Margin Evaluation
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Contents

Introduction:

The purpose of Guidelines is to provide standardized methods used to evaluate tumors in animals and accrue data so that, over time, large data sets with comparable information can be assessed and studies validated uniformly. Ultimately this will enable meaningful conclusions and accurate prognostic information that will improve patient care. New methods and modifications of present methods are encouraged and should be described in such detail so results can be replicated and validated. Guidelines and protocols are “living” documents which will be modified as new information becomes available to authors of each Guideline and or protocol. This Guideline is for general application, but future editions will include versions that are focused on unique margin requirements for an anatomical location and or a specific tumor type.

Margin assessment is one of the most important histological parameters evaluated in oncology.[1] Patient management decisions often hinge on the results of margin assessment and clinicians may value margin assessment as highly or more than a diagnosis. Adequacy of margins in surgical resection of a neoplasm is critical but there is little information in the veterinary or human medical literature that provides outcome - evidence based information apart from some neoplasm specific reports.[2-4] For the evaluation of surgical margins, the four main members of the cancer treatment team are the oncologist, surgeon, laboratory technologist and pathologist. A greater use of online communication and digital pathology facilitates remote interactions between

these team members. Communications between each member would be improved by standardization. The histopathology report represents the primary form of communication between pathologist and clinician and is critically important in the decision making process[5-7] The fundamental observations and measurements in margin assessment by the pathologist are: 1) relationship of neoplastic cells to the surrounding tissue including presence of a capsule, tissue compression, invasion or infiltration and lymphovascular invasion; 2) the distance from neoplastic cells to the narrowest or closest inked lateral and deep margin, which is the histologic tumor free distance (HTFD)[8,9] (Note A). and 3) the relationship of neoplastic cells to the boundaries of the *compartment* in which the tumor is located, especially along the deep borders (Note D). Metric measurements laterally are considered standard but for the deep margin a metric distance and the type of tissue present at the deep border should be reported. Histologic margin evaluation is only needed on tumors where the aim of surgery is to completely remove the neoplasm (achieve local control). Samples where there was no intent to totally excise the entire tumor, including intralesional or incisional biopsies, marginal resections, debulking or cytoreductive surgery, are for diagnosis and grading purposes.

Many of the procedures important in the assessment of margins and their methods are listed below. At the end of the Guideline there is a discussion and a section called “future considerations” for how margin assessment can be improved. The purpose of this Guideline is to provide the most important information necessary for assessment of margins and which should be included in studies evaluating margins. This checklist is divided into those details provided by each team member involved, one component of which is the pathologist. A “Cliff’s-notes” version will be added on the website, to make it easy for pathologists to find and then decide on its utility.

1. Clinician/surgeon

Anatomical site:

_____Specify exact site of tumor

Cytology results

_____ Provide cytology findings (summarize or attach cytology report)

Intent of surgery:

_____ Complete excision/Curative intent

_____Narrowest planned preoperative margin, measured by the surgeon before incision or excision (mm, cm)

_____ No intent to excise or cure: sample for diagnosis

Diagnostic Imaging:

_____ Report if imaging performed, technique and results. This may provide important data for neoplasms that are known to be infiltrative

_____ Provide link to online digital images

Ink:

Designation of margins, other anatomical landmarks or other areas of concern/interest with ink or other labels at the time of surgery is recommended. (Note C)

_____ Specimen was incised to improve fixation. Indicate location of incision.

_____ Specimen was inked prior to arrival at laboratory

_____ Specimen was not inked prior to arrival to lab

_____ Ink applied by laboratory technician

_____ No ink ever applied

_____ Sutures, tags or other markers present and labelled

_____ Yes

_____ Indicate what was identified by labels and what is to be evaluated

_____ No

_____First excision (primary) yes no

_____Re-excisional sample – attach report or provide link

_____ Weeks from first (primary) excision

_____ Evidence of tumor visible grossly?

_____ If yes, mark or indicate location of mass

Any previous incisional biopsy or excision histopathology results?
If there are prior histopathology results, attach report or provide link.

