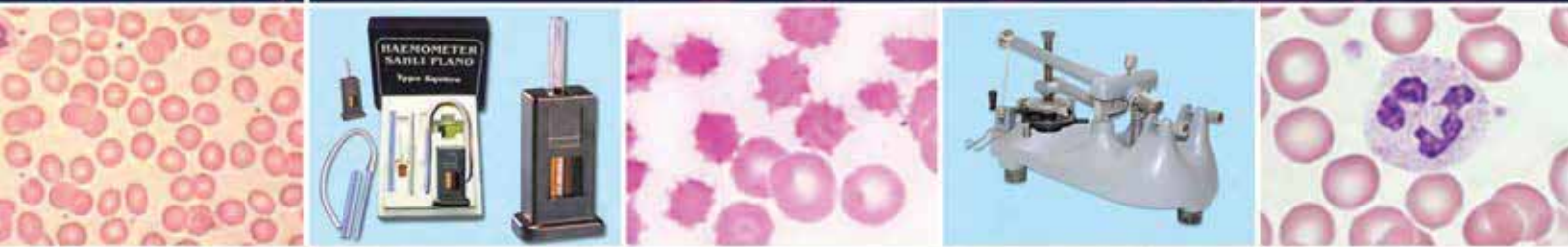


Principles & Interpretation of Laboratory Techniques in Pathology

Hematology, Cytology, Histopathology,
Museum and Autopsy Techniques



Shameem Shariff



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Museum Techniques

INTRODUCTION

“The dead teach the living” is the English translation of the Latin “mortui vivos docent” used to justify dissections of human cadavers in order to understand the cause of death. The same can be applied aptly to all pathology and anatomy specimens displayed in museums.

An institutional museum is a place where a record of operated and resected specimens is kept. All teaching colleges and hospitals have museums attached to the department of pathology which serve many functions, including a permanent exhibition of surgical specimens for undergraduate and postgraduate teaching purposes, a display of the operating skills of the institutional surgeons, and an important repository for congenital and developmental anomalies. The saying “Here the dead teach the living” is apt for the museum wherein there are present organs and organ systems to enrich the knowledge of medical and science students. Organ demonstrations still hold value for teaching although e-learning is slowly replacing this.

In order to set up a worthwhile museum familiarity with techniques of preserving and mounting specimens is mandatory.

BASIC MUSEUM TECHNIQUES¹⁻⁴

Any specimen for a museum should be handled by the following steps:

- Reception
- Choice of specimen for museum/mounting
- Preparation and fixation
- Restoration and preservation
- Mounting procedure
- Presentation and display

Reception of the Specimen

Any specimen received in the museum for purposes of mounting should be recorded in a reception book and given a specimen number—numbering should be, serial number, followed by year (e.g., 02/2017 is the second specimen received in the year 2017 for the museum). This number is the permanent specimen number for that particular specimen in the museum whether it is used as a wet specimen or mounted and displayed on the racks. This number is written on a tie-on type label in permanent ink and is stitched onto the specimen. The first column in the reception book will contain this number against which all necessary information about the specimen (surgical biopsy no., clinical, gross, and microscopic findings will be entered). The specimen may be used for display on the museum rack or as a collection as a wet specimen for the trainee or even mounted and given for display in other clinical departments.

Choice of Specimen and Categories for Museum/Mounting

Specimens in the museum may be channeled for various purposes. The ultimate use of the specimens will determine the best way to select or choose a specimen for the museum. The senior teaching staff of a department should be made responsible for the choice of the specimen for a specific purpose. For instance:

- Specimens intended for placement on museum racks (rarities and those for display) should be mounted in Perspex or glass jars which are sealed, and the specimen number and label be fixed on the jar.
- Specimens for demonstration/teaching of undergraduate medical students (where no handling of wet specimens by the students is needed); should be

completely enclosed in portable and nonbreakable containers, such as plastic bags (**Fig. 1**), or they may be mounted in plastic jars which are sealed in order to avoid leakage and kept aside for this purpose. Specimens in plastic bags are easy to handle by students.

