REVIEW ARTICLE

Grossing in Oral Pathology: General Principles and Guidelines

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Abstract

Grossing of surgical pathology specimens form the first and an important step in tissue processing, leading to diagnosis. It forms a connecting link between the patient and the pathologist. It involves a close coordination between the surgeon, pathologist and the histotechnologist. A correct grossing procedure helps in minimizing the processing errors while at the same time providing useful information about the specimen helping in the achievement of correct diagnosis. However, the importance of this step is often overlooked and neglected. The purpose of this article is to provide adequate insight about the grossing procedure of the pathologic specimens of head and neck region. Our effort is to present this article as a hands-on experience for the student population and the technicians.

Keywords: Grossing, head and neck specimens, inking, safety measures.

INTRODUCTION

Gross tissue evaluation of a pathology specimen forms an indispensable, but often neglected, component for complete pathologic evaluation along with microscopic examination. "Grossing"- a term that refers to examination and dissection of surgical specimens, along with preparation of sections from those tissues requiring processing, is the initial step in surgical pathology dissection.[1] An accurate diagnosis from this tissue is dependent upon correct identification, handling, and processing in this busy area. A pathologist, resident, physician assistant, histotechnologist or a biomedical scientist can gross specimens.[2] The objective of this article is to emphasize the importance of grossing in diagnostic oral pathology and provide guidelines to carry out the procedure in a sequential order.

GROSSING ROOM REQUIREMENTS

The room should be large, well-illuminated and properly ventilated with exhaust fan. It should contain shelves for specimen containers, ready access to formalin, large table for dissection of specimens, and sink with provision for hot and cold water. Other facilities ideally required are photographic facility, X-ray unit with view box, balances and refrigerator.[3]

GROSSING APPARATUS

The apparatus which must be present in the gross room include a cutting board placed inside a metal box designed in such a fashion that all the fluids flow directly into the sink. Box of instruments including heavy and small scissors, different-sized smooth and toothed forceps, malleable probe, scalpel handle, disposable blades, a long knife, ruler, pins for attaching specimens to a cork surface, box with cassettes and labels, and a dissecting microscope.[3]

A new grossing knife with two parallel blades for preparing uniform thickness tissue sections has been introduced by Dr. J Yang et al (2008). It has a handle and a head with two parallel slots for supporting two essentially parallel blades. The gap between the blades is predetermined at 3 mm to form a tissue receiving gap. The knife can not only be used for sampling uniform tissue sections from hollow structures and cystic specimens, but also from solid organs by changing different length of blades.[4]

GENERAL PRINCIPLES OF GROSSING

The major components of tissue grossing include reliable and rapid transfer of the specimens from the surgery to pathology.[2] The grossing of the specimen can be done either before fixation or following it. It is ideal to gross larger specimen in a fresh state and smaller following fixation. Prior fixation is preferred if rapid transportation of the specimen is not feasible.

A properly completed surgical pathology requisition form containing the patient's identification, age, sex,

essential clinical data, operation, surgical findings, tissue submitted and the site of biopsied specimen should accompany every specimen.[3,5] If more than one specimens have to be placed in the same container, they must be clearly marked, which is most readily done by means of sutures; do not rely on describing the shapes of the pieces of tissue submitted because when they are fixed this will probably have altered.[5] Description of additional specimens received from the same patient must be mentioned.[2] Additionally, on the request form, it is desirable to have previous biopsy numbers to enable comparison to be made if necessary. For example, to comment on the progression or regression of a dysplastic lesion.[5] Nontissue materials such as bullets, implants, foreign bodies should be recorded as it may be essential for medicolegal cases.[2]

Any discrepancies in specimen identification or labeling are resolved prior to processing. Only labeled specimens should be accepted. The label should be firmly attached to the body of the container so that it cannot be separated; labels should not be attached just to the lid of the container. Incorrect identification of any specimen results in the wrong diagnosis and incorrect treatment to potentially two patients.[2]