2. Laboratory technologist

Method of trimming and gross margin assessment:
Grossing technicians should describe the anatomic location of the specimen, the relationship of the tumor to the margins, including measurements of the distance to the narrowest surgical margins, the landmarks demarcated by ink or other labels and the sectioning technique (radial, parallel, tangential). Labelled photographs or diagrams of submissions are helpful.[5,10] *Photographs are recommended and can be archived with the case as a representation of the gross specimen (description is still recommended). Detailed pictograms of trimming methods for tumors in different systems in the body are published.*[10]*Small pictograms are included below, however readers are encouraged to review full sized images and descriptions in the reference cited.*[10]

_____ Radial sections made perpendicular to each other

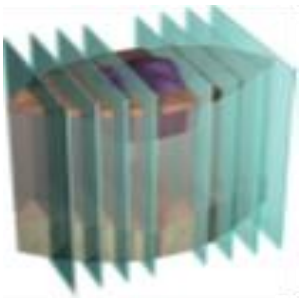
_____ Tangential (since HTFD cannot be assessed in tangentially sectioned margins)

_____ Combination radial and tangential (specify)

_____ Bread loaf (parallel)

_____number of sections

_____distance between sections



Gross margin distance:

If tumor is at a margin, report the distance as "0". Report distances as whole numbers in mm/cm.

_____ Tumor is at margin

_____ Distance (mm/cm) from tumor to narrowest surgical margin

_____ Distance from tumor to lateral and deep margins for skin tumors

3. Pathologist

Histologic Margins:

The responsibility of the pathologist is to report (1) the relationship of the neoplasm to surrounding tissue, (2) to measure the histological distance between neoplastic cells and the closest margin/s (lateral margin and the distance and type of tissue

between neoplastic tissue and the deep margin for skin tumors). Report HTFD as a whole number (no decimals) in mm/cm. (Note A), (3) List the presence of tissue barriers (fascial planes)

HTFD:

for skin and subcutaneous tumors indicate lateral and deep margins separately

_____ Neoplastic cells at inked margin

_____ Shortest HTFD (mm/cm tumor to margins; indicate site specific margins for example for skin tumors indicate both lateral and deep margins)

Skin and subcutaneous tumors indicate both lateral and deep margins

Lateral margins

_____ Narrowest HTFD (mm tumor to margins)

_____ Tumor is at margin

Deep margin

_____Narrowest HTFD (mm tumor to margins)

_____ Tumor is at margin

_____ Type of tissue at deep margin (collagen, adipose, etc.)

_____ No neoplastic cells in histologic sections of margins. Provide gross margin measurement

_____ Tangential (HTFD cannot be assessed in tangentially sectioned margins)

_____ Neoplastic cells present at margin

_____ Neoplastic cells not at margin

_____ Margins are not assessed (Explain why not)

Tumor Capsule:

_____ Not present

_____ Present

_____ Tumor does or does not extend beyond capsule

Peripheral Growth Habit:

_____ Well-circumscribed at leading edge of tumor

_____ Compression of surrounding tissue

_____ No compression of surrounding tissue

_____ Peripheral invasion

_____ Present

_____ Absent

Tissue barrier (fascial plane, compartment); (Note D)

_____ Present

_____ Location, type,

_____ Tumor cells penetrate tissue barrier (yes, no)

_____ Not present, could not be visualized

Lymphovascular Invasion LVI (LVI Guideline):

see LVI Guideline for details, notes, images and references

Lymphovascular Invasion (report format below)

_____ Not identified

_____ Equivocal (Notes A, B; Guideline 4)

_____ Present

____Thrombus adherent to intravascular tumor

_____Tumor cells invading through a vessel wall and endothelium

_____Tumor cells within the wall of a vascular structure covered by endothelium

_____Viable neoplastic cells within a space lined by lymphatic or blood vascular endothelium

_____ Neoplastic cells in a structure that has been confirmed to be a lymphatic or blood vessel using immunohistochemistry (Note C; Guideline 4)

Number of LVI foci (within a minimum of one representative section of tumor and peritumoral tissue. Report the number of foci of LVI within all sections examined.)

