Preparing Fresh Tissues for the Microscope

ORIGINAL ARTICLE

A Method for the Rapid Preparation of Fresh Tissues for the Microscope

Louis B. Wilson, MD

JAMA. 1905;45(9):1737

While engaged in general pathologic work I shared the common distrust of frozen sections of fresh tissues for microscopic diagnosis. On taking charge recently of the laboratories of the Drs. Mayo, surgeons, I carefully tested the various methods hitherto published and found them either too slow for results while the patient waits under the anesthetic or else giving poorly differentiated cell detail. After considerable experimentation the following technic was discovered, and for the last six months it has given uniformly excellent preparations:

1. Bits of fresh tissue not more than $\frac{2}{10} \times \frac{10}{10} \text{ mm.}$ are frozen in dextrin solution and cut in sections of from 10 to 15 microns thick.
2. The sections are removed from the knife with the tip of the finger and allowed to thaw thereon.
3. The sections are unrolled with camel's-hair brushes in 1 per cent. NaCl solution.
4. The sections are stained from 10 to 20 seconds in neutral Unna's polychrome methylene blue.
5. They are washed out in 1 per cent. NaCl solution.
6. They are mounted in Brun's glucose medium.

The microtome which I use is the Spencer automatic with a CO2 attachment in which vulcanite is substituted for brass in the wall of the freezing chamber, thus insulating the freezing plate. Thawing the section on the finger prevents to a great extent the formation of bubbles. The well-made camel's-hair brushes used by artists are much more useful for handling tissues than those usually furnished by laboratory supply houses. A heavy, shallow watch glass over a black surface is the best receptacle in which to unroll sections. Sections are best handled in the stain folded over a lifter made of a small glass rod drawn out and bent at convenient angle. The section is kept constantly moving while it is in the stain. The stain is contained in a minute cup to facilitate the rapid recovery of the section should it slip from the lifter. Washing out is done in several ounces of salt solution in a white porcelain dish and is continued only while the stain comes away freely. Brun's glucose medium (which is made by mixing distilled water 140 c.c., glucose 40 c.c., and glycerin 10 c.c., and adding camphorated spirit 10 c.c. and filtering), is held in an oval dish of porcelain (an "undecorated match safe") of such a size that a three-inch slide will rest in a slanting position, with one end in the bottom of the dish and the other on its edge. The section is spread out on the slide while it is in this position. The slide is then carefully withdrawn from the dish, the excess fluid removed, a coverslip dropped over the section and the specimen is ready for the microscope.

The whole process can be gone through in one and a half minutes from the time the tissue is placed on the freezing plate of the microtome until the stained specimen is on the stage of the microscope. The resulting coloring is uniformly good with the tissue elements sharply contrasted in red, purple and dark blue.

A diagnosis may be made from such preparations in a large percentage of surgical cases in which a diagnosis is possible by a study of sections of the same thickness cut from fixed tissues and stained with hematoxylin and eosin.

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Commentary by Gary Keeney, MD, and Kevin Leslie, MD

IN 1905, JAMA PUBLISHED A BRIEF REPORT AUTHORED BY Mayo Clinic pathologist and bacteriologist Louis B. Wilson in which he outlined a method whereby fresh tissue removed during surgery was examined immediately for diagnosis by microscopy. This was not the first exposition of the rapid preparation of fresh tissues for microscopy, but Wilson's refinements allowed the method to be applied to every surgical specimen removed at Mayo Clinic. This operational change for surgeons and pathologists heralded the beginning of the “frozen section” and, in turn, what is known today as “surgical pathology,” a subspecialty discipline within the broader context of postmortem pathology.

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The historical details related to initial and subsequent events were described by Gal and Cagle in 2005, in celebration of the centennial anniversary of the article by Wilson. In this Commentary, we provide a brief perspective of frozen section practice at Mayo Clinic over the years and venture an assessment of the future prospects of this technique.

As the reverberations of Wilson’s practice expanded beyond Mayo Clinic, the reactions were not always favorable. Many academic pathologists questioned the appropriateness and accuracy of such rapid intraoperative diagnoses, although later studies showed that the frozen section practice at Mayo Clinic is at least as accurate as frozen section procedures performed at other institutions. A number of additional factors influenced a continued negative reaction, not the least of which was the need for pathologists to be available continuously during surgery and the fact that most surgical suites then (and even today) were not designed for the presence of pathologists and their equipment for immediate microscopic examination of tissues.

