined a histological quality score of 1-4 (1-poor; 2-fair; 3-good/no noticeable differences; 4-excellent/better than traditional methods) based on comparison to lymph nodes harvested using traditional techniques.

Results

The specimens included in this study reflected a range of patient demographics, in addition to T and N stages (**Table 1**). Based on the initial gross examination, 8% of cases had less than 12 lymph nodes, with an average of 23 lymph nodes, histologically identified per case.

The LNL300 compressed tissue to approximately 9% of its initial weight and produced cassette-sized pieces of tissue averaging 2.7 x 2.2 x 0.4 cm (**Fig. 2**). With the LNL300, an average of 20 additional lymph nodes were identified in the remaining adipose of each specimen, totaling an average of 43 lymph nodes per specimen. The size of the lymph nodes identified with the LNL300 ranged from <1 mm to 8 mm in diameter. Lymph node metastases were identified in 6/25 cases (24%), which increased the N stage in 5/25 cases (20%) (**Table 2**).

The LNL300 preserved the quality of both lymph nodes and tumor deposits (**Fig. 3**). The histological quality of the lymph nodes retrieved with the LNL300 was most frequently noted to be good, with no noticeable differences compared to lymph nodes isolated using manual palpation (**Fig. 4**). An under-processing issue, however, was identified in several of the compressed specimens. Tissue cassettes for 18 of 25 specimens were placed on a 12-hour processing cycle, revealing areas of under-processing in 10 of the 18 cases (55.6%) during histological assessment. Upon recognition of the under-processing issue, the remaining specimens were placed on a 16-hour processing cycle which led to a reduction in the number of cases with areas of under-processed tissue (1/7; 14.3%).

Original N Stage	LNL300 Findings	Updated N Stage
N0	Tumor deposit	N1c
N1a	Tumor deposit and two metastatic lymph nodes	N1b
N1b	Two metastatic lymph nodes	N2a
N2a	Two metastatic lymph nodes	N2b
N2a	One metastatic lymph node	N2b

Table 2: Patient N stage based on lymph nodes identified at the initial gross, findings with the LNL300, and updated patient N stage based on the findings with the LNL300.

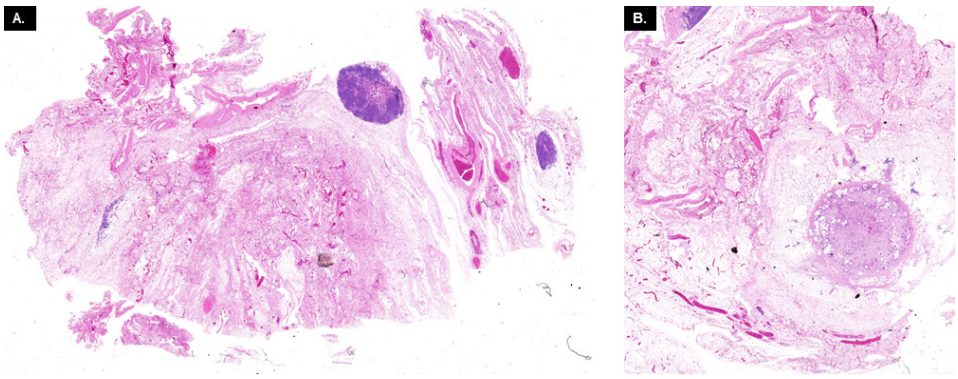

Fig. 3: Representative histological images of tissue compressed with the LNL300 and stained with H&E, demonstrating a lymph node (A) and a tumor deposit (B).

The time taken to compress the specimen with the LNL300 was defined as the time between when the specimen was removed from submersion in acetone until the tissue was placed into a cassette. The time required to prepare the specimen the night prior is not included in this value, as some specimens had previously had the fat stripped while others had not. On average, the compression time with the LNL300 took 25.4 minutes per case. The amount of time required strongly correlated to the weight of the adipose tissue as the LNL300 chamber accommodates approximately 150-200 g of tissue per compression cycle (**Fig. 5**). Up to three cycles of specimen compression were required for large cases (>400 g of adipose tissue). Disassembly, cleaning, and reassembly of the LNL300 took between 3-16 minutes between cases, with an average of 9.7 minutes. The first three specimens used with the LNL300 were designated as training specimens as the use of the device was guided under the direction of the manufacturers of the LNL300 during a virtual session. The times associated with the training specimens are not included in the time results.

An average of 14 tissue cassettes were required to submit the lymph nodes identified by manual palpation in the specimens prior to the study. On average, the compressed adipose tissue for all specimens was completely submitted in 15 tissue cassettes (**Fig. 6**). The number of cassettes required correlated to the amount of adipose tissue present.