The first step is the general inspection of the specimen, with identification of all of its normal and abnormal components. The type of specimen, structures included, dimensions, weight, shape and color must be recorded.[3] The pathologist must look beyond the basic task and anticipate any special investigations like immunohistochemistry, enzyme histochemistry, cytogenetics, gene rearrangement studies required for the case and preserve the tissues as per the requirement.[2,3]

The identification markers for orientation such as sutures in soft tissues must be carefully considered.[2] Surgical margins should be identified correctly after orienting it in the anatomic position. The pathologist must use sharp cutting instrument to avoid artifacts.[3]

Contamination of the specimen can occur anytime during the handling of the specimen, for example, floaters, cutting board metastasis which must be carefully eliminated. Under no circumstances should any portion of the specimen be discarded before the case is signed out.[3,6]

INSTRUCTIONS TO THE SURGEONS

For biopsy of mucosal lesions suspected of premalignancy or malignancy, particularly for excisional biopsies, the use of a laser or an electroknife should be avoided. These techniques may produce a coagulative artifact that hampers histologic interpretation of the samples, particularly the assessment of the margins.[7] The anesthetic should be administered to the area adjacent to, but not into the biopsy site as it can cause distortion and artifactual tissue edema in the specimen by producing hemorrhage and separation of connective tissue bands with vacuolization.[8,9,5] Squeeze artifacts are a form of tissue distortion resulting from even the most minimal compression of tissues that groups crush, hemorrhage, splits, fragmentation and pseudocysts. These are usually caused by forceps, by using a stitch for traction or by a dull scalpel blade which should be avoided.[10-13]

The biopsy sample should always be accompanied by pertinent clinical information, if multiple samples of the lesion are taken, each sample must be submitted in a separate, clearly labeled container.[8] The surgeon should provide details like identification of the position, anatomic landmarks, surgical margins and any other structures of significance. The surgically excised specimen should be immediately sent to the pathologist.[3] All the tissue material excised during surgery should be submitted and not selected portions.[14] In case the pathologist encounters any difficulties during orientation of the specimen, the surgeon should oblige and assist the pathologist.[3] Drawings and photographs to indicate the source of sections must be provided.[2] Samples and accompanying documentation should be sent by courier to minimize delays in diagnosis and prevent freezing artifacts that can occur if the samples are placed in mailboxes or transported by carriers without temperature regulation in the winter.[8]

FIXATION OF TISSUE SPECIMENS

To date, 10% neutral buffered formalin is the most common fixative used for tissues submitted for routine examination including tooth specimen. However, mineralized samples such as bone or tooth may require decalcification before it can be processed.[5,15]

To facilitate uniform penetration of fixative, it is imperative to fix small volumes of tissues (5 mm to 1 cm). The volume of the fixative should be in excess of 20 times the volume of the tissue. In practice, it is assumed that these processes require at least 1 hour per mm of tissue thickness, but routinely the tissues are fixed for 24 to 48 hours.[15] It may be necessary to replace the fixative with fresh solution when the specimen contains high percentage of blood.

Covering large specimens with fixative soaked gauze or cloth may help penetration and reduces surface drying.[2]

SPECIMEN HANDLING

It is good practice to have a separate gross sheet where the type of specimen and how it should be oriented is noted.[2] Dermatological specimens and mucosal biopsies are often small and should be handled carefully. Small specimens should not be cut, bisected, or inked while fresh and unfixed as they can reduce in size during processing and fixing.[2,16,17] They are easily lost in handling and are always difficult to orient.[10] For this reason, they are processed in cassettes either with a fine mesh, in lens paper, or in a 'tea bag'.[2] Curling artifacts are common in samples that are too small, making the correct orientation difficult during the embedding procedure. Curling is sometimes less problematic when thin lesions have relatively thick keratotic surfaces.[9] Curling can be prevented if, after the biopsy, the tissue is placed with the mucosal surface up (epithelial surface down) on a piece of sterile paper. This specimen can be allowed to remain unfixed for a short time while the incision is being sutured.[18] Since curling is seen in thin biopsy specimens, adequate depth of the biopsy specimen can help in preventing this artifact.[10]

With our personal experience we recommend, grossing of larger specimen prior to fixation as it will help in proper identification of representative areas for sampling. Few pointers for selection of representative tissue from the specimen are: avoid areas of hemorrhage, necrosis, traumatized areas, ligated areas, select areas with nodular outgrowths, calcified specks, variations in color and consistency.

