4. The Importance of Correct Specimen Handling and Transport in Histopathology

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4.1 Introduction

Types of specimens taken at surgery include small biopsies (eg. endoscopic biopsies) and larger samples including part of or entire organs (eg. liver, uterus). Collection and transport of these specimens for histopathological examination involves a series of essential steps from the time it is taken from the patient at surgery up to its reception in the laboratory. These include putting the specimen in an appropriate container immersed in an appropriate type and amount of fixative; accurate identification and labelling of the specimen container with corresponding patient details in the request form; and completeness of information in the request form including relevant clinical details.

Correct specimen collection and transport for histopathological examination is important for a number of reasons :

- Incorrect patient or specimen identification and labelling errors may lead to issuing of erroneous reports
- Tissue architecture and especially cellular detail can be obscured by improper fixation, making proper tissue diagnosis virtually impossible; this may sometimes result in a need for a repeat biopsy
- Incorrect orientation or lack of orientation of a specimen, lack of proper identification of orientation sutures in the request form, or lack of clearly excised margins (eg. mesorectum) makes it difficult for the pathologist to comment on surgical excision margins, which would especially be important in case of excision of a neoplasm
- Results of ancillary investigations maybe necessary in order to make an accurate histopathological diagnosis
- If the report is urgently required, it should be indicated in the request form. In a busy histopathological practice, there maybe delays in sampling, processing or reporting
- Some types of specimens may require a special fixative, or need to be sent unfixed (fresh) for certain investigations (see guidelines)
- It maybe necessary to inform the laboratory prior to sending specimens for special or urgent investigations (eg. intraoperative frozen section – see guidelines)

It is with these in mind that the following guidelines have been formulated by the College of Pathologists of Sri Lanka, under the auspices of the Health Sector Development Project of the Ministry of Health funded by the World Bank.

These are meant for the information of theatre and ward staff dispatching these specimens, the laboratory staff receiving them, clinicians, clinical postgraduate trainees, pathologists and trainee pathologists who are overseeing the process. It is their collective responsibility to see that these guidelines are adhered to in routine practice to offer a satisfactory histopathological service to the patient.

4.2 Guidelines for handling and transport of histopathology specimens

These include instructions for collection and transport of histopathology specimens of different sizes to ensure optimal tissue fixation as well as accurate documenting, and inclusion of clinical information in the request form that may be needed to aid histopathological diagnosis.

4.2.1 Sending small biopsy samples :

The specimen should be collected into a wide-mouthed container with a well-fitting lid containing an adequate amount of 10% formol saline (formalin) to completely submerge the specimen (GRADE X).

The container should be accurately labelled including patient name, age, sex, ward and BHT/clinic number (GRADE X).

If multiple biopsies are sent from the same patient, each specimen should be sent in a separate container indicating the sites of biopsy (GRADE X).

Endoscopic biopsies should be placed on a filter paper and then submerged in fixative [**figure 1**] (GRADE X).

Figure 1. An endoscopic biopsy sample on a piece of filter paper.

The request form should provide accurate and adequate details regarding the patient and the sample (see box below) (GRADE X)

Details to be provided in the request form:

- Type and site of sample
- Patient identification details (identical to those on the specimen container)
- Relevant clinical details (radiological findings for bone tumours, liver function tests for liver biopsies, renal function tests for renal biopsies, PSA levels for prostatic biopsies etc.) and where available, the clinical differential diagnosis
- Reference numbers and diagnosis of any previous relevant fine needle aspiration (FNA) or histology reports
- An **indication if the report is urgently required**
- *If* orientation sutures are placed, the margins they represent should be clearly indicated

4.2.2 Sending large specimens :

The specimen should be placed in a wide-mouthed container with a well-fitting lid, containing an adequate amount of 10% formol saline (formalin) to completely submerge the specimen [**figure 2**].