Discussion

Acetone compression using the LNL300 is effective in isolating small lymph nodes, lymph nodes with metastasis, and tumor deposits with comparable histological quality to lymph nodes retrieved using manual palpation. Use of the LNL300 eliminates the need for future resampling and ensures a patient receives the most accurate diagnosis during initial diagnostic assessment of specimen lymph nodes. Studies have noted variation between grossers in the number of lymph nodes identified using manual palpation, citing a combination of factors such as familiarity with lymph node harvests and time dedicated to a thorough lymph node search.[40,41] Submitting all the adipose tissue with the LNL300 standardizes the process of lymph node harvests, eliminating inter-user variability. The results of this study demonstrate the ability to submit all compressed lymph node containing tissue from resected CRC specimens in an average of 15 tissue cassettes, compared to an average of 14 tissue cassettes for traditional lymph node submission. Histological assessment of all the compressed tissue ensures small lymph nodes which are traditionally challenging to identify by manual palpation, particularly in neoadjuvant cases, are identified and assessed for metastases. The identification of lymph node metastases has significant prognostic and therapeutic effects on patients with CRC.[6–8] Lymph node metastases and/or tumor deposits were identified in 24% of specimens included in the present study, changing the N stage in 20% of cases. The results of this study, therefore, suggest that the application of the LNL300 as a part of standard protocol in the assessment of CRC specimens would improve patient care.

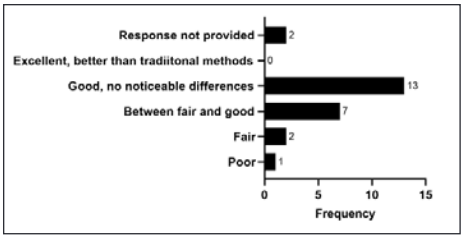

Fig. 4: Histological quality of lymph nodes isolated using the LNL300 compared to lymph nodes harvested using manual palpation, n=25.

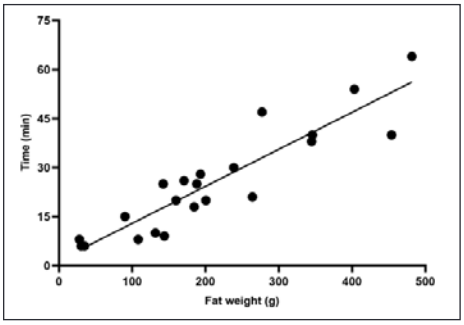

Fig. 5: Scatterplot and regression line of the initial weight of the adipose tissue and the time taken to compress the tissue with the LNL300, n=22.

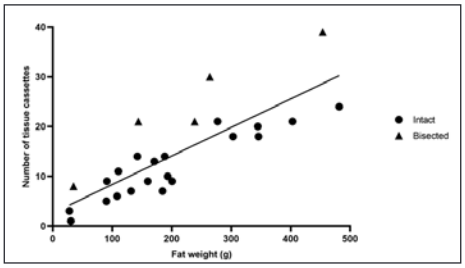

Fig. 6: Scatterplot and regression line of the initial weight of the adipose tissue and the amount of tissue cassettes required to submit the compressed tissue produced by the LNL300. Following use of the LNL300, tissue that was not further sectioned (intact; n=20) is designated by circles, while tissue that required bisection (n=5) is represented by triangles.

These results correlate to other studies which demonstrate acetone compression as an efficacious technique in isolating lymph nodes from other neoplastic specimen types, such as gastric, esophageal, and axillary contents from breast specimens.[34,42–44]

When considering implementation of a new technique within the laboratory setting, factors such as the cost of reagents, materials, and pathologists' assistant's time must be considered. Alternative fat eluents include Carnoy's solution, which has been demonstrated effective in isolating lymph nodes from adipose tissue more effectively than manual palpation without altering the morphology and histological quality of the lymph nodes.[45] The efficacy of Carnoy's solution was not investigated in this study due to the increased purchase cost of Carnoy's compared to acetone. A formal comparison of time taken to perform a lymph node search by manual palpation versus acetone compression was not included in this study. Manual palpation times are highly variable and depend on several factors, including dissector experience

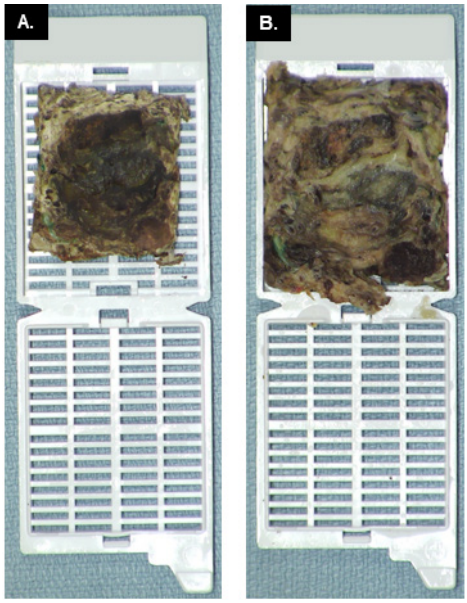

Fig. 7: Tissue produced by the LNL300 in standard-size tissue cassettes before exposure to formalin (A), and after being placed in 10% neutral buffered formalin for approximately 60 minutes (B).