SPECIMEN PHOTOGRAPHY

Photographs of fresh or fixed anatomic pathology specimens are obtained to aid in the documenting of pathologic lesions. To meet the needs of the medical staff for conferences, teaching, and patient care activities, photographs of gross surgical and autopsy case specimens are taken.[19] Color pictures are best taken either fresh or after brief (few to ten minutes) fixation. The brief immersion reduces distracting glare from reflected light. The color though may be altered if the immersion into formalin is prolonged (hours or days). If immersion is prolonged, return of some color can be achieved by placement in 70% ethanol for 10 to 15 minutes.[20]

Before photographing the tissue must be prepared and trimmed by washing to remove blood, blood clots and fat, opening the ducts and vessels and removing other unnecessary tissues around the lesion. The background should be spotlessly clean with no texture and should be well illuminated. A grey toned neutral intensity color is preferable (e.g. light blue). The use of drapes, sponges, gauzes should be avoided. Distracters like hands, forceps, probes, scissors and paper clips should be removed. Specimen identification can be done by the use of labels. Reflective glare should be avoided by properly placing the illumination system, by turning off the room lights and by blotting the cut section of the specimen with gauze. Several photographs of the lesion using different exposures are recommended. A clean ruler with metric system should be used for obtaining reference to size. The specimen should be properly oriented in the anatomical position and centered. Photographs of the external surface of the intact specimen as well as the cut surface should be taken. If possible, include normal structures in the photograph to serve as frame of reference for the lesion (Fig. 1).[21-27]

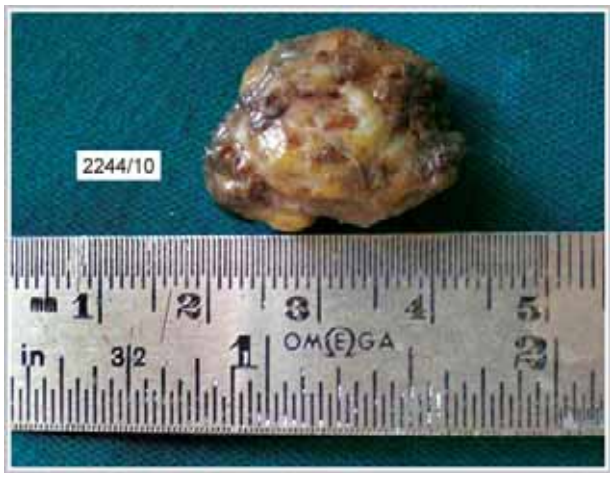
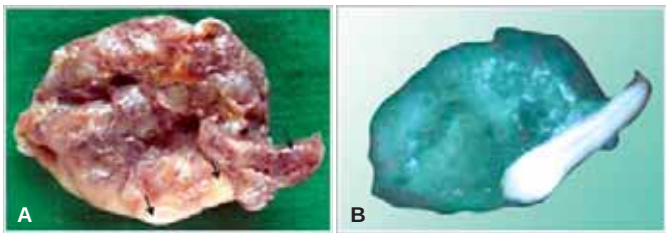


Fig. 1: A gross tissue specimen

SPECIMEN RADIOGRAPHY

Radiographic examination of surgical specimens sometimes provides important information. Specimens suitable for this type of examination include bone lesions, calcified soft tissue masses, lesions with embedded tooth (Figs 2A and B), radiopaque foreign bodies such as metal clips.[3] Radiologic—pathologic correlation can be made by perfusing radiopaque material within the lumina of ducts or vessels, radiographing the specimen and comparing the results with both the clinical X-ray film and gross specimen. Some pathologists have found specimen X-ray films useful for locating lymph nodes in radical resection specimens.[28-30] Others have used them to perform a microradiographic analysis of bone.[31]