Figure 2. A specimen in a suitable wide-mouthed container; however the amount of **formalin added is inadequate** to submerge the specimen. This will result in poor fixation.

The container should be larger than the specimen, preferably a bucket (Do *not* squeeze the specimen into the container) [**figures 3 and 4**] (GRADE X).

Figure 3. Do **not** squeeze a specimen into an inadequate container as shown here!

Figure 4. An adequate container for a large specimen, with a well-fitting lid. However the **label is not firmly affixed** to the container and may drop off in transit.

The container should be accurately labelled including patient name, age, sex, ward and BHT/clinic number (GRADE X).

When required, orientation sutures should be placed and clearly identified in the request form (GRADE X).

Slicing the specimen is *not* recommended in general, *except* where specifically allowed in certain situations (see guidelines for reporting of tumours of individual systems). This is because the sliced specimen

distorts on fixation, and accurate measurement of distance of the lesion (especially a tumour) to excision margins etc., maybe difficult to assess once the specimen is sliced.

Specimens should be transported to the laboratory as soon as possible for proper fixation procedures to be carried out (GRADE X).

Details to be included in the request form would be mostly similar to those in section 1.1. The **type** of specimen as well as its **site** and / or **side** (eg. left breast) should be indicated (GRADE X).

4.3 Special situations in which the laboratory should be informed prior to specimen collection and transport

4.3.1 Frozen sections (GRADE Y)

This investigation will be carried out only in centres where facilities are available. It is used mainly for intraoperative diagnosis which will influence the course of the operation. [1] Tissue sent for frozen section needs immediate and quick processing and reporting, for which the laboratory needs to be ready. Therefore, the pathologist and the laboratory staff should be informed beforehand regarding the date and time the specimen will be sent, and the type of specimen.

The specimen should be sent in the fresh state, without any fixative. The request form should include the relevant clinical details of the patient and the contact telephone number of the surgeon.

4.3.2 Specimens for microbiological tests (culture, etc.) (GRADE Y)

Microbiological tests will be performed where an infective aetiology is suspected.

These should be sent in the fresh state in sterile containers to the microbiology lab, as soon as they are sampled. The specimen should *not* be sent in formol saline.

4.3.3 Immunohistochemistry (GRADE Y)

Immunohistochemistry is a useful technique if available, for confirming a diagnosis, treatment options and certain prognostic indicators.

Most immunohistochemical stains can be performed on routinely processed paraffin-embedded tissue blocks, while some require frozen sections. The pathologist at a centre where these stains are available should be contacted for advice on sending tissue for same. If a tumour or other tissue is sent for immunohistochemistry, block selection must be done with great care to include adequate, well-fixed (non-autolysed) tissue from the area in question.

4.3.4 Immunofluorescence (GRADE Z)

Immunofluorescence is a useful adjunct to the diagnosis of certain skin, renal and other pathologies.

This is performed on frozen tissue, therefore the lab should be informed and specimens sent fresh without any fixative, immediately on sampling.

4.3.5 Molecular Biology (GRADE Z)

Molecular biological techniques are not widely available in Sri Lanka at present. If the necessity arises, the reference laboratory should be contacted for details on how to submit samples.

At present, techniques available include PCR, DNA sequencing (eg. detection of aneuploidy, screening for Duchenne muscular dystrophy), gene mapping, chromosome culture and karyotyping.

4.4 Situations where the specimen should be sent in a special fixative

1.1 Testicular biopsies

These should be sent in Bouin's solution if possible, for better preservation of microscopic detail (GRADE Y).

1.2 Electron microscopy (GRADE Z)

The main applications of electron microscopy are in the fields of renal and tumour pathology. [2]

The lab should be informed beforehand regarding arrival of the specimen (since this is not routinely done). The specimen should be transported in 2% glutaraldehyde in 0.1M phosphate buffer [3], obtained from the laboratory providing the service. If there is a delay in transport it should be stored (in fixative) in the refrigerator.

4.5 References

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