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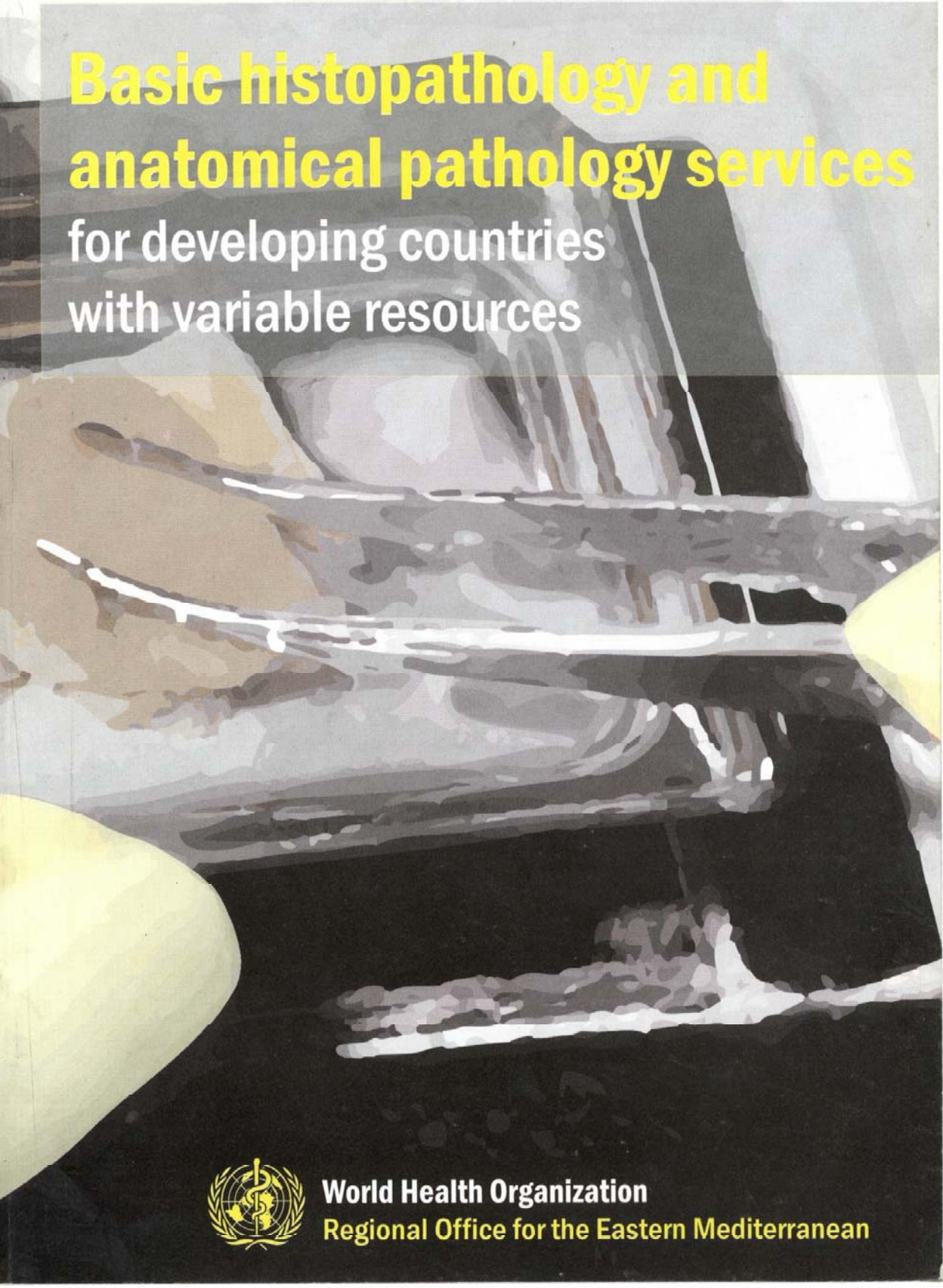
Basic histopathology and anatomical pathology services

for developing countries with variable resources



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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.

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.

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,

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.

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.

- Fixation should be carried out at room temperature. The fixative should not be heated.
- After the use of fixatives containing dichromate, the tissue must be washed free of excess fixative in water before dehydration
- After the use of fixatives containing mercury salts, precipitates must be removed from the tissue with 1% iodine in ethanol before staining.

The following three recipes (one uses concentrated formalin, another is a commercial product and another third alternative) are given as alternative examples to provide flexibility if the laboratory has a product.

Buffered formalin

Buffered formalin is a general-purpose fixative for tissues. It is the most practical and commonly used fixative.

Formalin should be buffered and 10% concentration for use.

For extremely small biopsies (such as gastric biopsies) add 6 drops of 4% *aqueous* eosin to 1 L of 10% buffered formalin. Prefill containers as set out below. This enables the specimen to be located in the container and also in the wax.

Preparation

Formalin in phosphate buffer pH 7 (24 hours+)[1]

Solution A: disodium hydrogen phosphate, anhydrous (Na_2HPO_4) 28.39 g; distilled water to 1 litre

Solution B: sodium dihydrogen phosphate 1-hydrate ($NaH_2PO_4 \cdot H_2O$) 27.6 g; distilled water to 1 litre

solution A 305 mL
solution B 195 mL
formalin (40% formaldehyde) 100 mL
distilled water to 1 L

[1] This is the preferred fixative.

If distilled water is not available in abundance, one may wish to use tap water, unless it has a high salt concentration (which may happen in a developing country). If the latter is the case one should use distilled water or get rid of the salt in the water by ion exchange treatment to obtain water with low salt content. The low salt content does not significantly affect the phosphate buffer.

Commercially prepared buffered concentrate

Dilute 5 L of commercial concentrate with indicator to 25 L with tap water (Large volumes are required as this is aliquoted into containers and sent to theatre. Large dispensers are routine in the mortuary and in the histology laboratory).

The pH is 7.3 initially and is very stable following contamination. When the inbuilt blue-green indicator changes to yellow, the pH is 6.7 or lower; the solution is no longer buffered and will result in formalin pigment being deposited on the tissue.

Formalin in isotonic saline (24 hours+)

commercial formalin 10 mL
sodium chloride 1 g
tap water[2] 90 mL

Keep over marble chips to absorb acid.

Safety

Gloves and eye protection should be worn when dispensing, preparing or working with formalin. Contact lenses are not to be worn without safety glasses.

Formalin vapour has respiratory effects. Formalin may have carcinogenic effects.

Hydrochloric acid and formalin should be avoided in combination.

[2] If tap water is not potable or otherwise unsuitable, distilled water should be used.

Prefilling containers

Containers should be prefilled from the bulk container, and each container should carry an adhesive label with a hazard warning. The adhesive label should also double as a label for affixing the patient and specimen identification needed. Filling of the container with fixative should be performed by laboratory staff under controlled ventilated conditions. This will reduce the handling of chemicals and hazardous fixatives by untrained staff in the theatre and in the ward.

Marker dye

A number of containers should be designated for use with marker dye. For extremely small biopsies (such as gastric biopsies) add 6 drops of 4% *aqueous* eosin to 1 L of 10% buffered formalin and then prefill containers as set out above. This dye enables the specimen to be easily located in the container and also in the wax at the time of embedding.

Hazards[3]

The hazards are detailed above under formalin and in Annex 1 for other fixatives but it should also be remembered that the laboratory cannot supervise the staff using the fixatives. Hence containers should carry warnings, and surgical and nursing staff must be educated on the hazards associated with their contents.

Request and documentation

Details of the patient and referring doctor are required on the request slip. The referring doctor should specify:

- the surgical operation that was performed, anatomical location, site and nature of tissue and anatomical side (left or right)of specimen
- diagnosis suspected by clinician.

[3] Cornell University's Environmental Health and Safety website http://www.ehs.cornell.edu/ offers, among other things, free labels detailing hazards of chemicals and first aid needed in case of intoxication (you download them and print them on to sticky labels, then affix the labels to laboratory chemical containers).

Reception and registration of specimen

Laboratory numbering system

Various laboratory numbering (accession numbers) systems are in existence. The usual features are:

- patient name
- year code, e.g. 2002
- case number (accession number), e.g. 6011
- block identification, e.g. A
- slide identification as to level of cut through the paraffin block, e.g. level 3
- slide identification as to special stain, e.g. PAS + diastase.

The finished identification on the slide thus might be

Smith JH 2002/6011 A level 3 PAS + diastase

The accession number is unique to the specimen or series of specimens from a given operation and is used to identify that specimen permanently; it will never be reused.

Request slips container and top

It is an advantage to label both the container and the top of the container with the accession number as small specimens sometimes adhere to the top. It also assists when storing and retrieving specimens.

Day book

All specimens must be recorded in the laboratory daybook. The following information must be recorded:

- patient's full name (first name, father's name and family name)
- other patient demographics, such as date of birth, so that at least two identifiers are available for any one patient
- ward and/or referring doctor
- nature of specimen(s)
- case accession number.

Transportation

Care must be taken with transportation to avoid formalin spills.

Specimens from out-of-hours operations should be treated as usual—placed in formalin and sent to the histopathology laboratory specimen reception area (there is no need for refrigeration unless the specimens are received fresh, i.e. without fixative).

Specimens received without fixative

Appropriate inspection, description and a decision made by the pathologist as to what necessary special investigations or special procedures are to be performed if available in the laboratory such as:

- photography
- microbiology culture
- cytology imprint, e.g. lymph node
- snap frozen specimen for special histochemical techniques (some techniques require specimens that are not formalin-fixed)
- urgent frozen section
- electron microscopy.