On the other hand, fixed and washed specimens are suitable for examination by pathologists-in-training (**Fig. 2**), clinicians, and sometimes undergraduate medical students. These should be fixed and tagged with labels and kept aside in big bins with fixative for removal as and when needed. When put aside in bins, each specimen should have a long strong thread attached to it with the tie-on type of label in indelible ink attached both to the specimen and the loose end of the long thread (**Fig. 3**).



FIG. 1: Specimen mounted and sealed in a plastic bag for easy handling by the student.



FIG. 2: Wet specimen handled by the trainee (postgraduate) in pathology.

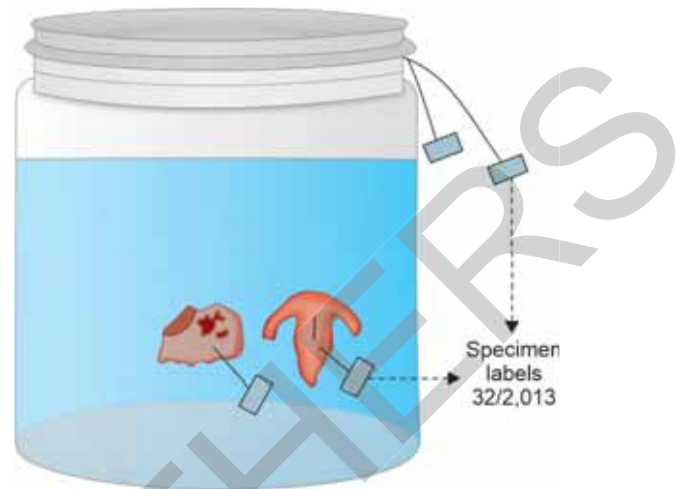


FIG. 3: Wet specimens stored in bins with labels attached to both the specimen and the end of a long thread.

The long end of the thread with the label should hang outside the closed bucket/large plastic container in order to read the number and identify a specimen easily. These specimens should be kept in running water for at least a few hours before handling and then reinserted after use in the same bucket with the thread tags hanging out. Bins or buckets should have adequate fixative, which should be checked and refilled from time to time.

Occasionally specimens are sent to the pathology department by surgical/gynecological departments with a request for mounting and transfer back to the clinical departments. Such specimens too should be entered in the reception register, mounted in sealed jars, and the detail mentioned against them with regard to its transfer to the said department, after mounting.

Preparation and Fixation

An ideal specimen for the museum is one that is obtained in a fresh unfixed state. However, most museums receive specimens from the pathology laboratory after being handled for grossing and so in formalin-fixed state. If an interesting specimen is seen at grossing to be used for a museum, the department policy should be that part of it (maybe one half) can be kept without disturbing it during grossing, e.g., in a kidney, it can be bisected, and one half kept aside for museum purposes; similarly, uterus and cervix. However, no compromise on the quality of the report should be made in order to preserve one-half. Any extra tissue/fat may be trimmed in order to optimize the specimen and its pathology. Fat-dissolving solutions such as chloroform and acetone may be used.

Fixation of the Specimen¹⁻⁴

It is essential that all museum specimens be adequately fixed. Proper fixation preserves specimens in a life-like

fashion for several years. The universal museum fixative is based on the principle of formalin fixation and derived from the “Kaiserling technique” and its modifications. Subsequently, many laboratories have had their own modifications (Meiller’s, Lundquist’s modifications, etc.).

As per *Kaiserling’s technique*, the initial fixation is in a neutral formalin (K-I solution) and then the specimen passes through Kaiserling II and is then transferred to a final preserving glycerin solution (K-III) in which it is displayed. Color preservation is maintained with these solutions.