Figs 2A and B: Gross photograph (A) of a specimen which revealed an embedded tooth on radiographic examination (B) *Courtesy: Dr. Rajiv Desai. Professor of Oral Pathology*

THICKNESS OF THE SPECIMEN

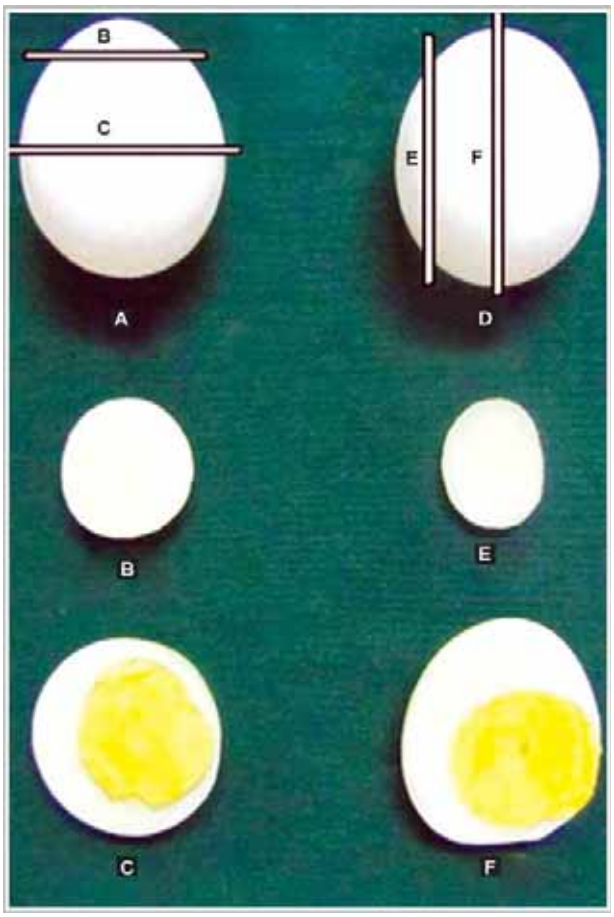
A uniform thickness of the specimen is an obvious requirement for processing, but it is difficult to achieve under conditions of insufficient fixation or inadequate hardening of specimen. The microwave-assisted accelerated processing has placed even more demands on uniformity of sections. The uniformity of sections is still an unresolved challenge at the practical level for grossing.[32] The tissue section should ideally be 2 to 4 mm in thickness.[4]

SECTIONING OF SPECIMEN

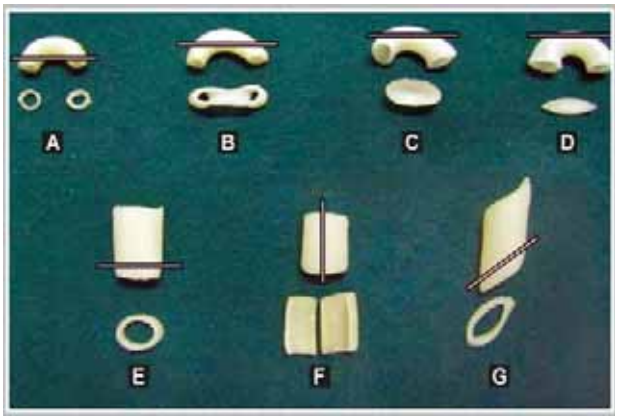
Any cut parallel to the longest dimension of a structure produces a longitudinal section, and any cut that is perpendicular to it produces a cross-section. A cut at any angle between these two planes produces an oblique section. A cut through the middle of a spherical structure produces a cross section and one that only grazes the surface produces grazing section, otherwise known as tangential section.[33]