_____ Few (< 5 foci)

_____ Moderate (5 – 10 foci)

_____ Many (> 10 foci)

Type of vessels invaded

_____ Muscular wall evident

_____ No muscular wall evident

Site of lymphovascular invasion

_____ Intratumoral (number of LVI foci)

_____ Peritumoral (number of LVI foci)

Discussion:

To ensure a diagnosis and margin assessment is possible, representative tissue must be collected, properly preserved, and processed. With small masses that fit into a standard 2 cm × 2.5 cm × 5 mm processing cassette, this is not a problem. However, many specimens exceed this size and must be trimmed to fit within the cassette. It is this critical step which ensures that both diagnostic tissue and the surgical margins are included in the biopsy and properly oriented in the cassette so that the pathologist can complete the evaluation, make a diagnosis, and accurately assess the surgical margin so the clinician can formulate a prognosis and the appropriate treatment plan.[30] The clinician / surgeon uses the surgical, clinical and ancillary data, including the histopathology report to decide if the margin is adequate for the tumor type and anatomical location. Only submissions (surgeries, biopsies) with intent to cure need margin reports. Certain tumors or the anatomic location of a tumor near vital structures dictate that excision for local control will be attempted but the surgeon realizes that adjacent structures limit how much margin can safely be taken. Therefore, margin assessment by the pathologist may not be critical to the surgeon, and communication between surgeon and pathologist will clarify what the surgeon needs to determine adequacy of the margin (e.g. thyroid, anal sac tumors, adrenal glands). This

communication can be augmented by intraoperative images to better explain the boundaries noted at surgery.

The aim of surgery is to use the smallest margin with the highest probability of achieving a local cure and to provide adequate tissue for a diagnosis. The type of neoplasm, the relationship of the tumor to the surrounding tissue (capsule, histological growth characteristics, invasiveness, lymphovascular invasion), the HFTD and the relationship of the neoplastic cells to potential tissue barriers (fascial planes) are critical factors used by clinicians in determining the adequacy of margins.

The distance between neoplastic cells and the inked margin measured by the pathologist in histologic sections is the HTFD. However, what the pathologist sees in histologic sections is not the same as what the clinician saw during surgery. The HTFDs are different from the distance to the surgical margin planned by the surgeon due to a combination of microscopic tumor cell invasion into tissue that appears grossly normal (non-neoplastic) to the surgeon intraoperatively and tissue shrinkage post-excision and during histologic processing. Tissue shrinkage is caused by surgical excision, tissue fixation and tissue processing and has been reported up to approximately 50%.[11] Therefore, tissue shrinkage partially explains why the actual margin taken at surgery is greater than the measured HTFD.[4,11] The likelihood of a false negative HTFD is mitigated by sampling of the margin that appears macroscopically to be the shortest distance between the mass and the inked margin. Thus, histologic sections are subject to sampling bias. In many cases, measuring the HTFD alone is not enough to determine the adequacy or appropriateness of surgical margins, yet it is the parameter that is often used to determine ‘completeness’ of excision by clinicians and pathologists. Only a small portion of the circumferential surgical margin is evaluated histologically (approximately 0.1- 0.01% of the total margin).[12] Other methods of margin evaluation which allow larger portions of the margin include tangential sectioning or parallel slicing.[5,13] Denoting which trimming method was used provides valuable information for clinicians when interpreting margin results. Validation studies that compare these different methods of sectioning are needed[14,15] A study that examined completeness of

histologic mast cell tumor reports found that the type of margin trimmed by the histology technologist was only reported in 17% of cases.[7]