Kaiserling’s Technique Fixation of Specimen

The specimen should be immersed in 10–20 times the volume of fixative in a spacious container that can accommodate the specimen and volume of fixative. The specimen is stored in the Kaiserling I solution for 1 month depending on the size of the specimen. Care should be taken such that the specimen does not rest on the bottom of the container, or else an artificial flat surface will result in hardening during fixation.

Kaiserling I solution:

- *Formalin*: 1 L
- *Potassium acetate*: 45 g
- *Potassium nitrate*: 25 g
- Distilled water makes up to 10 L

Restoration of Specimen¹⁻⁴

After fixation, the specimen is transferred to Kaiserling II solution for restoration of color that it lost during fixation. Before immersing in solution II, the specimen is washed in running water and then transferred to 95% alcohol for 10 minutes to 1 hour depending on the size of the specimen. The specimen is then kept in solution II and observed for restoration of color for around 1–1.5 hours. Longer periods than this will fade the color and this change is irreversible. After this step, the specimen is ready for preservation.

Kaiserling II solution (rejuvenator solution)

- *Pyridine*: 100 mL
- *Sodium hydrosulfite*: 100 g
- *Distilled water*: 4 L

Preservation of Specimen

The recommended solution for preservation is Kaiserling III. This is the final solution in which the specimen will remain for display. It is a glycerin-based solution.

Kaiserling III solution

- *Potassium acetate*: 1,416 g
- *Glycerin*: 4 L
- Distilled water up to 10 L

- Thymol crystals were added as a preservative to prevent contamination with molds.

Leave solution to stand for 2–3 days before using to ensure proper mixing of chemicals. Add 1% pyridine as a stabilizer. This solution acts as a permanent fixative. This solution over time changes color to yellow and needs to be replaced to restore the color of the specimen.

A modification of K-III solution made by Pulvertaft³ replaces solution K-II and is as follows:

- *Pulvertaft–Kaiserling mounting fluid III*:
 - Glycerin: 300 mL
 - Sodium acetate 10% (pH 8): 100 g
 - 10% formalin: 5 mL
 - Tap water: 1,000 mL
 - Camphor/thymol can be added to prevent the growth of molds.

Immediately before sealing 0.4% sodium hydrosulfite is added. The amount of hydrosulfite should not normally exceed 0.4%. If color restoration must be rapid, 0.6% may be added, but this is to be avoided, as a white precipitate may form.

Mounting the Specimens

To support the specimen within its jar in the preservative, it is attached to a specimen plate (of Perspex or other plastic) by drilling holes into the Perspex sheet and tying the specimen with nylon threads through these holes. A rectangular bent glass rod can also support the specimen in the jar. This can be done by tying the specimen with nylon threads to the rod. Proper orientation should be supervised by the teaching staff.

Display/Presentation of the Specimen

Previously museum specimens were mounted in cylindrical jars and sealed with sheep bladder walls. Later they were replaced by rectangular glass jars; which afforded better viewing of the specimens. They were covered by rectangular glass plates and sealed. Nowadays, Perspex jars are available, which are lighter than glass jars and nonbreakable. Due to the convenience of usage, most laboratories use these for their mounting. However, they cannot be used to store specimens fixed in alcohol as this reacts with plastics over a prolonged period of time. It is advisable to bulk order these Perspex jars in various sizes and store them. They can be used from time-to-time depending on the size of the specimen. Departments with large museums often buy plastic Perspex sheets and train personnel to make jars in the department workshop itself; this is cost-effective. Specimen jars should not only have the specimen number clearly written on the jars but also provision be made in one corner for shelf no. (cupboard), rack no. (upper,

middle, or lower horizontal rack), and placement serial no. starting from the right or left side of the horizontal rack. This helps in the easy replacement of the specimen even by personnel not familiar with placements after the specimen's usage. Separate shelves may be used to segregate specimens frequently taken out for undergraduate student demonstrations.

Other Methods of Specimen Display

Thin slices impregnated with gelatin or sealed between glass sheets, plasticated bronchial tree specimens, etc.