Specimens from the alimentary tract and urinary bladder cystectomy specimens are opened, cleaned with tap water (to gently remove faecal material, etc.), pinned on corkboard then fixed by floatation, specimen down, in neutral buffered formalin.

Pulmonary lobectomy or pneumonectomy specimens should be inflated with neutral buffered formalin via a cannula into the main bronchus followed by submersion of the whole specimen in a large volume of fixative.

Macroscopic description (gross or cut-up specimen)

There should be never more than one container open at a time to ensure that each specimen is returned to its correct container and there is no chance of mix up of tissue.

Qualified and trained staff should do sampling of specimen.

Macroscopic description and cut-up protocol should be established and documented. A useful basis can be obtained by reference to Rosai J. *Ackerman's surgical pathology*, 8th ed. St Louis, Missouri, Mosby, 1996: appendix E, p 2569.

The weight of some specimens is important. Specimens that should be weighed include:

- prostate (including transurethral resection specimens)
- testis
- thyroid
- lung
- kidney
- spleen
- ovary (if separate or abnormal)
- other specimens or tumours where indicated.

Sampling of specimens

Facilities

Light

Must be adequate and preferably natural daylight.

Fume extraction

Must be a strong extraction fan to remove fumes at bench level.

Running water

Must be available to wash out and clean tissues, and to wash bench and instruments.

Method for recording of description

Handwriting requires an assistant to be present to take the notes as writing materials and request slips should be kept free of contamination.

A foot-operated or voice-activated tape recorder is used in many laboratories to record the macroscopic description and the blocks taken.

Equipment

- scales (tare)
- face masks for infectious cases
- cutting board with absorbent paper—one new sheet for each case

- disposable wipes should be available to clean instruments between each case, thus avoiding carry over of tissue
- tissue-processing cassettes (to secure tissue samples taken from the main specimen)
- rubber gloves
- protective waterproof apron
- scalpels and blades
- scissors, knives and forceps
- ruler with millimetre scale
- magnifying lens.

Eye protection must be available and used (contact lenses should not be worn as they are a hazard—formalin splashes behind contact lenses can result in severe chemical burns to the eye).

Methods

Cross-contamination of tissue must be strictly avoided.

All instruments, containers, filters and cutting board must be washed and dried between each case.

Only one specimen container must be open at any time.

Tissue samples dissected from the main specimen must be placed immediately into labelled cassettes, surface to be cut facing down so that the specimen is embedded correctly and the correct side of the tissue cut. Where specific orientation is necessary for small specimens, which may move during machine processing, the specimen should be marked with diluted Alcian blue or Indian ink (alternatively with a nick or cut) in order to indicate the upper face.

Staples and sutures must be removed from tissue prior to processing as these will damage knives and disrupt the tissue.

Before and after examination specimens must be kept submerged in fixative and not allowed to dry out.

Where multiple blocks are dissected from an individual specimen, each cassette is identified with a suffix: A, B, C, etc. These codes should be recorded during the macroscopic description and serve to identify individual tissue blocks and their origin.

The number of pieces of tissue enclosed in the cassette should be recorded on the cassette. Multiple fragments too many to count should be recorded with an "*x*".

Small, friable and delicate samples should be supported between sponge pads (biopsy pads). This prevents specimen loss and reduces the possibility of loss through the holes in the cassette and cross-contamination during processing.

On completion of the cut-up procedures, scalpel blades and needles must be disposed of in a sharps container.

All instruments must be wiped free of blood and contaminants and then placed in a disinfectant solution.

The bench should be wiped with disinfectant and tidied.

Marking of margins

Indian ink can be used to mark the surgical excision margins prior to cutting.

Alcian blue solution is an alternative and is easier to apply to skin excision specimens or fatty specimens.

Commercial inks for histology margin marking are also available. These come in a range of colours.

Labelling of cassettes

A minimum of two patient identifiers is essential for the integrity of the case.

Identification of the specimen is by writing on the cassette with a pencil. The patient's name is written on the side of the cassette as the second identifier.

It is preferable not to prelabel cassettes as this predisposes to error.

Measurement and units

All linear measurements should be in millimetres only. Combining centimetres and millimetres in the same report can cause errors and misunderstanding; and a decimal point is not required if only millimetres are used.

Processing

Tissue processor

Specimens are processed overnight by a commercially available tissue processor.

The specimens in cassettes from the cut-up are placed into the tissue basket of the processor.

Times vary with types of processor. A typical schedule is:

Station	Time	Period	Solution
1			10% neutral buffered formalin
2	1700	1 hour	70% ethanol
3	1800	30 minutes	95% ethanol
4	1830	1 hour	95% ethanol
5	1930	1 hour	absolute ethanol 1
6	2030	1 hour	absolute ethanol 2
7	2130	1 hour	absolute ethanol 3
8	2230	1 hour	absolute ethanol 4
9	2330	1 hour	chloroform 1
10	0130	2½ hours	chloroform 2
11	0400	2 hours	paraffin 1
12	0600		paraffin 2

Xylene can be used as a clearing agent instead of chloroform. Many laboratories use the substitute xylene clearing agent limonene, which does not carry the hazards of xylene or chloroform.

It is important to ensure that processing of tissue is correct as in many cases a biopsy cannot be repeated.

Operation of tissue processing machines

For detailed instructions refer to the manufacturer's operation manuals.

Checklist for operation

- Check all process solutions and wax levels—top up as required.

- Wipe up any spillage immediately.
- Remove accumulations of wax, particularly from beaker covers and process basket clips.
- Check paraffin wax baths to ensure wax is molten and maintained with the correct temperature range up to 5 °C above the melting point of the wax, which is stated on the wax packaging).
- Check that the machine delay mechanism is correctly set. Use the delay facility for processing over public holidays, weekends, etc.
- Load specimens.
- Set timing mechanism and turn on.
- Have settings verified by senior staff member.

Maintenance of tissue processor

- Clean all wax spills daily.
- Check all solution and wax levels daily.
- Change all solutions and wax baths weekly.
- Clean instruments thoroughly weekly.
- Arrange for check of mechanical parts every six months.
- Arrange for check of electrical components annually.

Manual processing of tissue if a tissue processor is not available

- Problems can occur if a tissue processor is not used and specimens are processed manually.
- The turnaround time is prolonged as the solutions must be changed during working hours.
- The quality of the final histological section produced is not consistent.
- Manual processing times are limited to 8 hour divisible day periods and 16 hour night periods. A minimum number of solutions is used, and solutions that harden tissue on prolonged exposure are avoided during long breaks. Absolute ethanol does not unduly harden human tissue up to 72 hours immersion.
- Tissues are transferred with smooth-ended forceps through a series of solutions in stoppered bottles. At least 10 times the volume of solution to tissue is recommended and the Procedure is carried out at room temperature. An occasional shaking of the bottles between transfers

will hasten diffusion and a wad of absorbent material set in the base of the bottles will facilitate penetration of reagents to the tissue.

- More rapid schedules can be devised for tissue of smaller block size.
- There is no limit to the number of effective schedules that can be devised (see examples on following page).

Alcohol

In most countries a license for possession and use of absolute alcohol is required, and alcohol use must be documented and accounted for.

Tissue embedding

General

A tissue embedding centre is an important piece of equipment as it incorporates all the necessary features required to perform the embedding in order to obtain a quality product.

Tissue processing programmes such as that outlined above are designed to ensure that the processing cycle is completed by 0800 daily.

Delays may cause serious damage to specimens. Where a delay is imminent then the processing time must be adjusted accordingly.

The verification of the tissue processing times by senior staff is an essential quality procedure.

Biopsy embedding is to commence at the same time each day as dictated by the tissue processor.

The tissue embedding centre is switched on 2 hours prior to embedding and off at 1200 by an automatic time clock (after embedding has finished leaving the centre on is a fire hazard).

These settings can be overridden if tissue embedding is required outside these times.

Cassettes are removed from the tissue processor and transferred to the vacuum infiltrator. A vacuum not exceeding 400 mm Hg is applied for at least 5 minutes. Vacuum infiltration serves to remove air bubbles trapped in tissue and remove the clearing agent by increasing its rate of evaporation.

Typical schedules for sample preparation

Stage	Schedule 1		Schedule 2		Schedule 3	
Fixation *room temperature*	10% formalin pH 7 phosphate buffered	18–24 hours	10% formalin in isctonic saline	18–24 hours	10% formalin with calcium acetate	18–24 hours
Dehydration *room temperature*	70% ethanol	2 hours	70% ethanol	1 hour	70% acetone	1 hour
	90% ethanol	2 hours	90% ethanol	1 hour	90% acetone	1 hour
	Absolute ethanol	2 hours	Absolute ethanol	2 hours	Acetone	1 hour
	Absolute ethanol	2 hours	Absolute ethanol/ xylene (equal parts)	2 hours	Acetone	1 hour
	Absolute ethanol	17 hours			Acetone	1 hour
			Absolute ethanol	2 hours		
Clearing *room temperature*	Xylene	2 hours	Petroleum ether	16 hours	Acetone	2 hours
					Petroleum ether	17 hours
Infiltration *58–60 °C*	Paraffin wax	3 hours	Paraffin wax	3 hours	Paraffin wax	3 hours
	Paraffin wax	2 hours	Paraffin wax	2 hours	Paraffin wax	2 hours
Total time		56 hours		53 hours		53 hours

Where the vacuum is applied during the infiltration step on the tissue processing machine, this step is not necessary. Tissues processed on a machine with a vacuum facility should therefore be embedded without delay. If any delay is present then a vacuum step should be included.

