

The post-analytical phase of histopathology practice: Storage, retention and use of human tissue specimens

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ABSTRACT

There are several aspects to a histopathology practice besides the acquisition of biopsy specimens and histopathological diagnosis. Pathology Departments are home to an abundant source of knowledge in the form of stored specimens and slides. We attempt to highlight the importance of regulation of storage, retention, and appropriate use of human tissue material in research and ownership rights to the same. We also discuss requirement and waiver of informed consent for scientific work involving the use of such tissues, which in the absence of defined laws come under the purview of Institution Review Boards. Pathology Departments, under the binding of the parent institution, are conceded the responsibility of maintenance and retention of pathology specimens. This communication highlights some of the important aspects in human tissue material handling and research, underscoring the necessity for established regulations regarding the same.

Key words: Archival tissue, biospecimens, informed consent, pathology, storage

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INTRODUCTION

The basic tenets of a histopathology practice are the acquisition of a specimen that is biopsied by the clinician, gross analysis of the specimen and appropriate tissue sampling followed by routine processing, histopathological examination and finally, histopathological diagnosis. However, the story does not end here. There are several important aspects after despatch of the histopathology report such as storage, retention, and ownership of human tissue specimens.

Pathology Departments and museums are home to a vast number of human tissue specimens that remain after the completion of clinical and pathological investigations. Currently,

there is still ambiguity surrounding issues involved in handling of human tissue specimens. Different organizations may exercise individual protocols for retention of gross specimens, paraffin blocks, and slides. Also, pathology research often necessitates the use of archival specimens. However, there remains a lacuna in regulations addressing the use of such tissue material and the requirement of informed consent. There is also ambiguity as to who, in fact, has ownership rights over biospecimens.^[1,2] Therefore, in this communication we humbly attempt to highlight the subjects of storage, retention, ownership or custodianship of stored tissue specimens and the concerns revolving around such specimens in present or future research.

STORAGE AND RETENTION

It is ideal to store tissue materials indefinitely; however, practical issues such as constraint of adequate storage space in most Pathology Departments may not always permit so. Professional organizations such as the College of American Pathologists and the Joint Commission on Accreditation of Health Care Organizations (USA) recommend that tissue blocks and slides must be retained for a sufficient period of time as for appropriate care of the patient (10 years for paraffin blocks, wet tissue, histology slides, cytology smears and 7 days for peripheral smears).^[3] The Royal College of Pathologists, UK recommends preservation of blocks

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permanently, histology slides and smears for 10 years and wet tissue for at least 4 weeks after despatch of the report.^[4] In general, a period of 10–20 years is considered as a lower limit guideline for retention.^[5] It is routine for major institutions in India to preserve slides and blocks for 10 years and for 25 years in cancer referral centers.

The conditions of storage are equally important. Museum specimens and left-over gross specimens after appropriate sampling and diagnosis are stored in 10% neutral buffered formalin, whose volume should be at least 20 times the volume of the specimen. Neutral buffered formalin maintains the solution at neutral or slightly alkaline pH and is advantageous to counter the acidity of formalin on prolonged storage.^[6] By general experience, we propose that the following can be used as simple guiding points for replacing of formalin solution in long-term storage:

- When pH turns acidic. The pH can be easily tested periodically by using the litmus paper test. Change in color of blue litmus paper to red indicates a switch to acidic pH
- When paraformaldehyde precipitation is observed at the bottom of the container
- When the solution shows discoloration.

Paraffin blocks and slides should be stored below 27°C or at room temperature in humidity-free conditions with adequate pest control. Stained slides should also be kept away from direct light to preserve the intensity and quality of stains for a longer duration. In case unstained slides are required to be stored for future testing (immunohistochemistry, molecular hybridization, etc.) it is recommended that they be kept in absolutely dry conditions to prevent hydrolysis and for adequate preservation of proteins. Storage under refrigeration at 4°C has also been suggested as it was shown to produce improved quality of staining; however it is neither proven to be superior nor is practical in a routine laboratory setup.^[7]

OWNERSHIP OF TISSUE MATERIAL

The procurement of tissues from patients may either be in the course of routine diagnosis and treatment (e.g., pathology archives) or specifically for the purpose of use in research (e.g., blood samples collected prospectively). There is lack of established law addressing the issue of ownership or custodianship of tissue specimens used for research.^[1-3] In the absence of existing regulations, there is a conflict of opinion regarding ownership rights to tissues amongst patients, surgeons, pathologists and the institution housing the tissue specimens.