The distance from tumor to margin measured *prior to surgery* (by the surgeon) and with a high probability of achieving local control is known in human oncology as the surgical safety margin (SSM).[16,17] For tumors of the skin and subcutaneous tissues, the surgeon measures the margin desired from the edge of the grossly palpable tumor, and draws a line on the skin to guide surgery. Many practitioners estimate the distance and incise. Thus, there is considerable inter-surgeon variation in determining the gross edge of the tumor and the distance from the tumor they believe to be appropriate for the anticipated or known tumor type. Therefore, it is essential for the surgeon to ink the gross margins; and indicate any specific regions they want examined. There is no known SSM for many human tumors. Some human tumors have studies to determine the SSM that is specific to a subtype such as soft tissue sarcoma,[18,19] pleomorphic sarcoma,[8] renal cell carcinoma,[17] squamous cell carcinoma,[9] and human breast cancer[20] although these are still controversial. The SSM varies from 1mm to 5mm or even in some human breast cancer and soft tissue sarcoma studies[18] the standard is “the tumor should not touch the margin ink”. “Mohs surgery” is considered the gold standard for excision in some types of human skin cancer where removing the least amount of tissue is desired. The pathologist examines frozen sections and reports when the sections are free of tumor cells. This results in the narrowest margins possible and high cure rates. Serial sections of frozen sections during surgery are a consideration for future studies.

In surgical pathology, the HTFD with a high probability of preventing local recurrence (measured by the pathologist) is referred to as the histologic safety margin (HSM). The SSM and the HSM differ between tumors or within subtypes of the same tumor presumably because of differing biologic behavior. The HSM is not known for most tumors in veterinary and human medicine. If neoplastic cells are present at the margin after a tumor is excised (microscopic disease), it seems logical that recurrence is likely. Similarly, if the entire tumor appears to be excised based on histology, logic

suggests it will not recur. However, in dogs, low-grade cutaneous mast cell tumors do not recur even when there are neoplastic cells at the margins, and high-grade mast cell tumors do not have a safe HTFD that prevents recurrence (a HSM could not be determined).[21] It is similar for canine soft tissue tumors/sarcomas: greater than 95% of canine STT/STS do not recur if margins greater than 1mm are free of neoplastic cells and when margins are less than 1mm, approximately 75% do not recur[22-24] The biology of the tumor, its location in the host and the genetics of the host may be more important factors in predicting recurrence than are neoplastic cells at a histologic margin.

In veterinary oncology there are proposed distances for the surgical margins of canine cutaneous and subcutaneous mast cell tumor (MCT), and STT/STS from 2 mm to 5 cm and/or one or two fascial planes deep.[25,3] Until recently there is little research in veterinary medicine and no standardization of how to trim tumors, measure the margin width, HTFD, HSM or reporting of results. In a review of surgical biopsy reports of canine cutaneous mast cell tumors (n = 368), study findings suggested that while histologic margins are generally reported, details about the margins and consistency of how histologic margins are reported were generally lacking). For example, while margins were reported in 92% of cases, lateral and deep margins were described separately in 77% of cases, margin direction was only given in 16% of cases and descriptions of the deep margin component were only available in 11% of cases.[7] There are attempts to aid margin reporting by assigning a score based upon the HTFD or extent of tumor at the margin (M1-M4)[10] and a proposal to score the extent of residual tumor with scores of RX (residual tumor could not be assessed); R0 (no residual tumor); R1 (microscopic residual tumor); R2 (macroscopic residual tumor).[26,27] These systems should be evaluated in clinical patients with robust outcome data, as should the determination if there is value in estimating the amount of tumor along a margin (focal, diffuse etc). Future studies should attempt to determine if a HSM can be established for different tumor types in animals.