The operating temperature of the cold plate is usually preset and cannot be adjusted.

The temperature of the warm stage and wax reservoir are thermostatically controlled. These must be carefully set to avoid tissue damage.

Where the temperature differential between the wax and the cold plate is too high the wax will crack during solidification, disrupting the tissue and causing artefacts[4] (defects and tissue patterns produced as a result of poor processing) on sectioning.

Tissue orientation

Most tissue blocks are embedded so that the sections are cut from the largest surface area of the tissue, but there are many important exceptions.

Skin should be oriented at embedding for cutting after embedding in a plane perpendicular to the skin surface to obtain a section through the dermis and epidermis.

Gastrointestinal tract and other epithelial surfaces (gall bladder, walls of cysts, urinary bladder) are embedded at right angles to the mucosal surface so that the section includes all layers of the specimen.

Polyps should be embedded so that the stalk and the base of the polyp are included in the section.

Tissues of a tubular nature (e.g. vas deferens, fallopian tube, artery) are embedded cross-sectionally at right angles to the section so that the section includes all layers of the specimen.

Muscle biopsies are sectioned in both longitudinal and transverse planes.

If a feature is only on one face of the tissue this must be embedded face down so that the first cut is through the feature or abnormality.

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[4] A good reference, with pictures of artefacts and their cause and prevention, is Luna LG, ed. *Manual of histological staining methods of the Armed Forces Institute of Pathology*, 3rd ed. New York, McGraw–Hill, 1968: p 242–51.

Instructions requiring special orienting must be recorded clearly on the embedding notes.

Hard and tough surfaces should be embedded so that they are the last to meet the blade of the knife on cutting and do not drag through the adjacent soft tissue with resulting artefact.

Tissues should be placed in a central position in the mould and arranged along the long axis of the mould. They will thus be parallel to the knife edge at the time of cutting (if tissue edges are not parallel to the knife it is difficult to obtain an even section).

Embedding procedures

Remove the cassette from wax bath and place on the heated stage.

Select the base mould from the thermal console. The size should allow 1–2 mm margins as a minimum around the specimen to be embedded.

Fill the base of the mould with the molten wax and then place the tissue in the mould using warmed forceps. These must not be too hot otherwise charring of tissue will result. Tissues are delicate so minimal pressure on the forceps should be used.

Slide the mould on to the cold area of the embedding centre. The wax solidifies while holding the specimen in position. The specimen is now at the base of the mould and correctly oriented.

Press the processing cassette firmly on top of the embedding mould. This allows molten wax to enter the holes at the base of the cassette.

Fill the cassette with wax for support in the microtome chuck.

The original pencilled identification remains on the embedding cassette, which is now on top of the embedding mould.

Transfer the embedded specimen to the cold plate to allow solidification.

Clean the working area and wipe the forceps to prevent cross-contamination before opening the next cassette.

After a few minutes lift the specimen cast from the base mould in order to commence microtomy.

Remember:

- have only one cassette open at any one time while embedding
- prevent cross-contamination

- orient the tissue
- treat the tissue with care; it is delicate
- use a worklist to make sure all the tissue has been embedded correctly
- make sure that two identifiers remain with each specimen.

Embedding centre maintenance

Daily cleaning

- Clean outside only when equipment is cool.
- Peel off wax from the surfaces once cool.
- Clean the embedding centre with a cloth and a small amount of xylene.
- Minimize contact between xylene and painted surfaces.
- Xylene is flammable. Make sure equipment is cooled and all switches are in off prior to cleaning.

Periodic cleaning

- Drain the wax from the container.
- Allow the centre to cool.
- Thoroughly clean the inside with a clean dry cloth.
- Vacuum the air filter on the back of the unit.

Thermostat adjustment

The embedding centre has adjustable thermostats for the wax being used. The thermostat for the working platform and the wax bath thermostat are adjustable from 50 °C to 80 °C. The factory setting is 65 °C.

An additional thermometer is placed in the wax bath for checking the thermostat setting.

Temperature settings are dependent on the type of wax being used (indicated on the packaging).

Section cutting (microtomy)

Microtome operation and maintenance

See manufacturer's instructions.

Section cutting procedures

The rotary microtome is most common and is preferred to the less common sledge type as it is quicker, not as heavy and more compact.

Each cutting station is provided with section adhesive (such as 1% commercial histology adhesive), 20% ethanol, wide glass slides, forceps, camel hair brush, microtome knife (see below) and blade holders.

The blocks are assembled in order on a cold tray or with ice blocks.

The blocks are trimmed to expose the full surface of the tissue using a conventional microtome steel knife or a used disposable blade. The block is returned to the cold tray after first cutting a few sections to remove the coarse cutting effects (the microtome is set at a thicker cut for trimming and this distorts the tissue, hence cooling must occur before the fine sections are cut).

Once all the blocks are trimmed, routine 5 μm sections are cut using a new disposable blade. (New disposable blades must be thoroughly degreased and cleaned using xylene before satisfactory results can be obtained.)

Frosted-end glass microslides are labelled with the minimum of two identifiers that have been carried through the entire process using a lead pencil. If frosted-end slides are not available, glass slides can be labelled using a diamond pencil, with the identifiers cut into the glass.

Short ribbons of sections are laid on a clean microslide, then 20% ethanol is pippetted under the sections.

Groups of sections or individual sections may be separated by cutting along the wax junctions with a scalpel.

Sections are then floated on to the floatation bath set 5 –10 °C *below* the melting point of the wax (47 –49 °C is suggested).

The labelled microslide is then dipped into 1% aqueous commercial adhesive. This adhesive is recommended for paraffin sections.

Wrinkle-free, flat sections are picked up on to the labelled slide and positioned towards the non-labelled end.

The slides are transferred to a slide drying bench and left until they appear to be dry. The temperature of the slide drying bench should be adjusted so that the wax does not melt when the slide is supported in an inclined position.

Cutting thickness

Cutting thickness should be 5 μm section thickness.

Water bath maintenance and temperature

The water bath must be kept clean and its temperature constantly monitored.

Sections and tissue will develop artefacts if the temperature is not monitored constantly during the floatation process.

Preventing cross-contamination

Sections can be contaminated by previous cases if cleaning of the water surface is not done between cases. This is best done by dragging a piece of absorbent tissue paper across the surface after each case.

Specimen integrity

Specimen integrity is essential, and care must be taken to preserve this. There must be no possibility of specimen cross-contamination or mix up. The block should also checked to ensure that the surface has been cut deep enough to include the entire surface plane required.

Knives

Disposable knives are now the industry norm and are to be preferred. They produce better sections; and the process of keeping the now uncommon steel blades sharp is difficult, tedious and time-consuming, requiring extra capital outlay on knife-sharpening equipment.

Disposable knives no longer suitable for microtomy (when the blade is dull, sections do not cut as well, and lines and tears appear in the sections) can continue to be used for macroscopic tissue cut-up and gain a second life. They can also be used to remove folds in the sections and so on after floatation.

If stainless steel knives are used several knives should be regularly rotated for sharpening and then honed with a leather strop immediately prior to use.

Staining

The slides of paraffin sections, once cut, are ready for routine haematoxylin and eosin staining and are placed in racks for batch staining.

Drying

Slides are placed in the 60 °C oven for 30 minutes to dry the sections. This facilitates the removal of the wax.

Difficult specimens to cut

Hard tissue such as nail and tendon will cause problems such as:

- block detaches from mount
- only portion of block cuts
- excess compression
- alternate thick and thin sections.

Apply a softening agent (e.g. 5% commercial softening agent) to the block face for 5–10 minutes before sections are cut.

Surface decalcification of small amounts of calcium ions that may be present is performed by immersing the block in decalcifying solution for 15–20 minutes. Rinse in water, and then several sections can be cut before more calcification is reached and further decalcification is required.

Blood clot, which is present in large amounts for example in uterine curettings, can result in fragmentation on cutting. 5% ammonium hydroxide applied from paper on to the block surface will result in less fragmenting.

Troubleshooting[5]

Causes of fault in paraffin section	*Remedy*
Ribbon and sections are curved	
1. Trailing and leading edge of block not parallel	1. Trim with scalpel until parallel
2. Knife blunt in one area	2. Use different part of knife
3. Wax surplus on one side	3. Trim wax excess
4. Variable tissue consistency	4. Reorient block 90 degrees Mount individual sections Cool block with ice

[5] Bancroft JD, Stevens A. *Theory & practice of histological techniques*. London, Churchill-Livingstone, 1982.

