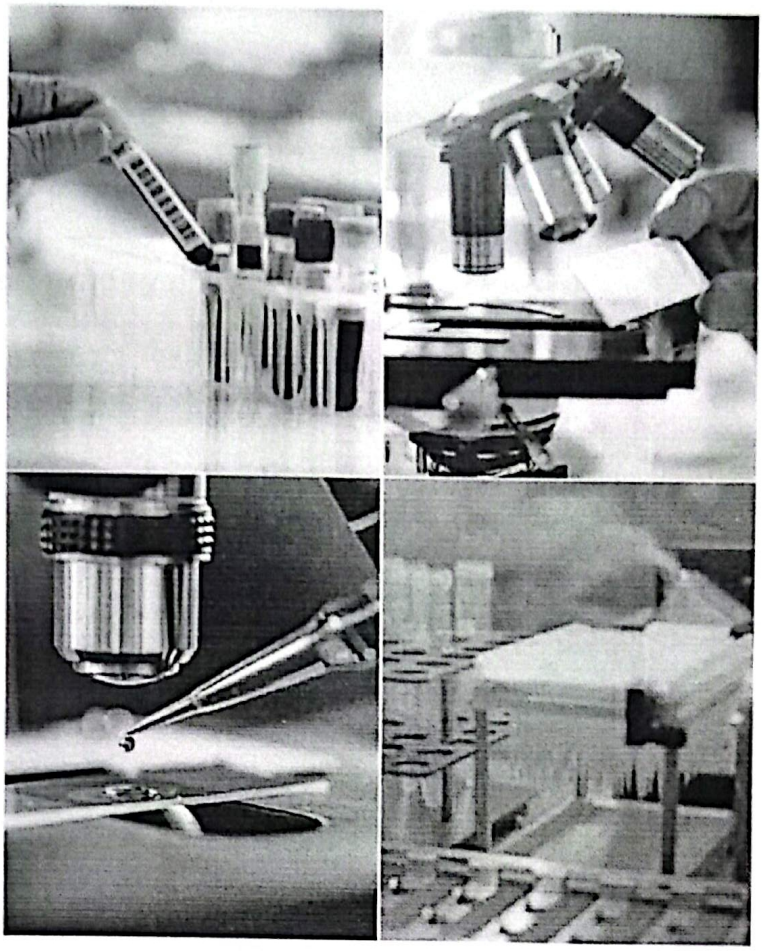

Histopathology User Manual of Service Laboratory

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Preface

Although some developing countries have quite well developed histopathology and anatomical pathology services, there is a shortage of pathologists and histopathology and anatomical pathology services in many others. Even within those developing countries with good histopathology and anatomical pathology facilities, satisfactory services are often restricted to just one well staffed and equipped central hospital pathology department, with, in some instances, a few other centres located in the capital and other main cities. Regional and remote small hospitals in these same countries may have less than basic facilities or none at all. These regional and remote small hospitals have poor access to histopathology expertise and services. Referral systems are often inadequate and are further complicated by difficult, slow and costly transport to or from hospitals. Consultations between pathologists and their colleagues in many developing countries are difficult to arrange and organize.

Generally the resources available for laboratory medicine and pathology services in developing countries are often insufficient. This shortage is often more evident in pathology and histopathology services to the extent that it is difficult to maintain basic pathology services. Furthermore, where these services are available, it is not unusual to find them inappropriate, unreliable and lacking quality assurance.

This book, although not intended to propose a system for histopathology and anatomical pathology services applicable to all developing countries, addresses a system covering basic principles which should be maintained at all levels and provides annexed self-assessment checklists, which can be useful in setting up quality systems at the work place.

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Introduction

The developing world does not embody just one level of sophistication. It comprises heterogeneous groups at different stages of development that may have different health care delivery systems, including histopathology and anatomical pathology services. Some of these systems are well developed with an established infrastructure whereas in other countries there is a lack of even basic facilities.

It would be difficult to propose a system for histopathology and anatomical pathology services applicable to all developing countries. It would be more practical to address various levels of recommended equipment and services. These levels may be considered even within the same country at central, regional, and district levels, depending on the remoteness of clinical services. It is important, however, that the basic principles of specimen identification, fixation and integrity be maintained at all levels, even if the specimen is to be transported to a central facility.