The lack of validation of HSM means that terms such as complete, clean, and clean but close are subjective and inconsistent interpretations that may not reflect the probability of local surgical control.[5] What a pathologist sees histologically (the HTFD)

and would call ‘complete’ may have a different meaning from what clinically may be an appropriate margin. Histologic samples do not reflect the life history of the neoplasm and do not take into consideration pertinent clinical and surgical details like clinical rate of growth and relationship to critical structures. It is only through consideration of all aspects of the clinical, diagnostic imaging, surgical, and pathologic details that the adequacy of surgical margins for local control can be determined with the highest degree of certainty. Subjective terms should therefore be avoided in a pathology report.[5] Although these terms are ingrained in the clinical lexicon, practitioners, surgeons and oncologists should also discourage their use and instead, use statistically valid assessments that are available. The pathologist contribution to margin assessment is to report the relationship of the neoplasm to the surrounding tissue, HFTD and if possible, the relationships to tissue barriers (fascial planes, compartments). Many more clinical outcomes or evidence-based studies using multivariable competing risk regression models are needed to determine the best trimming and margin evaluation methods.

The presence of neoplastic cells at a surgical margin, clinically called microscopic disease, presents a conundrum for all involved in cancer therapy. There are many examples of neoplasms where an apparent *incomplete excision* does not result in a recurrence.[22,21,23,24] Finding residual neoplastic cells histologically in a re-excision specimen from dogs likewise presents a conundrum. Presence or absence of tumor in re-excision specimens did not accurately predict recurrence in dogs with soft tissue sarcomas.[28] It is difficult to find microscopic residual tumor cells when a gross nodule is not present. If the surgeon sees a nodule grossly in a re-excision specimen, they should tag the nodule/mass and label it. How many sections should be taken in re-excision specimens is not standardized. There may also be additional de novo development of neoplasia. Biology and molecular makeup of tumors and their hosts are likely to be critical factors in predicting recurrence, transplantation, and metastases. Also poorly understood are the kinetics of spread of neoplastic cells within a surgical site. In general, potential contamination of any part of a surgical site means that any other part of the surgical site could be compromised. Surgeons expect to see a report of the distance from the previous scar tissue to the new surgical margin.

Future studies should be prospective, evidence based and use statistical analysis of not only clinical, molecular, or genetic features of tumors, but microscopic features such as relationship of neoplasms to the surrounding tissue, presence or absence of tissue barriers and HTFD. Studies must be described in detail so others can replicate them, and methods must be correlated with accurate outcome assessments. Suitable statistical analysis such as multivariable competing risk regression models are required.[29]

Notes:

A. The HTFD is the distance of the closest approach of the neoplastic cells to the inked margin. The reported distance should be the narrowest margin of the several sections examined. Given the variable nature of the periphery, the asymmetry of some tumors and shrinkage induced by removal and fixation we recommend pathologists report the closest HTFD as a metric whole number without decimals. To measure the HTFD more precisely ("*The surgical margin width is 2.6mm"*) implies a greater precision than is real from a formalin fixed paraffin embedded specimen and that the entire margin was examined.

- **a.** Total number of pieces of tissue taken at the margins that should be examined is not standardized. A minimum of 5 margins is traditionally examined in tumors of skin and subcutis.
- **b.** The HTFD is measured with the measuring tool of whole slide scanning software. An ocular micrometer or the diameter of a field of view should be used for microscopes. See Note E to determine diameter in the FOV.
- **c.** For primary care clinicians and surgeons, assessment of the deep margin of skin tumors is of utmost importance. While the lateral margins can be easily visualized during surgery, the deep margin is not as readily assessed. Pathologists must report the deep margin and lateral margins separately. Metric measurements laterally are considered standard but for the deep margin a metric distance and the type of tissue present at the deep border should be reported. More detailed descriptions of the deep margin should include the type of tissue (adipose tissue, dense connective

tissue, fascia, skeletal muscle) and quality of tissue at the deep margin (normal, necrotic, thermal damage) with descriptions of neoplastic cells in this location.