Thick and thin alternate sections

1. Wax soft for conditions and tissue	1. Cool block with ice or re-embed in higher melting point wax
2. Loose knife or block	2. Tighten
3. Clearance angle insufficient	3. Slightly increase angle
4. Faulty microtome mechanism	4. Check and get serviced

Sections will not join to form a ribbon

1. Wax too hard	1. Breathe on block to warm or re-embed in wax of lower melting point
2. Debris on knife edge	2. Clean with xylene-soaked cloth
3. Knife edge too steep or shallow	3. Adjust

Areas of tissue in block not in sections

1. Incomplete impregnation of tissue	1. Return tissue to vacuum bath for a few hours or reprocess if fault is excessive
2. Wax block becoming detached from cassette	2. Reattach with hot spatula

Sections are attached to block on return stroke

1. Insufficient clearance angle between block and knife	1. Increase angle
2. Wax debris on knife edge	2. Clean with xylene-soaked cloth
3. Debris on edge of block	3. Trim with sharp scalpel
4. Static charge on ribbon of paraffin tissue cuts	4. Place moist cloth near knife

Chatters (thick tissue alternating with thin tissue cut in bands in the same section parallel to knife edge)

1. Blunt knife	1. Replace or sharpen knife
2. Vibrating microtome	2. Service
3. Knife loose	3. Tighten
4. Knife angle too steep	4. Reduce angle but leave clearance
5. Tissue or wax too hard for sectioning	5. Use heavy-duty knife or softening fluid on the tissue
6. Calcified areas present in tissue	6. Rehydrate and decalcify or surface decalcify

Scoring or splitting of sections at right angles to knife edge	
1. Defect in knife edge	1. Use different part of knife
2. Hard, gritty areas in tissue	2. If Ca^{2+}, decalcify If other, remove with fine sharp pointed scalpel
3. Hard particles in wax	3. Re-embed in fresh filtered wax
Compression of sections	
1. Blunt knife	1. Sharpen or renew
2. Knife bevel too wide	2. Regrind
3. Wax too soft for tissue or sectioning conditions	3. Cool block with ice or use wax of higher melting point
Sections expand and disintegrate on waterbath surface	
1. Poor tissue impregnation	1. Return tissue to vacuum impregnation for two hours
2. Water temperature too high	2. Cool water bath
Sections curl and do not stay flat on knife	
1. Blunt knife	1. Sharpen or renew
2. Rake angle too little	2. Reduce knife tilt if clearance angle is excessive
3. Section thickness too great for wax	3. Reduce section thickness or use wax of slightly higher melting point. Breathe on block as sections are cut.

Labelling

Labelling is performed by using a diamond pencil to etch the slide permanently with the two identifiers used through the whole system. If one end of the slide is frosted, pencil marking is sometimes used but this is not as permanent.

Quality of glass slide

The quality of the glass slide varies considerably, and checks should be made before bulk purchases. There is a particular problem if the slides come dirty or have a grease film that requires special washing before use. This adds to the labour costs.

Staining

Nuclear stains

General information and formulae

There are many different stain recipes and variants, and the reader may observe differences between those presented here and those in other authorities. Use of stains is more of an art than a science, and experience is as valuable a teacher as any resource.

Haematoxylin

Haematoxylin is a natural product derived from the tree *Haematoxylin campechianum* Haematoxylin is not a dye substance by itself. It is purchased as a brown-to-violet powder which, when dissolved and oxidized, acquires a chromophore (colour) group and is converted to a reddish dye known as haematein (unrelated to blood haematin). Haematein combines with metallic mordants (a mordant is a metal cation, such as aluminium, ferric iron and less commonly other metals, used to increase the ability of a stain to highlight structure details) forming on mixing a blue to black insoluble dye-lake (a lake can be defined as: "a coordination complex formed between a polyvalent metal ion and certain dyes". This term is derived from *lac*, a mordant dye obtained from an insect in India, and from which shellac is derived), which, in one form or another, has dominated our choice as a nuclear stain since 1880, when it displaced carmine.

Combinations of haematein with aluminium in the formulae of Mayer, Ehrlich, Harris and Delafield are examples of commonly used nuclear stains. The formulations are not perfectly stable although some may last for a year or more.

Combined with ferric iron as in formulae of Heidenhain and Weigert, haematoxylin is both vigorously oxidized and mordanted. When applied to sections the lake so formed can be induced to impart a sharp black. stain to nuclear chromatin which is more resistant to acid decolourization than its aluminium haematein counterpart. These formulae and variants are used where a study of nuclear chromatin is involved in order to demonstrate presence or absence of certain parasites (such as amoeba) in tissue or show

cross-striations in fibres thought to be derived from voluntary (skeletal) muscle but occurring in pathological tumours.

Mayer acid haemalum[6]

haematoxylin 1 g
sodium iodate 0.2 g
potassium aluminium sulfate 50 g
distilled water 1000 mL

Dissolve overnight, then add:

chloral hydrate 50 g
citric acid 1 g

The stain is ready for use. It has a working life of one month and may be stored for six months.

Ehrlich haematoxylin

absolute ethanol 100 mL
haematoxylin 2 g

Dissolve together and add:

distilled water 100 mL
glycerine 100 mL
glacial acetic acid 10 mL
potassium aluminium sulfate 15 g

Plug bottle with gauze and leave in the light to ripen (oxidize) for several months. The staining solution will keep for years if stoppered in glass.

[6] The original formula of Mayer differed in detail from that given; however the recipe cited here has become accepted as Mayer's formulation.

Harris haematoxylin

10% alcoholic haematoxylin 10 mL
10% aqueous aluminium ammonium sulfate 200 mL

Mix and bring to the boil, add 0.5 g yellow mercuric oxide and continue boiling till the solution turns purple. Cool rapidly. Filter and add 4 mL glacial acetic acid. Ready for immediate use.

Delafield haematoxylin

absolute ethanol 25 mL
haematoxylin 4 g

Dissolve together and add 400 mL saturated aqueous aluminium ammonium sulfate. Leave the solution exposed to light and air in an unstoppered bottle for three to four days. Filter and add:

glycerol 100 mL
methanol 100 mL

Allow to oxidize for several months in a gauze-stoppered bottle before use. The solution keeps for years in a glass-stoppered container.

Heidenhain iron haematoxylin

Solution A: 5% aqueous ferric ammonium sulfate (lilac crystals). Ferric ammonium sulfate 5 g dissolved in 100 mL distilled water

Solution B: 0.5% aqueous haematoxylin. 0.5 g Haematoxylin in 10 mL absolute ethanol and then add 90 mL distilled water.

Ripen for 4 weeks before use.

Method of use for Heidenhain iron haematoxylin:

1. Bring paraffin sections to water.

2. Mordant in solution A for 1 hour at 37 °C or up to 12 hours at room temperature.
3. Rinse in distilled water (do not prolong this step).
4. Stain in solution B till quite black (about the same time as for mordant step).
5. Rinse in distilled water.
6. Decolourize.
7. Counterstain if desired, dehydrate the slides (e.g. by two changes each in 95% alcohol, 100% alcohol, and two changes of xylene),clear and mount.

Eosin as a counterstain

Most pathologists prefer eosin as a general counterstain (stains the cytoplasm and not the nucleus) to aluminium haemateins. This preference is conditioned by early training where colour values for pattern recognition are established using this combination.

Eosin Y is a member of a family of chemically and tinctorially related dyes varying from yellowish red to bluish red depending on the degree of halogen substitution:

Eosin Y—sodium salt of tetrabromofluorescein

Eosin ethyl—ethyl ester of tetrabromofluorescein

Eosin B—sodium salt of dibromodinitrofluorescein

Erythrosin B—sodium salt of tetraiodofluorescein

Phloxine—sodium salt of tetrabromotetrachlorofluorescein

Rose bengal—sodium salt of tetrabromotetrachlorofluorescein

Eosin Y is usually used. A yellowish red acid dye with a greenish yellow fluorescence, soluble in water 44.2% and in alcohol 2.18% at 26 °C.

Simple aqueous and alcoholic solutions of eosin Y lack sufficient affinity for tissue fixed in 10% formalin, and much colour is lost in the dehydration stages prior to mounting.

Two methods of overcoming this defect are:

1. The addition of acid or buffer to the eosin solution to increase the hydrogen ion concentration.
2. The preparation of the free acid (hydrochloric acid–precipitated) from eosin Y and its resolution in alcohol.

In both cases the addition of hydrogen ions to the solution creates a more active affinity for the acid stain. Below pH 4, eosin Y precipitates from solution.

Preparation of buffered alcoholic eosin

Eosin Y saturated in 95% ethanol 50 mL

0.2 M sodium acetate (27.22 g L^{-1} crystalline sodium acetate) in 95% ethanol 16 mL

M acetic acid (60 g L^{-1}) in 95% ethanol 34 mL

This results in a medium density stain after 10 minutes duration which is not greatly increased in depth by prolonged staining. The stain is retentive and allows for a leisurely dehydration.

Preparation of hydrochloric acid–precipitated eosin

An approximately 10% solution of eosin Y is prepared in distilled water and reprecipitated from solution by the dropwise addition of 20% hydrochloric acid. Filter off the supernatant fluid and discard. Wash the precipitate on the filter paper with repeated changes of distilled water over several hours until washings are chloride free. Dry the precipitate at moderate oven temperature and prepare as a 0.3% solution in 70% ethanol. Filter before use.

This stain is inclined to be dense if staining is prolonged. Staining time varies from 20 seconds to 10 minutes after prolonged use with multiple batches of slides. The free acid colour produced by this method is insoluble in water.

Routine haemalum and alcoholic eosin staining

Any fixative; paraffin wax sections

Treat sections with xylene to remove wax.

Treat with descending grades of alcohol to remove xylene.

Wash briefly in water.

Stain[7] in any haemalum till overstained (15–30 minutes).

Wash briefly in water.

Differentiate in acid alcohol (concentrated hydrochloric acid 0.5 mL; 70% alcohol 100 mL) until nuclei only are stained.

Rinse in water.