The quality of manual histological tissue processing is not as consistent as that produced by a tissue processor. Sending the specimens to a central point for processing and return of slides for interpretation and reporting should always be considered as an option for improved quality. To maintain quality and to aid cost–effectiveness the centralizing of the processing of anatomical pathology specimens to a well equipped and appropriately staffed facility should be considered. Internet and telemedicine facilities will become more important in the future and must be considered fully in any planning, particularly with regard to facilitating consultation and mentor opinion.

The specimen

The integrity and the quality of the specimen are vital to good histopathological diagnosis.

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Taking a specimen

Education of surgeons and referring practitioners is essential, so that each specimen can be fully exploited. This is best performed by using a procedure manual (laboratory handbook) directed to operating theatre staff as well as surgeons and by individual communication at the time of tissue audit sessions

Topics should include the following.

- The surgeon must be familiar with the conventions of, and use of, orientation-marker suturing on tissue (for example, lateral = long suture and superior = short suture).
- The nature of the tissue, site and side must be communicated.
- For certain diagnoses (for example, possible malignant lymphomas requiring impression smears or flow cytometry), fresh tissue is required and must be available.
- Desiccation of tissue should be minimized by immediately placing it into fixative.
- Small pieces of tissue should not be placed on gauze, which allows for desiccation before fixation.
- What clinical problems are present, and what does the surgeon wish to solve through histopathology?
- What previous histopathology has the patient had and where was it performed?
- Is the biopsy a resection of tissue for cure, or is it a biopsy for diagnosis? This should be communicated by the surgeon.
- What operation was performed? Was it a curette, shave biopsy, punch biopsy or excision biopsy? Do margins of excision require documentation?
- The specimen container should be large enough not to distort the specimen in the container.
- The fixative should have free access to all surfaces of the specimen.

Containers

Containers should be of glass with screw-top lids (reusable) or plastic, with leak-proof lids (disposable). There should be a range of sizes available,

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and containers should be clean and uncontaminated, particularly by other tissue, (labels should not be affixed to containers prior to placing the specimen in prelabelled containers is found to increase the risk of wrong labels as staff tend to label several cases and then line up the containers. There is a higher chance of mix-ups when this is done). Labelling should be performed after the specimen is placed in the container.

Labelling

Two patient identifiers are required through the whole processing of the specimen. These should be on the original specimen container as a minimum. This requires at least two of

- patient's name
- patient's date of birth
- laboratory number
- hospital number

to be on the container and remain on all the cassettes and slides in the subsequent processing.

Fixation

Good preservation of tissue is the most important factor in the production of satisfactory histology slides. One rarely has difficulty with the later stages of preparation if the tissue is well preserved; if however the preservation has been inadequate, good sections are absolutely impossible. The preservation required is of a special type known as fixation.

The aim of fixation is:

- to prevent autolysis or decomposition due to bacterial or osmotic change
- to preserve tissue as near to its original form as possible
- to protect tissue against subsequent changes during processing and embedding
- to give tissue a texture which permits easy sectioning
- to render the various constituents of the tissue reactive to the proposed stains.

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There are three essentials to good fixation: fresh tissue, proper penetration of the fixative, and the right choice of a correctly formulated fixative. Surgical tissues should be placed in fixative immediately they are removed and sent to the laboratory as soon as possible, where proper fixation can be supervised. In post-mortem cases refrigeration of the body will do much to retard autolysis and permit reasonably good histology.

Inadequate penetration of the fixative is the commonest cause of bad results. As a working rule it can be assumed that no fixative will penetrate a piece of tissue thicker than 10 mm, and in most cases even this thickness is excessive. For dealing with larger specimens the following methods are worth consideration:

- solid organs—cut slices not thicker than 5 mm
- hollow organs—open out or fill with fixative
- large specimens—inject fixative along vessels (or bronchi in case of lungs).

Specimen sections after trimming to be placed in cassettes should be thin enough to allow proper penetration of fixative and other reagents in processing. These thin slices are particularly important when the less effective manual or non-vacuum processing is used.

Type of fixative

From an almost infinite number of fixatives only formalin is mentioned in the main text of this volume. Other common fixatives are noted in Annex 1, but because of their potential toxicity, their use is not encouraged. They are appropriate for most ordinary purposes. Note the following.

- All fixatives are used once only.
- The specimen container must be an adequate size for the specimen, and there must be nothing to prevent any part of the tissue coming in contact with the fixative. The quantity of fixative must provide an excess of active agent for chemical interaction with the tissue. Two-thirds or more of the container volume should be fixative. Usually 10 times volume of fixative to tissue will suffice but this may be changed for fresh solution during the course of fixation.

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