In practice, the patient may request for his clinically-archived tissues (in the form of paraffin blocks or slides) to be

transferred to other pathology centers/laboratories for additional testing or consultation. Keeping patients' care in utmost precedence, his wishes should be honored whenever possible. Concurrently, it is also imperative to keep in mind that records are generally considered to be the property of the person(s) who make them; in accordance with which the pathologist may retain day-to-day control over archived tissue materials and may exercise independent judgments on sampling, retention and diagnostic value of the same.^[8,9]

There is a lack of guidelines regarding transfer, use and exhaustion of paraffin blocks in histopathology practice.^[8] When requested by the patient, precut unstained sections, or a diagnostic core sample may be released to the patient. The reverse could also be practiced wherein recut deeper sections may be retained by the Pathology Department and the remaining block(s) may be handed over to the patient. It is of utmost importance to maintain a chain of custody at all times, and document transfer of diagnostic tissue material at each step. Sometimes, exhausting of blocks may become necessary in the course of diagnosis, and patients must be informed beforehand that the diagnostic tests may entail the use of all of the removed tissue.^[5,8] This is especially relevant in incisional biopsies of suspected malignancies or cysts, where serial sectioning may be imperative to rule out or to confirm the diagnosis.

The parent care-providing institution may claim its right over tissue materials, particularly in the event of conflict. In a landmark case, Dr. William Catalona requested transfer of more than 3500 tissue samples which he had amassed during the course of his employment at Washington University, St. Louis, USA. He asked his patient-donors who had initially consented for removal of their tissues, to write to the university requesting the transfer. The university did not oblige, and a legal dispute arose. The courts finally ruled that the control lay with the university, and since the patients had made a gift of their samples, while they may ask for their samples to be destroyed, they did not retain the right to direct transfer of samples.^[2,3,10] It is generally accepted that the institution has an ultimate physical claim to specimens and tissue materials.^[9]

In another historic case, cells from a woman named Henrietta Lacks suffering from cervical cancer were utilized to develop the widely used immortal HeLa cell line, which went on to change the field of cell biology and contributed to a vast array of medical advances. Neither was consent ever obtained from Henrietta Lacks or her family nor did they benefit from any profits obtained from the use of the cell line. This raised fundamental questions about ethics, violation of anonymity through the nomenclature of the cell line, informed consent, and benefits sharing.^[11,12]

Cases like these brought to light the dilemma of ownership of tissues. In certain instances, courts have ruled that patients

relinquish any property rights in excised tissues.^[3,8] Especially in the case of left-over diagnostic tissues, which are in contrast to tissues collected prospectively for research, it has been suggested that patients forego rights to control the fate of such tissues or the products or profits derived from them. In the absence of specific laws, it is unclear if patients possess the right to refuse the use of their diagnostic tissue in research.^[8] While keeping in view patients' rights, it is also debated that recognizing property rights in tissues would threaten effective use of stored tissues for research and render it impractical, and ultimately deny greater benefit to the human society.^[3,10] It is yet to be determined within who rests the ultimate authority and control over the fate of excised tissues or biospecimens. Court cases involving overlapping claims underscore the importance of having policies or guidelines for biospecimen-based research in place.^[1] There is an urgent need to reach a broad consensus regarding ownership of human tissue specimens.^[2] Regulatory bodies will perhaps, describe upon these aspects in the future.