Some idea of difficulties involved in visualizing three-dimensional structures from the appearance of single section can be gained by mentally reconstructing a hardboiled egg (ovoid structure). Some of the misconceptions that can arise are illustrated in Figures 3A to F. Only slice F in Figure 3 contains enough information to reveal the true structure of the egg. In some cases, the internal microscopic structure of parts of the body is so complicated that it can only be understood by mounting photographic enlargements of serial (consecutive) sections on material of appropriate thickness and assembling them in a proper order to constitute large reconstruction. It is very important to think in three-dimensions, when trying to match the shapes of the areas seen in histological sections to the shapes of structures encountered in gross anatomy.[33]

The body abounds with tubes of various diameters; those most frequently seen in histological sections are blood vessels, lymphatics and tubular ducts. Tubes are most easily recognized if they have been cut in cross-section. When



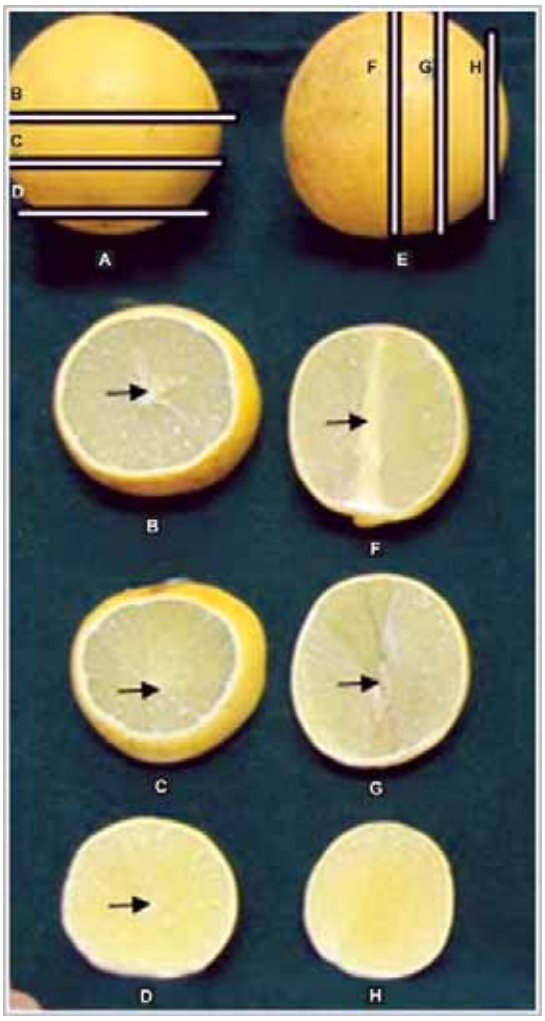
Figs 3A to F: Series of sections which shows the effect of sectioning on the interpretation of the lesion



Figs 4A to G: Appearance of sections of a curved tube (A to D) and a straight tube (E to G) at different levels relative to the center of the lumen

they have been cut longitudinally or obliquely, or have been sectioned in places where they are curved, it is necessary to visualize them in three-dimensions in order to recognize them as tubes (Figs 4A to G).[33]

The individual compartments of a glandular organ which may be comparable in size and shape may appear larger



Figs 5A to H: Series of sections showing the appearance of segments of a lemon (A to H) that has been cut in various planes of section. A is transversely cut, E is longitudinally cut. Although the segments are septum bounded compartments with similar dimensions, noticeable inequalities in size are seen in certain planes of section (C,D,G,H)

and some may appear smaller depending on the level of section. This is readily verifiable by cutting a lemon in different planes and observing the size of the specimen. Similarly, apparent differences in the size of nuclei or even whole cells can be due to differences in the level at which they are cut (Figs 5A to H).[33]

INKING

The purpose of inking is to determine the margins of the specimen, indicating specific areas of interest to the pathologist and the mode of embedding.[32] Inks of different colors designating each of six specimen margins (superior, inferior, medial, lateral, anterior and posterior) can ideally be used to maintain anatomic orientation of the specimen.[34]