Dip in Scott tap water substitute (potassium hydrogen carbonate 2 g; magnesium sulfate 20 g; distilled water 1 L) in order to accentuate the bluish colour to the desired degree (dilute alkali or alkaline tap water may be used in place of Scott solution) 5 minutes.

Wash in water 2 minutes.

Immerse in 70% alcohol.

Stain with alcoholic eosin to desired intensity.

Dehydrate in absolute ethanol.

Clear in analytical (purest) grade xylene.

Mount in liquid mountant.

Lugol iodine

iodine 1 g
potassium iodide 2 g
distilled water 100 mL

Dissolve potassium iodide in small quantity of water. Dissolve iodine in the solution and add remainder of water.

Treatment for tissue fixed in mercury-containing fixatives.

Between stages 3 (first wash with water) and 4 (overstain in haemalum), place sections in Lugol 1 minute followed by 5% sodium thiosulfate till colourless; wash in water.

[7] Modify for progressive staining by putting the slides in haematoxylin until the desired intensity of staining is achieved. In regressive staining the slides are left in the staining solution for a fixed period after which it is taken in a solution such as acid alcohol to remove part of the stain.

Modification for progressive staining

With carefully prepared Mayer haemalum used for 10 minutes or naturally ripened haemalums at early stages of oxidation, the time of staining may be adjusted to not overstain. Steps 6 (differentiation in acid alcohol) and 7 (wash with water) above may then be dropped.

General comments concerning dyes

Preparation

Numbered commercial dye lots are preferable as they are better quality.

Preparation should be done carefully and to specific volumes.

Care should be taken when weighing on analytical balance not to contaminate the balance

Protective clothing should be worn as stains are permanent.

Date and details of preparation must be on label of prepared stain.

Use-from and use-by dates to be added.

Date of maturing of the made-up solutions must be on the bottle and time allowed for maturation according to each specification.

Date the bottle was opened for use must be added.

Contamination

Fungal and bacterial growth should be regularly checked for.

Crystalline precipitation of the dye should be monitored and the batch discarded if precipitation or fungal contamination occurs.

Times for staining in each reagent

Times for staining in each reagent should be documented and monitored with internal quality control daily as a minimum.

Case integrity (float-offs)

Extreme care must be taken to avoid cross-contamination of tissue between cases. It is advisable where possible to alternate the type of tissue in adjacent cases, rather than working on the same type of sample sequentially; for example prostate, then uterine curetting, then gastric

biopsy. This will assist in identifying any float-off cross-contamination if unfortunately it does occur.

Coverslipping and slide mounting

Slides are removed from the staining process and held in a container of xylene until mounted.

Sections are mounted with No. 1 coverglasses using a mounting medium.

A coverglass of suitable size is selected from the range available which must fully cover the section(s) without being too large.

Stain debris, section remnants and incomplete sections must be wiped from the slide, usually with absorbent tissues, prior to mounting.

The mounting technique must ensure no air bubbles are trapped under the coverglass.

Bubbles form because:

- they are present in the mounting medium before mounting
- the mounting medium is left on the coverglass too long before mounting, allowing a skin to form on the surface
- the section is not saturated with xylene
- the section is pressed on to the mounting medium too quickly.

The following mounting technique should be followed.

- Apply a suitable quantity of mounting medium to the selected clean coverglass. The mounting medium must be free of bubbles.
- Remove the section from xylene and quickly wipe the back of the slide and around the section, remove all debris and section remnants.
- With the section saturated with xylene, lower gently on to the mounting medium. When mountant starts to spread out, lift the slide and turn it over.
- Gently press coverglass with a pointed instrument to remove air bubbles then centre the coverglass.
- Leave flat to dry.

The whole procedure must be accomplished as quickly as possible.

Slide labels and presentation of the case

Preprinted or written self-adhesive slide labels bearing the specimen accession number and department identification are provided for each specimen.

Every slide must be labelled.

Each slide label requires the following information:

- accession number (preprinted or handwritten)
- patient's surname
- block identification suffix (where multiple blocks are processed)
- coded information indicating levels, duplicates, special stains, etc.

Labels are placed over the frosted ends of the mounted slides after collating and carefully ensuring the information on the labels is accurate and consistent with the specimen.

Verification of sections

After microtomy, all blocks are collected and arranged in consecutive numerical order.

Arrange mounted sections in consecutive numerical order.

Verify sections by comparing them macroscopically with the blocks.

Place labels on the slides.

Confirm that slides are labelled correctly and the tissue sections correspond to the blocks taken in number and size by referring to the macroscopic report on the request form.

Verify nature of tissue, adequacy of section and quality of preparation microscopically.

Repeat unsatisfactory results.

Arrange slides on trays with paperwork for pathologist microscopy.

Microscopic assessment of slides with regard to quality

The control slides must give clear and precise demonstration of the tissue components both normal and pathological, demonstrated by that method. For example, if mucin is to be stained the control tissue must contain mucin.

The nuclear stain must be precise.

The section must be adequately stained, not over or under-differentiated.

The counterstains must be adequate.

The section must be dehydrated and cleared properly.

There must be no stain precipitation present.

There must not be air bubbles or water droplets present.

The stain must satisfactorily demonstrate the histological features intended.

Reporting

Format

Must be readable and useful to the clinician.

Headings should include:

- clinical notes
- macroscopic description
- blocks and stains submitted
- microscopic description.
- Summary diagnosis should include:
- tissue
- site
- side
- operation
- pathology
- diagnosis
- margins.

Protocols are available for reporting formats. Two references are quoted.

Recommendations on quality control and quality assurance in anatomic pathology

Document prepared by an ad hoc Committee of the Association of Directors of Anatomic and Surgical Pathology chaired by Juan Rosai. From *American journal of surgical pathology*, 1991, 15(10):1007–9. Quoted in

Rosai J. *Ackerman's surgical pathology,* 8th ed. St. Louis, Missouri, Mosby, 1996: appendix E, p 2569. Also at http://www.panix.com/~adasp/art1991.htm.

A generic document which allows for the unique nature of anatomical pathology compared to the more discrete measurement of parameters in clinical pathology. It recommends the monitoring of a number of defined quality parameters that can be documented by the laboratory.

Standardization of the surgical pathology report

A proforma to allow for a complete surgical pathology report to be produced. There is a series of items that are essential for inclusion in the surgical pathology report. The emphasis is on producing a clinically meaningful high quality report.

Reference document prepared by an ad hoc committee of the Association of Directors of Anatomic and Surgical Pathology chaired by Richard Kempson. From *American journal of surgical pathology*, 1992, 16:84–86. Quoted in Rosai J. *Ackerman's surgical pathology,* 8th ed,. St. Louis, Missouri, Mosby, 1996: appendix A, p 2519. Also at http://www.panix.com/~adasp/standSPrep.htm.

Frozen sections

Careful and detailed justification is needed for setting up a frozen section service. Administrative financial and medical need must be demonstrated as well as the presence of expertise to interpret.

The CO_2 method requires experience and skill in order to obtain high quality sections.

A cryostat requires careful maintenance and training and skill to obtain quality sections. It is the best equipment for frozen sections but may not be economic for developing country laboratories.

Paraffin sections must always be completed and correlation of findings made.

Retention of documents, slides and specimens

Filing of paraffin wax blocks

Paraffin wax blocks should be filed immediately the sections are verified.

Blocks are filed according to accession number in partitioned filing boxes designed for this purpose.

Filing boxes are housed in metal filing cabinets in the laboratory file room.

Blocks are retained in the laboratory files for at least 12 months and then put into permanent storage

Permanent storage for filed blocks should be available in correct conditions (see below).

All blocks must be retained and the files maintained to ensure blocks are readily retrievable.

Filing of slides

Slides are filed according to accession number in metal microscope slide filing cabinets.

Slides are not filed wet.

Individual slides are separated by spring inserts in some laboratories when first filed.

Slides remain separated for 2–3 months in order to allow time for the mounting medium to fully dry.

Each slide filing cabinet drawer is labelled with the accession number of the first and last slide filed in it.

At least 10% of the drawer space must be left vacant to allow for inclusion of additional and withheld slides.

Slides are retained in the laboratory file room for a period after which they which they are transferred to a secondary file for permanent storage.

All slides are retained permanently in a readily accessible manner.

Conditions for filing

- Dry.
- Room temperature not more than 40 °C.

- Vermin free (tissue can be eaten by vermin).
- The floor must be strong, as the combined weight of glass slides can damage the building.
- A paraffin seal should cover blocks before filing in order to prevent desiccation and to reduce exposure to vermin.
- Request slips reports and work sheets should be filed and cross-referenced where necessary.

Storage of wet formalin fixed tissue

- File current specimens on a vacant storage shelf.
- Unblocked wet tissue is retained for four weeks or until the report is completed and the surgeon has had time for queries.
- Shelves are provided for storage of wet tissue. One shelf for each days specimens—20 shelves in all.
- The area must be well ventilated to extract any formalin fumes.
- Specimens are discarded in rotation by clearing one shelf each day in preparation for the new day's specimens.

Equipment

Types of equipment

- Computer and peripherals.
- Waterbath: thermostatically controlled and monitored.
- Oven: thermostatically controlled and monitored.
- Embedding centre: see above under embedding.
- Microtome.
- Microscope.
- Safety cabinet.
- Refrigerator.
- Tissue processor.
- Reference textbooks.

Maintenance

- Inventory of equipment must be maintained with date of purchase and records of service.

- Records of service and any problems.
- Cleaning: regular documented schedules to be established.