In the context of ascertaining who can exercise a right over excised tissues, it is noteworthy to understand the differences between custodianship and ownership. The notion of ownership includes the property rights, where the owner usually possesses the right to use, sell, transfer, exchange or destroy his property and to prohibit others from doing so.^[3] On the other hand, custodianship involves charge and control of property (e.g., excised tissue) within specified legal guidelines.^[9] In general practice, custodianship of tissue material is a responsibility bestowed upon Pathology Departments under the ultimate binding of the Parent Institution. In fulfilment of their duties as legal caretakers, it is up to Pathology Departments to regulate the use of such materials for research, teaching or other purposes. Research on tissue blocks may be permissible as long as adherent to legal regulations. That said, it is important for pathologists to be aware of their responsibility to ensure that all of the available diagnostic tissue is not exhausted for research.^[8]

Human tissues are an indispensable source of knowledge. Institutions should be encouraging toward avenues of scientific inquiry and promote biospecimen use to fully utilize their potential in the interest of biomedical research initiatives and for the greater benefit of health science.^[8] At the same time, both the privacy of research participants as well as the intellectual investment of investigators should be accorded due respect.^[13]

ROLE OF THE INSTITUTION REVIEW BOARD

Research may involve use of tissues that are specifically obtained for the purpose of research, those collected within the context of diagnosis or treatment prior to pathological

assessment and remnant tissue from that which was collected for clinically-indicated procedures. Tissues may be used for different purposes such as research, as control tissues, for educational or academic use. Decision-making regarding use of human tissue specimens is usually the responsibility of the Institutional Review Boards (IRB) also known as Institutional Ethics Committees (IEC), who are designated by the Indian Council for Medical Research (ICMR) in India, to thoroughly evaluate the scientific content and ethical soundness of any proposed research prior to its commencement.^[14] The prime considerations in this regard are protection of patients' interest and privacy and informed consent in research.^[1] For long it has been ambiguous whether studies involving human tissues are subject to full review by the IRB and if the requirement of informed consent may be waived off.^[2] Broadly speaking, most of the research conducted by pathologists does not harbor the potential to cause any serious physical or mental harm to human subjects. It usually deals with assessment of histological parameters, investigating the pathological basis of diseases, laboratory techniques and prognostic information, all with the ultimate goal of improving patient care.

The IRB functions to screen research proposals and depending on the risk involved, categorize them into three types, namely, exemption from review, expedited review and full review. A research proposal may be exempt from review if it presents with less than minimal risk for the research participant. Exempt research does not require IEC screening and the requirement of informed consent can be waived off in certain situations in research subject to expedited review, at the discretion of the IEC.^[14] It has been suggested that any data or samples that have been detached from any identifiable information (anonymized) does not fall under the category of human subject research, and thus may be classified as exempt research.^[15] However, the level of anonymization of samples and data in existing pathology centers may be variable. It should be the prerogative of the medical records departments to bring institutions closer to this goal.

Some experts believe that since diagnostic tissues are considered to be abandoned by patients, they are different from biosamples obtained prospectively and should also be categorized as non-human subject research and thus, exempt from review.^[3] Expedited review may suffice for some studies such as those involving records or specimens that have been collected for non-research purposes or left-over samples after clinical investigations and pathology archival material. The IRBs have a crucial role in this regard as they can exercise discretion regarding tissue procurement, use in research and waiver of requirement of informed consent. They can also decide under what conditions re-consent or separate approval from patients is imperative.^[14,16]

Research involving genetic information warrants special consideration. Studies aimed at screening of genetic patterns or disclosures of genetic information are subject to the general ethical principles of protection from harm and voluntariness of participation. Individuals may have objections if their tissues are used in genetic studies involving the search for certain kind of genetic relationships, which may be against their cultural and religious beliefs.^[17] It is imperative to respect patients' privacy, obtain informed consent, maintain confidentiality and safeguard individuals from any psychological distress or social stigmatization.^[14]

INFORMED CONSENT IN RESEARCH

The ethical considerations of autonomy, beneficence and justice dictate the discussion of informed consent. All individuals have the right to self-determination and should be given the opportunity to choose what will or will not happen to them.^[2] In line with this, it is of utmost importance to obtain informed consent from the participants at the beginning of any study and explain the full implications of all performed procedures and research to them. Informed consent should also be sought when research is concerned with use of tissues obtained as part of clinical investigations or in subsequent research to be performed on previously obtained tissues.^[16] In scenarios where future research cannot be envisaged, it is recommended to include the possibility of future research in consent forms thereby informing the patients and providing them with the choice of non-participation.^[16] Experts are now attempting to formulate an acceptable format for consent which enables coverage of the broader aspects of research.^[1]

