Issues About Tissues, Part 2: Sampling In The Laboratory

Philip Bryant
Department of Pathology, Princess of Wales Hospital, Bridgend, Wales, United Kingdom

Abstract

This is the second part of 'Issues about tissues', which was presented at the 30th National Society for Histotechnology Symposium in Toronto, Canada. Laboratory sampling integrates macroscopic examination, gross dissection, and microscopy, and this work highlights issues based around these domains. Although custom and practice is diverse and may differ between laboratories, general guidelines and systematic protocols for sampling are available. The microscopically generated histology report is a powerful influence on the clinical outcome, therapy, and management of disease and ensures that the status of histopathology as a diagnostic tool remains undisputed. (*The J Histotechnol* 28:181, 2005)

Submitted May 16, 2005; accepted with revisions July 11, 2005

Key words: tissue sampling, dissection, resection margins, tissue sections

Introduction

One of the important skills in the histology laboratory is the ability to accurately examine, describe, and sample gross specimens. Although these tasks normally are performed by the pathologist, many histologists, technologists, and surgical pathologist assistants describe and process entire samples and biopsies on a regular basis. The examination of larger, more complicated resection specimens do require greater skill, and accurate descriptions of these samples must provide permanent histological records (1). These descriptions should include patient demographics, clinical information, observations made at dissection (such as the size and location), and a record of the samples taken (2). The difference between the amount of sample taken and the tissue as a whole is known as sampling error and, although, it may be small at gross level, it increases dramatically with magnification. With changes in some diseases remaining localized, sufficient slicing and examination of tissues in the laboratory is essential if diagnostic errors are to be minimized (3).

Address reprint requests to Philip Bryant, Department of Pathology, Princess of Wales Hospital, Bridgend, Wales, United Kingdom CF31 1RQ.

Laboratory Sampling

At the time of gross examination, most samples obtained from curative surgical procedures should be inked to aid orientation and determination of resection margins, although discriminating between what should and should not be inked must be considered with care (2,3). Ideally, margins should be inked and allowed to dry while the specimen is fresh, before fixation and preliminary dissection, but this is not always possible because most samples are invariably received in formalin. In the laboratory, the two methods for assessing specimen margins are parallel (*en face*) and perpendicular to the plane of resection (4). With the margin parallel to the plane of resection, the benefit is that a larger surface area can be examined, as seen with an entire circumference of a bowel resection margin. If tumor is identified in a parallel margin, then that margin is considered to be positive. Conversely, if no tumor is observed, then that margin is considered negative. One disadvantage of the parallel *en face* resection margin is that exact distances of any tumor to that margin cannot be assessed. The benefit of sampling a margin perpendicular to the plane of resection is the ability to determine precisely the distance of a tumor from that margin. Perpendicular margins are recommended when tumor approaches to within 2.0 mm to what otherwise would be considered a negative margin (4). However, the drawback of this type of margin is that only a small part of it is actually sampled.

There are many procedural guidelines available both electronically and in textbook format that are able to assist in the gross dissection of tissue samples (5,6). These manuals can help in the proper identification and orientation of specimens and provide descriptive texts and speech recognition programs (3,5). Generally, surgical resections for tumor are accurately described and measured, preferably with photographs and diagrams showing the sites of tissue blocks selected (3). It is from these samples that the histopathologist will diagnose the tumor type and report on the extent of spread and adequacy of resection (2). Samples that include the edge of a tumor usually are more informative and sampling of blood vessels should also be performed to show the

presence of vascular infiltration. Thorough sampling of lymph nodes should always be conducted to show the extent of tumor involvement. Although palpation of lymph nodes is the method generally practiced, it is best avoided because it biases the sampling in favor of involved nodes (1). Slicing the fat at regular intervals and processing all the nodes that are presented at the cut surfaces is a more commendable method (6). Taking an adequate number of blocks for histology from the large resection samples is vital, and tissue blocks should be thin enough for adequate processing to ensue (3). In small samples where only a single block is available, then it is equally important to examine deeper sections throughout the block to ensure that sampling errors are minimized (1).

Sectioning

For most diagnostic purposes, paraffin sections are cut at approximately 5 μm, although there are instances when sections may be cut thicker or thinner (1). Thick sections will reduce sampling error but generally are useless for diagnosis of most lesions because of superimposition of cells. Sections may be cut at 10 μm or greater yet still provide a diagnosis particularly with staining methods associated with the nervous system. However, 20 μm sections can be a useful way to find pathogens such as bacteria or asbestos fibers provided focusing the microscope through all planes of the section is carried out. Although paraffin wax sections can be cut thinner than 5 μm, it is preferable to use tissues that have been embedded in acrylic or epoxy resins since they provide greater support (3). Current applications include renal biopsies, lymph node biopsies, and bone marrow trephines where the clarity provided by a 1 μm plastic section is a consequence of the elimination of cellular superimposition (1).

Discussion and Conclusion

Microscopic examination of tissues is the principal method for histological diagnosis, yet interpretation does not necessarily become easier or more accurate as the magnification increases. In reality, it is low power appearances that often provide the means of differentiating benign tumors from malignant ones, and it is in this capacity that the pathologist contributes his role (2). Diagnostic histopathology is important for both clinical practice and the management of patient care. The evidence provided by the many processes involved has shown that the interaction between the clinician and the laboratory is as vital as the ability of the histopathologist to correctly interpret different abnormal states.

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