Decalcification

Introduction

Decalcification by the use of acid inevitably results in tissue disruption and the removal of minerals, particularly haemosiderin. Where mineralized sections are required (for example for the study of metabolic bone disease), undecalcified sections are prepared by embedding in resin, which should be referred to a specialized laboratory.

Calcified tissues for paraffin embedding must undergo decalcification prior to processing.

Specimens must be adequately fixed, usually in neutral buffered formalin (48 hours), prior to decalcalcification. Each specimen is recorded on a decalcification list at the time decalcification commences. Specimens are checked daily, using a chemical test, to determine the end point of decalcification.

Procedure

Record date and specimen accession number on decalcification list.

Drain off fixative and replace with Kristensen decalcifying solution(see formula below). Use 10× to 20× specimen volume.

Next morning decant decalcifier and discard; rinse specimen in tap water to remove residual solution then replace with a small volume (5× specimen volume) of fresh decalcifier.

Stand 2 hours then test for presence of Ca^{2+} (see below)

Positive test indicates decalcification is not complete, so top up solution and repeat test next morning.

When Ca^{2+} is not detected in the decalcifier, the specimen may be processed after washing in running tap water for several hours.

Chemical tests for calcium ions

Dipstick commercial calcium test

Dip the test strip briefly in the decalcifying fluid.

Immerse the reaction zone for 45 seconds in a solution of 1 mL 30% hydrogen peroxide in 20 mL of 1 N sodium hydroxide.

Compare the reaction zone with the colour scale provided with test strips.

Calcium oxalate test

Add an equal volume of 5% ammonium oxalate to an aliquot of used decalcifying fluid.

Stand 30 minutes.

A white precipitate of calcium oxalate forms, indicating a positive reaction. Calcium oxalate is insoluble at the pH of Kristensen decalcifying solution, so neutralization of the fluid prior to the addition of ammonium oxalate is unnecessary.

Kristensen decalcifying solution

sodium formate 136 g
formic acid (90%) 680 mL
distilled water to 4 L

Special stains

Readers should consult methodologies in a standard histological method textbook such as Bancroft JD, Stevens A. *Theory & practice of histological techniques*. London, Churchill-Livingstone 1982.

Giemsa

Giemsa should be available for lymphomas and for staining of *Helicobacter pylori* in gastric biopsies.

Periodic acid Schiff and diastase

Periodic acid Schiff and diastase should be available for fungi and for mucin identification.

Immunoperoxidase staining and other advanced staining

Immunoperoxidase staining and other advanced staining should only be considered for the largest centre. Referral of the blocks should be facilitated where required.

Acid-fast bacillus stain (Ziehl–Neelsen)

Acid-fast bacillus stain should be available for histological sections as well as for routine microbiology.

Safety and cleaning

Reference is made to El-Nageh M, Maynard J, Cordner S. *Quality systems for anatomical and forensic pathology laboratories: guidelines for implementation and monitoring*. Alexandria, Egypt, World Health Organization Regional Office for the Eastern Mediterranean, 1999 (WHO Regional Publications, Eastern Mediterranean Series, 18).

This publication provides a checklist for laboratories which will assist with safety matters as well as establishing a quality system (see Annex 2 below).

Safety manual

A process must be in place to advise with regard to safety matters and to monitor incidents. The safety manual is an essential document complementing a quality manual, and linked to it through document control. See *Quality systems for anatomical and forensic pathology laboratories: guidelines for implementation and monitoring* above.

The following items should be covered in the safety manual. Some items are covered by national or local regulations and should be amended accordingly. There may be other items which should also be considered.

General guidelines

Accidents should be avoided by preventive actions:

- choose methods with minimum hazards
- avoid flammables in method selection
- avoid carcinogenic and other toxic substances
- indicate all hazards in method documentation.

All staff are to be issued with and made aware of these laboratory safety orders on appointment.

All specimens and bodies must be regarded as potentially hazardous or contagious.

Admittance to the laboratory should be restricted.

Medical emergency planning: procedures for responding to cardiac arrest should be implemented.

Laboratory and mortuary staff

Potential hazards should be identified and hazard minimization procedures communicated.

Regulations which apply to the materials and chemicals being used should be known and followed.

Gowns or protective coats:

- must be worn when working in the laboratory
- must not be worn outside the laboratory (if necessary use a second coat).

Protective gloves should be removed before handling telephones, keyboards, and so on.

Pipetting by mouth is not allowed. A rubber bulb system or equivalent must be used if a glass volumetric pipette is required.

Automatic samplers and diluters should be used whenever possible.

Eating, drinking or smoking is not permitted in the laboratory or mortuary.

Drinks and foodstuffs must be kept only in a refrigerator set aside uniquely for this purpose.

Lipstick or cosmetics should not be applied in the laboratory.

Labels must not be licked.

Pencils and pens must not be placed in the mouth.

Instruments or machines connected to power and water supplies must not be touched or turned off, except by authorized staff.

Specific instructions for packaging and transportation of biological material must be observed.

Sitting on laboratory benches or mortuary trolleys is prohibited.

Non-slip footwear is to be worn.

Storage and handling of hazardous chemicals must be done by qualified staff, and documentation should be complete and detailed. (See *Quality systems for anatomical and forensic pathology laboratories: guidelines for implementation and monitoring.*)

Wax on the floor is a hazard and should be minimized by cleaning wax flakes at frequent intervals during section cutting.

Special items of safety in histology

Tuberculosis

Care must be taken with decontamination of equipment; frozen sections should be avoided.

Creutzfeldt–Jacob disease

Suspect Creutzfeldt–Jacob disease tissue should not be handled or processed except in specially set-up laboratories.

A checklist should be used to monitor correct procedures for

- chemical hazards
- storage
- flammable and explosive materials
- carcinogenic chemicals dyes
- physical hazards
- thermal
- sharps
- protective clothing
- eye protection
- masks
- gloves
- gowns.

A useful checklist is included in *Quality systems for anatomical and forensic pathology laboratories: guidelines for implementation and monitoring* (reproduced in Annex 2 below).

Tissue review and clinical correlation

Tissue review and correlation should be part of routine feedback and communication with clinicians and surgeons. This assists in improving the quality and relevance of the service.

Training and education

Mentors

There needs to be formal contact between the laboratory pathologist and a pathologist who can give advice and opinions as a mentor. This is particularly important in any developing country laboratory. It allows for and facilitates consultation on difficult and problem cases as well as continuing education for both.

Sources of information

Reference textbooks are an essential part of any laboratory.

Website access can provide a library and this can often complement mentor communication.

External quality assessment programmes

The World Health Organization has a mentor programme for one laboratory in each developing country to participate as the contact laboratory in setting up an in-country anatomical pathology quality assurance programme.

It is important for all laboratories to participate in an external quality assessment programme in order to assist in technical and diagnostic standard maintenance.

Annex 3 contains a list of web addresses of external quality assessment organizations.

Annex 1

Some commonly used fixatives other than formalin

Bouin fluid

Bouin fluid is used when enhanced staining for connective tissue is required. An excellent premordant fixative for chromatin to be demonstrated if using iron-haematoxylin methods. Prolonged exposure of tissue to the fixative reduces the nuclear staining, so this fixative should be issued only after consultation.

Preparation

formalin (40% formaldehyde) 50 mL
saturated aqueous picric acid 150 mL
glacial acetic acid 10 mL

Safety

Gloves and eye protection should be worn when dispensing, preparing or working with formalin. Contact lenses are not to be worn without safety glasses.

Formalin vapour has respiratory effects. Formalin may have carcinogenic effects.

Hydrochloric acid and formalin should be avoided in combination.

Picric acid is poisonous and explosive when solid and dry.

Acetic acid is corrosive.

Zenker fluid

Zenker fluid is a mercury-based fixative and presents problems with the safe disposal of the mercuric chemicals. It has poor storage capabilities once it is made up.

Preparation

mercuric chloride 25 g
potassium dichromate 12.5 g
sodium sulfate 5 g
distilled water 500 mL

Add 5 mL acetic acid to 95 mL Zenker fluid before use. (The solution does not keep well after the addition of the acetic acid.)

Safety

Mercuric chloride is a poison.
Acetic acid is corrosive.
A chemical disposal company is needed to dispose of mercury waste.

Carnoy fluid

This is a rapidly penetrating fixative (three hours for normal-sized 4 cm × 4 cm × 1 cm thick tissue pieces). It gives good nuclear fixation but haemolyses red blood cells. The use of Carnoy fluid is not encouraged because it contains chloroform.

Preparation

absolute ethanol 60 mL
chloroform 30 mL
glacial acetic acid 10 mL

Safety

Use of this fixative is not encouraged due to its chloroform content and associated hazards.

Annex 2

Laboratory self-assessment checklists[8]

Introduction

A quality manual must address certain issues and in some cases refer to norms and regulations to fulfil its purposes. In previous sections, most of these issues have been identified. In this annex, a number of questions have been formulated to allow the laboratory to review itself and decide if any action is necessary. The relevance of the questions to a specific laboratory will depend on its responsibilities and tasks. Therefore not all questions need result in a positive answer for every laboratory.

Questions are listed in different sections focusing on specific areas of laboratory services and management. More than one section of the checklist will be relevant for the reviewing process. For example, the section on equipment applies to all kinds of laboratories, independent of their area of responsibility. Likewise, similar questions may occur in different sections of the checklist.