B. The surgeon determines the most appropriate distance between the mass and the intended surgical margin. This distance should be measured (mm or cm) and the measurement recorded so that comparison with the HTFD can be performed. There is an Enneking surgical dose categorization scheme that is used by secondary and tertiary care surgeons that defines intralesional, marginal, wide, or radical surgical procedures (and there is some variation in the literature in what constitutes a 'wide' margin).[30] Primary care veterinarians generally do not use this scheme.

C. Ink should be used to identify margins after surgery and is optimally applied at the time of surgery. Inking the margin by the clinician/surgeon immediately after tumor excision is required if a HTFD is expected. However, margins are often not identified at the time of surgery. In one survey of pathology reports, approximately 34% of surgeons or oncologists identified the margins in tissues submitted for pathological examination.[7] If ink is not present when the sample arrives at the lab this should be noted. If the gross specimen is not inked by the clinician or the ink cannot be identified in the sections examined (Figure 10), the significance of any measured margins is questionable. If there is no ink on a margin reported to be inked, deeper sections should be obtained to include the inked margins. The tissue should be blotted dry, ink applied and allowed to air dry prior to fixation. Different colors of ink can be used to designate anatomical landmarks or areas of interest or concern. ***Black ink*** should be avoided on oral and digital lesions, and especially if the mass is pigmented. Surgical margins must not be incised prior to or after application of ink and no tissue should be trimmed from the margins before fixation. Tissues larger than 2 cm diameter which will not reach the laboratory within 24 hours can be incised to assist fixation, but the surgical margins must be avoided.[5] If tissue is trimmed from the

specimen by the clinician this should be described and recorded on the pathology requisition. Submission information should include detailed descriptions of the inked landmarks as well as any specific requests regarding tissue sectioning. Laboratories must ink those margins in specimens received without designated margins.

D. Surgeons consider the concept of compartmental boundaries important to plan and perform surgical removal of tumors[30,31] that are potentially invasive. Surgical margins in a well delineated anatomic compartment (eg bone, joint, muscle) may be planned differently than a poorly demarcated anatomic compartment (eg subcutaneous tissue, intermuscular spaces).[30,31] These anatomic compartments may provide natural barriers to tumor extension. Therefore, surgeons would like the pathologist to report the relationship of the tumor to surrounding anatomic structures that make up compartments. Compartments, fascial planes, and fascia vary depending on anatomical location therefore information about location and gross tumor growth patterns (confined, infiltrative) should be provided on the pathology requisition. Surgeons should ink and label the gross specimen and state on the pathology requisition what precise structures are important to them. Pathologists will identify the tumor, structures labelled and relationship of the tumor to lateral and deep margins (HTFD) and report what tissue is present along the deep margin (adipose, muscle, fascia etc).

The terms “fascial plane” or “tissue barrier” are routinely used by surgical oncologists. There are recent reviews describing fascia for the dog and horse[32] and surgically identifiable fascial planes for the dog.[33] The thickness and tensile strength of fascia is markedly variable in different anatomical locations. Some are thin, barely discernible, torn easily (subcutaneous superficial fascia) while others are thicker, visible grossly and histologically (deep fascia on muscles).[32,33] Fascial planes are described in the surgical literature and can be used to dissect along to isolate and excise tumors from the skin, subcutis, and musculoskeletal system, more so than when removing tumors from internal organs. It is recommended that excision of potentially aggressive tumors from the

subcutaneous tissue include at least one uninvolved fascial plane, deep to the tumor.[25,3,34] The intact fascial plane below the tumor provides a physical barrier of normal tissues such as collagen, muscle and other tissues that may help prevent extension of the tumor. Pathologists rely on the morphological features of anatomical structures to identify fascia and boundaries of the fascial plane indicated by the surgeon.