and dedication, pathology department expectations, site workload levels, and prior neoadjuvant treatment of the specimen. Using 15-30 minutes as a reasonable estimate for lymph node harvest by manual palpation, the 25.4 minute average time per acetone compression is comparable and arguably superior, considering the submission of all possible lymph nodes. The number of tissue cassettes and time required to compress the tissue with the LNL300 was correlated to the amount of adipose tissue present in the specimen. As such, the weight of the adipose tissue could serve as an accurate predictor of the compression time required per case, which could aid pathology laboratories in allocating grossing resources. The LNL300 could also be deployed in combination with manual lymph node harvesting techniques, such as in cases of neoadjuvant therapies or after a low yield initial manual search.

This study revealed some minor issues and challenges when using the LNL300. One issue involved the swelling of compressed tissue in cassettes which were held in 10% NBF prior to processing (**Fig. 7**). Rehydration of the tissue in formalin caused expansion to all edges of the cassette borders, creating challenges during embedding and cutting after processing. This issue was remedied by bisecting the tissue in the cassette and placing the resulting pieces in two separate cassettes as contiguous sections. This increased the number of cassettes, added additional grossing time, and remains an issue requiring resolution. A possible solution may be to avoid leaving the cassettes in formalin for an extended period, immediate processing of the tissue cassettes, or creating a unique processing cycle that minimizes rehydration of the tissue. A second issue identified was the time required for disassembly and cleaning of the LNL300, which averaged 9.7 minutes between cases. The eluted adipose exits through 2 mm holes on the sides and top of the LNL300 device. Thorough cleaning of these small holes is required to evacuate any trapped tissue, which could serve as a source of possible contamination. The authors were very impressed by the build quality of the device and are confident the LNL300 would have many years of service after numerous cycles of compression and cleaning.

This study has limitations. This study used specimens stored in 10% NBF for a minimum of two months for all LNL300 lymph node isolations. The effects of long-term formalin fixation on the efficacy of acetone to elute fat is unknown. The quality of lymph node harvests by the LNL300 compared to tissue placed in acetone prior to formalin fixation is unknown. In addition to a fat eluent, acetone is a known tissue fixative. As such, it is possible for the fresh mesenteric tissue to be stripped from a specimen at the time of receipt in laboratory, placed in acetone overnight, and the compression performed the following day. This would improve turnaround time by allowing the grosser to submit the compressed tissue along with the routine tissue blocks taken after formalin fixation. There are risks to this approach, however, as removing the mesentery prior to tissue sampling may disrupt the tumor, prevent accurate sampling and measurement of radial margin, and interrupt sampling of large lymph nodes. Tumor extension into the surrounding fat must be considered prior to removing the fat from the specimen. Large lymph nodes (>1 cm) and lymph nodes abutting the radial margin should be removed from the specimen using manual palpation and submitted in tissue cassettes before the remainder of the adipose tissue is placed in acetone and compressed with the LNL300.

Additional theoretical limitations are the possible double sampling of larger lymph nodes during the compression process and the possible under-sampling of very small lymph nodes within the compressed block. Adipose placed in the LNL300 compression chamber needs to be cut into strips. If these cuts bisect lymph nodes, there is a potential to double sample nodes after compression. This issue was not identified or suspected during this study. Each cassette sized piece of tissue produced by the LNL300 was histologically assessed at a single level. The average thickness of the cassette-sized portion of tissue was 4 mm. Since lymph nodes as small as <1 mm in diameter were identified in this study, it is possible that occasional small lymph nodes located in the middle of the tissue block could have been missed. Cutting deeper levels through the block would resolve this issue.

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Lymph Node Locator 300 cont. from page 13 >

Should acetone compression become a widespread, standardized practice in pathology laboratories, there is a possibility that guidelines for the minimum number of lymph nodes necessary for accurate diagnosis of metastases may increase. Techniques that routinely lead to harvests of 30-50 lymph nodes may change our understanding of pN stage in colorectal and other GI carcinomas. It remains an area of future study to determine whether an increase in identification of metastases in cases using acetone compression would have an impact on long-term patient outcomes.

In summary, this study demonstrates the LNL300 as an effective and valuable tool for a high volume, acute care pathology service. The LNL300 was shown to isolate an average of 20 lymph nodes per CRC specimen which were initially missed by traditional manual palpation techniques. Lymph node metastases and/or tumor deposits were identified in 24% of cases, affecting N staging in 20% of cases. Acetone compression with the LNL300 offers potential to save pathologists' assistant time at the grossing bench, standardize lymph node harvests, and reduce the likelihood of missing positive lymph nodes, ultimately leading to an improvement in patient care.

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