Terms such as "adequate" are used in the checklist; details of what is regarded as adequate will vary but, in general, facilities and procedures will be regarded as adequate if they do not limit the quality performance of tests or procedures and are compatible with applicable safety and staff amenity standards.

It would be beyond the limits of this document to provide an exhaustive checklist. Questions related to the detail of specific technology may therefore be added by the reviewer.

[8] Adapted from El-Nageh M, Maynard J, Cordner S. *Quality systems for anatomical and forensic pathology laboratories: guidelines for implementation and monitoring.* Alexandria, Egypt, WHO Regional Office for the Eastern Mediterranean, 1999 (WHO Regional Publications Eastern Mediterranean Series 18).

General checklist

- Is there a formal system of document control?
- Is there a quality manual?
- Does the quality manual address:
  - the internal organization of the laboratory
  - daily surveillance of results (routine and urgent)
  - limits of acceptability of results (analytical goals)
  - recording, documentation and archiving of all quality control results
  - the use of calibrators and controls
  - internal audit procedures
  - safety regulations?
- Is there an organization chart?
- Have the operational responsibilities of each member of the laboratory been documented?
- Is there a designated quality officer(s) responsible for monitoring quality?
- Have documentation procedures been defined and are they distributed throughout the laboratory?
- Is there documented evidence of quality of any laboratory to which work is referred (subcontracted)?
- Has the person-in-charge approved the use of the outside referral laboratories?
- Are there procedure manuals available for:
  - all measurements and observations
  - maintenance of instruments?

- Is there a comprehensive instruction for writing procedure manuals?
- Are copies of the procedure manuals available in the work areas?
- Are all procedure manuals continuously revised to reflect changes in methods of operation?

Internal quality control and external quality assessment

- Are analytical goals specified for all quantities?
- Is there a documented internal quality control programme?
- Are quality control results checked for each batch of results before they are reported?
- Are there written criteria as to whether batches of results or slides are to be accepted or rejected (control rules)?
- Are quality control documents and records kept up-to-date at all times and regularly reviewed?
- Is evidence available to show that corrective measures are being taken when necessary?
- If some measurements are done by more than one procedure, are checks made to ensure that the results are comparable?
- Does the laboratory take part in external quality assessment (proficiency) programmes?
- Are records kept of all external quality assessment results?

- Are results from external quality assessment programmes available to laboratory staff?

- Is evidence available to show that corrective measures arising from the internal audit are being taken?

- Does the laboratory have an established collaboration with a mentor laboratory?

Health and safety

General

- Does the laboratory have a designated and properly trained person in charge of safety?

- Is the person in charge of safety known to all laboratory staff?

- Is there a safety education programme comprising:
  - chemical hazards
  - microbiological hazards
  - physical hazards
  - use of safety glasses and other measures for personal protection
  - personal hygiene
  - use and removal of protective clothing (including gloves and enclosed shoes)
  - emergency equipment and procedures
  - first-aid?

- Does the laboratory comply with:
  - fire regulations
  - electricity regulations
  - water and sewage regulations
  - hazardous materials regulations

- noise and ventilation regulations
  radioactive substances regulations
- postal and other distributor's shipping rules?

- Are personnel immunizations current?
- Do staff have regular occupational health checkups?
- Is a safety manual (or list of special precautions) available?
- Do staff exposed to radioactivity or X-rays wear monitoring devices?
- Is the laboratory checked for background radiation, when work is done with radioactive reagents kind chemicals?
- Are monitoring devices regularly checked and the results monitored and archived?
- Is there a recognized mechanism for reporting all laboratory accidents and for documenting action(s) taken in response?
- Are personnel required to wear a gown and gloves for specimen preparation and handling?
- Do personnel wear closed shoes?
- Are masks and eye protection available in dissection areas?
- Are there instructions for precautions to be taken with unfixed tissue, known or suspected infectious tissue, and tissue submitted for frozen section (e.g. hepatitis, tuberculosis, HIV)?
- Are safety glasses and gloves used when handling liquid nitrogen?

- Are staff instructed to remove gloves before handling any non-contaminated surface, stationery or equipment?
- Are material safety data sheets made available from reagent suppliers?
- Are there written instructions for the safe handling and disposal of specimens, used glassware, biological media and animal remains?
- Are there written instructions for handling spills of contaminated materials?
- Are these instructions for handling spills known to the laboratory staff?
- Are decontaminating solutions appropriately used for their specific purpose?
- Is the laboratory equipped with fire extinguishers?
- Are all personnel familiar with operation of the fire extinguishers?
- Are there regular fire drills?
- Is the functioning of the fire extinguishers regularly inspected?
- Is the laboratory equipped with first aid equipment?
- Are personnel instructed in the use of safety and first aid equipment that might be available (e.g. emergency showers, gas masks, etc.)?
- Are there regular checks of the measures to be taken in case of emergency?

- Are volatile and/or flammable chemicals stored in areas or containers specially designed for the purpose?
- Is bulk storage of flammable liquids located in a separate building within the complex?
- Are areas where volatile fluids are used suitably ventilated?
- Are vessels containing flammable liquids kept covered when not in use?
- Are limits set to volumes of flammable liquids allowed on the bench and in the laboratory?
- Are staff instructed in the safe handling of acids, alkaline solutions and corrosive or hazardous chemicals?
- Are fume cupboards provided when needed and:
  - do they function properly
  - are they checked regularly?
- Are all specimens treated as potentially infectious?
- Is mouth pipetting prohibited?
- Are suitable devices available to avoid mouth pipetting?
- Are measures taken to minimize the formation of aerosols?
- Are there documented procedures for disinfection of instruments and workspace?
- Does a protocol exist whereby service personnel can be informed that an item of equipment requiring maintenance or repair may be contaminated internally by infectious material?

- Are there designated and adequate sharps containers?
- Are gas cylinders handled according to regulations?
- Are staff informed about the hazardous nature of ultraviolet light and radioactive materials?
- Is smoking, eating and drinking prohibited in laboratory work areas?
- Is all waste disposed of daily in a way that poses no direct or residual hazard to the community?
- Is recycling of reagents used where possible?
- Is all contaminated and potentially infectious material adequately sterilized before disposal or cleaning?
- When handling concentrated formaldehyde solution, do staff use:
  - fume cupboard or flame extraction system
  - respirator approved for use with organic vapours
  - gloves
  - gown
  - safety glasses?
- When handling fixatives containing mercuric chloride is
  - skin contact avoided
  - metal corrosion avoided?
- Are xylene or xylene substitutes handled in such a way to prevent inhalation and skin contact?
- Are staff educated with regard to precautions to prevent exposure to contact with toxic substances and known carcinogens?
- List any laboratory design features creating a hazard

Mortuary safety

- Are the mortuary and related areas secure so that unauthorized persons cannot gain physical access to these areas?
- Are all visitors and tradesmen needing access to these areas given specific access authority and is this authority recorded?
- Are all authorized staff properly identifiable?
- Are there documented procedures for out-of-hours access to the mortuary and related areas?
- Are the mortuary staff disciplined and informed about safe laboratory and mortuary practice?
- Are all bodies, tissues and samples regarded as potentially infectious?
- Are both staff and management involved in:
  - reviewing safety procedures in the laboratory and mortuary
  - reviewing and preparing the documentation about safety procedures
  - developing staff training and the education in relation to safety
  - developing, reviewing and documenting procedures in the event of chemical, biological or fire emergencies?
- Have some staff members completed an approved first aid course?
- Are first aid kits available and do staff know their location?

Facilities

- Is adequate space provided for:
  - specimen collection

  - analytical work workbenches
  - instruments storage for chemicals storage for consumables
  - refrigerated storage specimens administration records?

- Do the specimen collection area, surgical pathology dissection area, histology laboratory and autopsy room conform to local standards and regulations for:
  - lighting
  - power points stable electrical power ventilation
  - temperature and humidity control
  - water supplies (hot and cold tap and deionized/distilled)
  - drainage/sewerage
  - biological waste disposal chemical waste disposal?

- Is there a staff library?

- Is there a meeting room?

- Are there a sufficient number of adequate staff and rest rooms?

- Is the laboratory appropriately and regularly cleaned and maintained in good order?

- Is there an emergency power supply to maintain essential services?

- Is there a direct outside telephone for emergency use?

Staff

Person in charge/laboratory manager

- Is the person-in-charge involved in:
  - staff training programmes

- staff appraisal
- assessment of methods and approval of their changes
- review of quality assessment programmes?

- Is the person in charge readily available for consultation with:
  - laboratory staff
  - medical doctors
  - administrators?

- Is there a suitable relief arrangement in case of absence of the person in charge?

Other staff

- Staffing policies:
  - is there a defined staff structure
  - is there a staff manual including all staff rules, procedures and privileges
  - are all the staff aware of their duties, privileges and responsibilities?

- Do current records include:
  - résumé of training and experience
  - formal qualifications or necessary registration
  - dates of employment etc.
  - job description
  - work injury records
  - tasks performed in the laboratory
  - continued education?

- Are the staff properly educated?

- Do staff perform tests without proper training or experience?

- Is there a formal certification of staff qualified to make specified measurements?

- Is there a supervisor assigned to new or non-qualified staff?

- Do less experienced staff have access to technical advice from senior staff at all times?

- Is the after-hours service provided by qualified and experienced staff?

- Is the quality of work carried out after-hours checked at the earliest opportunity?

- Are staff not directly involved in routine work but using the same equipment (e.g. research staff) certified?