If one or more fascial planes of tissues are removed with a tumor, the surgeon should state on the pathology requisition what tissues are to be examined in relationship to the tumor. If the surgeon wants to know how close a tumor is to the deep margin and what tissues are present along the deep margin, then those regions need to be inked grossly and labelled. The pathologist reports if the tumor extends to the deep margin (which the surgeon inked) and the composition and integrity of the deep margin. A report that states the HTFD is 4 mm to the deep margin but there is no fascia present, is interpreted quite differently by the surgeon and medical oncologist from a report that states the HTFD is 4 mm and sections from the deep margin include dense collagen, fascia which is free of neoplastic cells. This information helps the surgeon and oncologist decide if the margin is adequate and consider treatment options. Pathologists can attempt to identify fascia with H&E, consider histochemical stains for collagen or may visualize the fascia in the context of its adjacent skeletal muscle.

E. The evaluation of margins for benign tumors and what should be reported needs to be addressed. It is logical that the type of tumor, anatomical location in the body and purpose of surgery are factors in these decisions. One philosophy is margins do not need to be reported as the tumor is benign. Another philosophy is, if the clinician removed the tumor with the intent to cure local disease (remove the entire tumor) margins should be reported regardless of benign vs aggressive assessments. For the majority of excisions, the clinician does not know the diagnosis at the time of surgery, and it is very likely they want to know if their surgery removed the entire tumor and how wide is the area of non-neoplastic

tissue around the tumor. What depth of the intestinal, gastric, or urinary bladder wall did the polyp, adenoma extend? The level of certainty that the tumor is benign is a factor e.g. mammary adenoma, hair follicle tumor or plasma cell tumor. Furthermore, margin assessment of benign tumors shares the same concerns encountered in malignant tumors regarding how adequately radially sectioned tumors reflect the surgical margins. It is likely that many clinicians will request margin evaluation even if the histological diagnosis is a benign tumor. Until studies provide guidance for how the types of tumors and anatomical locations influence margin evaluation it seems logical that practical considerations such as this are left at the discretion of the pathologist and the diagnostic lab. In this version of Guideline 3 we believe if the aim of surgery was to completely remove the neoplasm then margins should be reported, and they will be expected by the clinician.

Practical Considerations:

Distance measurements in field of view (FOV) with microscope:

With Whole slide imaging (WSI) measure the HTFD with the measuring tool (Figure S28). With microscope use an ocular micrometer or use the table below to approximate the distance.

FN 22mm/40 X objective = 0.55 mm diameter FOV

FN 20mm/40 x= 0.5 mm diameter FOV

FN 22mm/20X objective = 1.1 mm

FN 22mm/10X objective = 2.2 mm

FN 22mm/4 X objective = 5.5 mm

FN is the field number in mm engraved on a side of the ocular. FN divided by objective magnification is the diameter in the field of view (FOV) at specimen level. Knowing the diameter in the FOV at the different objective magnifications is a helpful aid.

Future Considerations:

1. Evaluate radial, tangential, and parallel (breadloaf) trimming to determine recommendations based on anatomical location of tumor or tumor type

2. Emphasize clinical outcome and evidence-based studies.

    a. Correlate tumor relationship with surrounding tissue with outcome of surgery for each tumor type.

    b. Correlate the presence of tissue barriers (fascial planes) with outcome assessments, especially distance and type of tissue at deep border (quantity, quality).

    c. Correlate HTFD in the presence and absence of tissue barriers (fascial planes).

    d. Determine the value in reporting margins as R0-RX or M1-M4 based on statistical valid evidence and outcome.

    e. Develop equations for calculating HSM in animal tumors (allowing for shrinkage)

    f. Recurrence should be monitored for 2-3 years

3. Future studies should attempt to determine if HSM can be established for different tumor types in animals.

4. Define criteria for describing tumor cells that remain at the margins

    a. Determine if there is prognostic value in estimating the amount of tumor along margins e.g. focal few, diffuse numerous etc.