Routinely used dyes for inking include India ink, eosin and hematoxylin. Davidson's Marking System (Bloomington, MN) dyes are commonly used for inking the specimens. The color options are green, black, orange, red, yellow and blue. Green dyes generally do not interfere with stains and is always visible while black dyes are more securely fixed to the tissue as they are protein based. Cotton tips and wooden picks may be used for dye application. Izak B. Dimenstein (2009) has recommended flat angled, 17 mm, smooth, nonwettable Stratagene's Strata Tips (Stragene Inc, La Jolla, CA) as best for inking. A color enhancer or a fixer such as acetic acid may also be used. A common practice includes the following: dry the tissue vigorously until the paper is not bloated, apply the dye, air-dry for a while, apply a fixer to the inked surface, and dry gently with a sponge, then process.[32]

Any unstained area should be facing down in the embedding mould, and the ink should be facing up if the specimen has been cut. If the specimen has not been cut, the inked area should be at the periphery.[32]

The disadvantages of using ink are that it obscures the view of the trimming pathologist and as colored inks are pigments suspended in an aqueous medium, they spread onto the cut surface of the specimen and stain the trimming board.[35]

V Shinde et al (2008) has recommended use of plain gelatine instead of colored ink or colored gelatine. The advantages of plain gelatine are: it is a colorless, translucent substance through which structures such as lymph nodes can be identified during trimming; its viscosity prevents its spread onto the cut surface of the specimen; it does not stain the trimming bench and tools; and as it is made up of proteins, it takes up eosin during the staining process and appears bright pink, making it easy to see the surgical margins under the microscope.[35]

ORIENTATION

Tissue should be oriented to determine the depth of invasion of the lesion and the margins of resection.[2] The ultimate goal of grossing is correct, diagnostically sound sections with proper orientation. Various techniques have been used for orientation such as sticking specimen to gelfoam using cyanoacrylate or strips of cellulose acetate. Agar, dehydrated plane cucumber slabs and Histogel can also be used.[32] In case of incisional biopsies, the specimen may be orientated by placing a suture at a known margin, for example the anterior or superior margin. This would enable the pathologist to confidently indicate the precise location of any residual tumor. The same applies to surgical resection specimens.[5]

EMBEDDING TECHNIQUE

Correct embedding and orientation is the last step where grossing can directly influence specimen processing.[32] Diederichsen C. and Whitlatch S (1999) have recommended sectionable cassettes with fluoropolymer platforms.[36] Silicone pads for automatic embedding orientation are also proposed but they are still at the stage of testing. The rest is in the hands of the histotechnologists who do the cutting and staining, although poor fixation, wrong thickness of the section, and incorrect placement in the processing cassette can significantly compromise the cutting and staining process.[32]

HEALTH HAZARDS AND SAFETY MEASURES

Staff members working in the gross room area encounter many possible risks including infections, chemicals which may be flammable, toxic, allergenic or carcinogenic, electrical and physical hazards as well as cuts and needle stick injuries;[2] the most common hazard being needle stick injuries.[37] Bone dust, as well as bone fragments and crumbles, disseminated in the working environment are potentially biohazardous.[38] Formalin fumes are also known to be a health hazard. It is a severe eye and skin irritant and is toxic by ingestion and inhalation.[2]

These can be minimized by proper tissue handling and fixation of the specimen before grossing. All tissues must be considered potentially hazardous and universal precautions must be taken as per occupational safety and health administration regulations. Adequate protective measures to protect from infection must be undertaken such as disposable gowns, gloves, facemasks and eye gear. Contact with chemicals should be minimized and the protective gear should be disposed off in correct manner. The laboratory personnel should clean the instruments and wash hands regularly to avoid spread of infection.[2]

CONCLUSION

Gross examination of pathology tissue specimens forms an important part in reaching at a correct diagnosis. Accurate gross description and observation of the pathology specimen can give many clues to aid in final diagnosis. The final report must include macroscopic and microscopic findings along with the final diagnosis. Thus, it is imperative for the pathologist to undertake this step meticulously.

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