CATALOGS

These should be prepared so as to enable one to see the detail on a particular specimen. Each specimen should have in the catalog the specimen number with

the following details: Shelf no., rack no., surgical biopsy no., gross specimen photograph, salient microscopic description with microphotograph, and finally the diagnosis. Most universities require such a complete picture to be maintained at least for the teaching specimens with a number of catalog copies to be made available for the students.

CONCLUSION

It is imperative that all laboratories strive to have a good display of all their surgical specimens not only for record purposes but also to enhance and motivate teaching to the younger generation. In order to have this documentation of the procedures used to enhance the quality of the specimens displayed should be undertaken in order to preserve these rare exhibits.

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Principles & Interpretation of Laboratory Techniques in Pathology

Salient Features

- Hematology techniques with principles, procedures, and methods
- Approach to investigation of various anemias
- Cytology sample collection and processing techniques of all fluids
- Reporting criteria on urine analysis, synovial fluid, and semen analysis as well as "The Bethesda System of Reporting Cervical Cytology" with recent advances and recommendations
- Histology techniques with the journey of specimens from "receipt in the laboratory to the finished report"
- Advanced procedures used in pathology
- Principles of and functioning of various types of microscopes
- Automation in pathology
- Photography, detail on museum techniques, and autopsy techniques.

Shameem Shariff MD PhD is a Senior Consultant, Surgical Pathologist and Cytopathologist. She recently retired as Professor and Head, Department of Pathology, MVJ Medical College and Research Hospital, Bengaluru, Karnataka, India. She also headed the Research Division of the Institution. Prior to this, she was heading the Department of Pathology and Cytology, St John's Medical College and Hospital, Bengaluru.



She graduated from the Government Medical College, Bellary, Karnataka, and did her postgraduation in Pathology from Bangalore Medical College, Bengaluru, in the year 1981. In 1995, she was awarded PhD in Pathology, first in the State of Karnataka. She served St John's Medical College, Bengaluru, for 20 years, from 1982 to 2002. She served as a Senior Consultant in the Ministry of Health University Hospitals in Bahrain and Sultanate of Oman from 2002 to 2008. In 1987, she trained at the prestigious Memorial Sloan Kettering Cancer Center, New York, and at the Memorial Medical Center, Long Beach, California, USA on an Endocrine Foundation Travel Fellowship. On her return, she established the FNAC division at St John's Medical College.

She has several awards to her credit—in 1984, the Khanolkar Award (young scientist award) by the Indian Association of Pathologists and Microbiologists for the best research paper of the year, at the Annual Conference of the IAPM; in 1996, the Excellence in Teaching Award; and in 1998, the Ernest Fernandez Award.

She has presented papers at several National and International conferences—the 15th International Conference of the International Academy of Pathologists held at Miami, Florida, USA (1984), and the 11th International Conference of Tropical Medicine and Malaria, Alberta, Canada, to mention a few. She has over 110 national and international publications. She has edited and authored three textbooks targeting postgraduates and surgical pathologists. These are among the recommended books by Rajiv Gandhi University of Health Sciences (RGUHS) for postgraduates.

A well-known Pathologist and Cytologist, her name featured among the cytologists of the country in the manual "Cytologists of Repute in the Country" published at the Annual Cytology Conference in 2009. She is invited all over India as guest speaker in Cytopathology and Histopathology. She has been an undergraduate and postgraduate Examiner to several universities in India as well as a PhD guide in Pathology at the RGUHS. She has been member of the PhD Board of Studies at the RGUHS.

She is the Founding Member Secretary of the Academy of Pathology, presently the President of the academy. She was on the editorial, Journal of Cytology; and is presently on the Editorial Board, National Journal of Basic Medical Sciences; and Advisory Board, Journal of Medical Sciences and Health. Her website www.shameempathology.com is an innovative teaching platform for surgical pathologists where she has laid open her collection of original cases in the form of recorded videos, tutorials and online classes.

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