        i. Example: Clusters of cells away from the main mass vs cells still attached to mass; size of clusters, etc.

        ii. Example: Focal - few cells seen at margin vs diffuse: numerous tumor cells at margin

Figures:

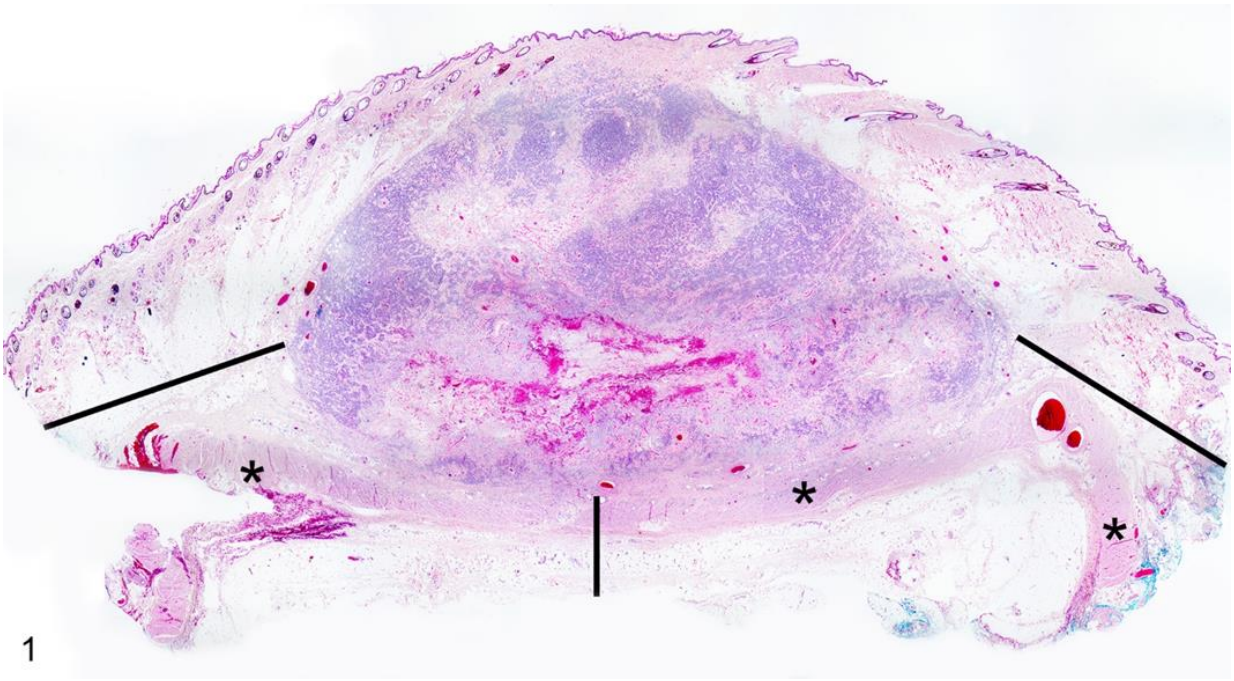


Figure 1: Canine cutaneous mast cell tumor involving the dermis and subcutaneous tissues. The histologic tumor free distance (HTFD) is depicted with horizontal and vertical black lines and can be measured with manual or digital means. Note that ink can be observed at the lateral (or peripheral) margins but is not visible at the deep margin. Therefore, the deep margin measurement represents an approximation given the lack of ink. Additional sections into the formalin fixed, paraffin embedded block may resolve this issue. A potential tissue barrier within the subcutaneous tissue is the striated muscle (also called panniculus carnosus or cutaneous trunci in the truncal region, denoted by the asterisks). This muscle is not always visible in histologic sections of cutaneous and subcutaneous tumors; it has variable distribution and continuity in different body regions.[32,33]

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