One option available to researchers is that of a "one-time" consent covering all prospects of future research, known as blanket consent. A national survey in the USA revealed that 48% of the respondents preferred "one-time" blanket consent while 42% would want the opportunity to re-consent for every subsequent use of clinical material.^[18] It is considered best practice to obtain tiered consent which provides participants them with a list of choices to which they may decide to consent for. However, tiered consent has its disadvantages and is shown to result in lower quality of decision-making.^[17]

Contrary views also exist, that favor waiver of consent for tissue-based research. Experts have argued that the provisions aimed at protecting the privacy and autonomy of patients do not necessarily do so and additionally, increase burden on researchers.^[1] Courts have also taken note of the fact that biomedical research cannot be performed efficiently if individuals were given absolute control over fate of their

tissues, especially pertaining to excised tissues which are considered to be abandoned by individuals.^[3] It has been contended that in case of left-over body tissues, it is better to use them in research than to discard them. The consent system for such cases is debated on the grounds that less material will be available for scientific research and it also leads to abandonment of several potentially useful research projects. The lack of consent has also not been known to cause many problems in the past.^[19,20] Moreover, studies have found that that most individuals are willing to contribute their tissues to research and do not object to their tissues and health data to be stored for future research as long as they are used appropriately and will be used to obtain useful information.^[1,18] The percentage of persons objecting to use of tissues has been found to vary from 1 to 18%.^[20,21] In one study, a vast majority (82%) was found to be willing for their tissues to be used in cancer research whereas only 26% said they would consent for genetic research.^[21]

Informed consent lies in a delicate balance with individual's autonomy on one side and scientific research and social welfare on the other. Stored tissues are a rich source for teaching, research, diagnostic and prognostic tests and control assays. The abundant knowledge available today would not have been possible without past research using tissue materials and data.^[19] Given the reasoning that the fruits of medical knowledge derived from tissue-based research is beneficial to all individuals and even future generations, the society may justify the potential use of such tissues for research.^[2]

It is of course the pathologist's responsibility to safeguard patients' interests at each step and follow best practices.^[19] Research should not pose any physical or psychological risk to subjects, and should be reasonable in the context of anticipated benefits and the importance of knowledge that may be expected.^[15] Pathology Departments are recommended to preserve adequate diagnostic material before using it in any kind of research.^[20] Ideally, tissue material ought to be coded and used anonymously.^[19] Informed consent should be sought from research participants as appropriate. The College of American Pathologists recommend that patients should be asked to indicate if they wish to "opt out" of use of resected tissue for research, during obtainment of consent for surgical procedures.^[20] In addition, informed consent should provide provisions for unanticipated biospecimen use.^[13] Concerned institutions may be advised to have in place, prior agreements regarding biospecimen use and the authority to control decision-making pertaining to such tissues. In the field of Human Genetics, National Agencies such as Central Ethical Committee (ICMR) and/or National Bioethics Committee may be advised to conduct open discussions to reach consensus on debatable issues.^[14]

NEED FOR LAW

It is rightly said that with great power, comes great responsibility. The recent years have seen a rise in the number of medico-legal cases. The medical category ranked at the ninth position in all consumer cases filed in India as on March 2010.^[4] It is important for all persons affiliated to a histopathology laboratory to have thorough knowledge of the standard of practices in their regulatory environment and remain cognizant of local laws and any possible medico-legal issues that may arise, for, e.g., incorrect trimming of specimen, incorrect diagnosis etc.^[15] In such instances, the pathologist has the moral responsibility to immediately inform the physician and patient. Institutions are advised to review its maintenance and storage policies regularly and follow best practices.

Courts are now faced with the controversies regarding use of tissues of the body and their derivatives that are scattered among pathology laboratories, museums, archives, etc.^[3] Disputes arise regarding human tissue use in research and overlapping claims where multiple parties claim right over clinical material. Some countries have formulated law regulating the use of human tissues. For example, The Human Tissue Act, 2004 is in place in the UK and The Common Rule within the Code of Federal Regulations governs human tissue use and protection of research participants in the USA. In the Indian context, the ICMR has provided ethical guidelines for biomedical research on human participants. However, regulation for use of human tissues needs further definition. This paper highlights some of the important aspects in human tissue material handling and research, underscoring the necessity for established regulations regarding the same.

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