Most surgical pathology practices ultimately adopted the cryostat method for performing frozen section procedures, a technique that involves slower tissue freezing and presents more technical challenges and potential artifacts (especially when dealing with fatty tissues). Mayo Clinic Rochester continues to perform the method used by Wilson with the freezing microtome that is faster and uses a colder freezing temperature, allowing the operator to obtain readable sections of tissues containing fat (breast biopsies, breast margins, and fatty lymph nodes), typically in 1 minute or less. Notwithstanding the numerous advantages of this method for frozen section procedures, the time-intensive and “emergent” nature of the frozen section procedure, the costs and specialized personnel involved, and the need for extreme diagnostic stringency on the part of the involved pathologists undoubtedly played significant roles in creating some prejudice against the model of surgeon and pathologist “joined at the hip” during surgery.

Despite the hurdles limiting a more generalized applicability of the method used by Wilson, the close working relationship established in 1905 between Mayo Clinic surgeons and pathologists flourished over the years and remains alive and well today. Like the navigator of an airplane, pathologists continue to play an ever-present role during surgery. Techniques have evolved with equipment advances (such as the replacement of aerosolized carbon dioxide with an enclosed refrigerant) but the underlying philosophy remains the same: the diagnostic information from the pathologist is vital to the surgeon while the operation is in progress. In fact, not only have critical diagnoses been rendered intraoperatively for more than a century, for most specimens the entire evaluation is performed during the surgical procedure, with the intent of having the complete and final pathology report in the record before the patient is returned to the hospital room. Speed in producing tissue sections and comprehensiveness of the pathology assessment during surgery remain the goal. In contrast, typical frozen section techniques at other hospitals take significantly longer to perform and are intended for very limited application and very focused questions (eg, is this a tumor? is this tissue adequate for diagnostic purposes?).

The dedication to the frozen section method at Mayo Clinic Rochester came with tangible benefits for Mayo Clinic pathologists. With so many tissues being examined routinely by the frozen section method, the operators became highly proficient and the pathologists’ expertise as diagnosticians increased progressively. The process, being quite labor intensive, requires experience and appropriate mentorship. To underscore this point, Dahlin, a Mayo Clinic pathologist, stated in an editorial in 1980 addressing the first 75 years of frozen sections at the Mayo Clinic: “Persons poorly trained in the method usually deprecate the technique. Essential for the correct interpretation are a capable pathologist and sections of good quality.”

The substantial challenges associated with the technique were emphasized when Mayo Clinic branches were established in Florida (1986) and Arizona (1987). Both locations found that duplicating the process exactly as it is performed in Rochester, Minnesota, was not possible or cost effective. At these locations, frozen sections are still performed more liberally than in most centers across the United States, but not with the same equipment, intensity, or staffing levels of those at Mayo Clinic Rochester. It would seem that the phenomenon initiated in 1905 is origin specific.

As to the future of frozen section practice, the challenges faced at Mayo Clinic today reflect a worldwide trend toward ever-earlier subspecialization in pathology training. With new generations of diagnostic pathologists emerging with great knowledge focused on narrower specialty themes, the generalists left to staff the frozen section suite may be a vanishing breed. The unlikely alternative would require multiple (10 or more) subspecialists, optimally in duplicate, awaiting specimens from their preferred organ system. Mayo Clinic has adapted to this problem, in part, by using the expertise offered by numerous subspecialist pathologists at work in their offices nearby via live videomicroscope connections—an adaptation limited by consultant availability.

Another critical issue is related to particular diseases (eg, lymphomas) that require special diagnostic testing before a final diagnosis can be rendered. Mayo Clinic Rochester currently assesses the adequacy of such tissue samples and final diagnosis for these cases is rendered after surgery and the completion of immunohistochemical and molecular diagnostic testing. It is not a stretch to envision a time when pathology of nearly every organ system (certainly tumor pa-
thology) will require specialized postsurgical analysis to provide information for primarily nonsurgical targeted medical therapies. At Mayo Clinic Rochester, the goal remains to finalize the pathology diagnosis as often as possible during surgery and report the results of ancillary tests as an addendum the next day.

Two key questions remain: Is intraoperative pathology feedback to the surgeon really necessary today, and does this practice lend itself to meeting the ever-increasing demands of the current molecular genetic age, in which more and more information for diagnosis, therapy, and prognosis is being gleaned from smaller patient tissue and blood samples? Despite the challenges outlined, we believe that “guiding the surgeon’s hand” will remain an integral part of high-quality surgical pathology practice. Without this collaboration important opportunities would be lost, such as determining the adequacy of tumor margins, tissue assessment for immediate intraoperative therapy, confirming the presence of diagnostic tissue allowing immediate triage for special studies, and identifying whenever possible the underlying nature of a pathologic process.

Financial Disclosures: None reported.

REFERENCES