Training programmes

- Are the objectives of the training clear and understood by trainee and supervisor?

- Does the training programme involve:
  - orientation of new personnel
  - scientific and procedural matters
  - occupational health and safety
  - bench training access and use of teaching aids
  - seminars, external courses, projects, etc.?

- Is the training evaluated against objectives?

- Are technicians appropriately trained on specific instruments?

Equipment, instrumentation, reagents, methods and reports

General

- Are operating and maintenance manuals available for all types of equipment?
- Are all staff who use the equipment familiar with the detailed methods of operation?
- Is the equipment adequate for the choice and number of tests being performed?
- Is there an equipment inventory?
- Is electrical equipment properly connected, earthed and checked regularly?
- Are equipment and measuring devices (pipettes, diluters, photometers, etc.) calibrated and tested regularly?
- Are fundamental quantities (temperature, wavelength, straylight, absorbance, etc.) of complex instruments regularly checked?
- Are performance and tolerance limits defined for each analytical instrument, component or procedure of a system?
- Is there a reference thermometer available to check the bias of all working thermometers in use?
- Are regular temperature checks made of water baths, incubators, refrigerators, freezers, heat blocks, etc.?
- Is the equipment well maintained?

- Is equipment that is out of order clearly identified?
- Are calibration, maintenance and service records kept on file and steps taken to rectify faults?

Autoclaves

- Is the autoclave of adequate size for the workload?
- Are autoclaves checked regularly for efficient functioning by means of spore strips?
- Are autoclaves checked by thermograph and temperature tape on each run?
- Are heat-proof gloves available for loading and unloading the autoclave?
- Are face shields and protective aprons available while working at an autoclave?
- Is regular maintenance of the autoclave undertaken and logged?

Incubators

- Is there a sufficient number of incubators in relation to the daily workload?
- Are there incubators working at 25 °C, 30 °C, 35 37 °C, 42 °C, with controlled CO_2 atmosphere?
- Are all incubators clean and well maintained?

Microscopes

- Are staff properly instructed in how to use a microscope?
- Are microscopes properly cleaned after use and well maintained?
- Are workstations for microscopy ergonomically designed?
- Are there sufficient binocular microscopes available with oil immersion objectives?
- Are there low power stereo microscopes available?
- Is an ocular with a calibrated micrometer for the microscope available?
- Is the illumination adequate on all microscopes?
- Is a dark ground microscope available?
- Is a multiple headed teaching/consultation microscope available?
- Is a fluorescence microscope available?
- Are the hours of use of high-energy light sources recorded?
- Are ultraviolet globes changed in accordance with the maker's instructions?
- Is the ultraviolet light source in the fluorescence microscope adequately shielded to protect personnel?
- Are microscopes stored under appropriate conditions?
- Are microscopes serviced at regular intervals?

- Are the correct spare fuses and bulbs kept for each model of microscope used in the laboratory?

Tissue processors

- Are solutions changed regularly?
- Is a written record kept of solution changes?
- Is the temperature of the wax baths checked regularly?
- Is a record kept of the type of wax used?
- Is the processor ventilated?

Microtomes

- If steel knives are used are they regularly sharpened and correctly stored?
- If disposable knives are used how are they disposed of?
- Do microtomes have safety shields over the knives?

pH meters

- Are pH meters and their electrodes properly maintained?
- Are pH meters regularly checked with buffers before use?
- Are calibration buffers of adequate quality?

Balances

- Are staff properly instructed in how to use a balance?
- Are the balance pans and the environs of the balances clean?
- Are balances mounted on a vibration-free stand?
- Are balances located in an area free of draughts and without marked temperature fluctuations?
- Are certified weights available for daily checks?
- Are balances regularly checked, serviced and calibrated?

Centrifuges

- Are there appropriate centrifuges for the specific needs available in the laboratory?
- Are centrifuge bowls decontaminated daily?
- Are centrifuges provided with safety locks?
- Are there necessary precautions to avoid aerosols?

Pipettes and volumetric glassware

- Is all volumetric glassware of the required degree of accuracy, and where necessary verified?
- Are pipettes and diluters calibrated periodically?

- Are safety devices available to prevent the need for mouth pipetting?
- Are damaged pipettes discarded?

Refrigerators/freezers

- Is sufficient refrigerator and freezer storage for specimens and reagents needing cold storage available?
- Is cold storage available at the following temperatures: 0–4 °C, –20 °C, –40 °C, –70 °C or below?
- Are refrigerators used for storage of biological materials and reagents equipped with an alarm system?

Safety cabinets

- Is there a safety cabinet for the handling of contagious specimens or organisms?
- Does the biological safety cabinet meet minimum requirements for the protection of workers from infectious agents and protection of cultures from contamination? •Is the functioning of the cabinet tested each day of use?
- Are filters maintained according to a regular schedule?
- Are ultraviolet lamps replaced in accordance with the manufacturer's instructions?
- Is the cabinet checked annually by an inspecting authority?

Specimens

- Does the laboratory provide written instructions for the collection and transport of specimens which:
  - are available to all users
  - outline patient preparation and collection techniques
  - give details of specimen storage and preservation
  - give transport requirements?

- Are appropriate containers, swabs, etc., provided by the laboratory or other approved source?

- Does the laboratory request form include remarks for clinical history (including vaccination when relevant), and source of specimen?

- Are validated measures followed to ensure that the appropriate preservative has been taken for the collection and transport of specimens for a specific laboratory investigation (anticoagulants, enzyme inhibitors, antimicrobials, buffered formalin, etc.)?

- Is there adequate space for receiving and entering specimens?

- Is there adequate space for the safe storage of
  - large buckets or glass jars
  - smaller containers?

- Is there a protocol for the retention of specimens?

- Are the specimens kept for a mandated period?

- Are specimens numbered and recorded as soon as they enter the laboratory?

- Does the laboratory assess the acceptability of specimens received?

- Does the laboratory check that all specimens received are properly labelled and authorized?

- Do written instructions exist to deal with poorly labelled specimens?

- When specimens are received, is a registration made of
  - the name or other sufficient identification of the patient
    the specimen identification
  - the name of the person referring
  - the date (and time when relevant) of specimen collection
  - the date and time the specimen was received in the laboratory?

- Is adequate identification of specimens provided through all phases of laboratory analysis?

- Are specimens adequately stored within the laboratory from time of receipt until final discard?

- Is refrigeration used to preserve specimens:
  - at the collection site
  - in the laboratory
  - during transportation?

- Are incubators/refrigerators/freezers available for out-of-hours storage of specimens when appropriate?

- Are there suitable facilities for storage of specimens in the laboratory?

- Does the storage area for specimens have a separate fume extraction system?

- Is the fume extraction system an exhaust fan, down draught, or other?

- Is a microwave oven used in the dissection area and is microwave fixation monitored?
- Is there a protocol for the examination of gross specimens?
- Is the protocol manual for a cut up readily available in the dissection area?
- Is the gross specimen examination recorded in a uniform manner?
- Are all measurements in millimetres?

Reagents, media and reference materials

- Are all reagents, stains or media properly labelled and dated with the time of receipt or preparation?
- Are old and outdated reagents discarded in a safe manner observing environmental regulations?
  NOTE. There may not be regulations for handling reagents used. The laboratory is obliged to find environmentally safe disposal routines.
- Are reagents correctly stored?
- Is distilled or deionized water available and used as appropriate?
- Is the quality of purified water specified and monitored?
- Are reagents or media with appropriate quality used?
- Are the manufacturer's recommendations for reconstitution, storage and expiry followed for all reagents?
- Are reagents tested with normal and abnormal controls before use?

- Are biological reagents with reputable purity and potency used?
- Are new biological reagents checked against the old before being taken into use?
- Are reagents, stains and/or media subject to quality control procedures?
- Are sufficient quantities of reagents and/or media kept in stock?
- Are the conditions for storage specific, safe and appropriate for the reagents and media?
- Is the quality of stored, diluted reagents (e.g. pH, colour, ion concentration, absence of water etc.) routinely checked before use?
- Are reference materials and/positive controls used and do they comply with the method of investigation?
- Are reference materials used for control of each batch of stains?

Methods

- Does the nomenclature of quantities and units follow that nationally agreed?
- Is there an operational procedures manual including the description of all procedures in the laboratory?
- Are copies located in the work areas?
- Does the procedures manual follow a standard protocol for preparation?

- Are the manuals reviewed and updated regularly?
- Who reviews procedure manuals?
- Do procedure manuals include:
  - when the procedure was introduced to the routine and the latest data reviewed or updated?
  - instructions for the collection, preservation and transport of specimens
  - sufficient information on reagent and kit preparation
  - step-by-step outline of the procedure
  - internal quality control and external quality assessment schemes
  - any hazards of the procedure notes on interfering substances
  - instructions for reporting results
  - alternative procedures as "back-up"' for automated tests, or instructions for referral of specimens?
- Are all written instructions rigorously followed?
- Are relevant current textbooks and periodicals available?
- Are copies of relevant original literature related to methods available?
- Is there access to a medical library?

Reporting and recording

- Is there a record of the outcome of every specimen received?
- Is there a documented procedure for accelerated handling of
  - seriously abnormal results
  - urgently requested specimens?

- Is there a recognized system of nomenclature used for reporting results?

- Are there procedures to check for transcription, calculation, or data entry errors? •Are results legible?

- Are final reports despatched without undue delay?

- Does the report include
  - the patient's name, age and sex
  - the patient's record number (for hospitals)
  - the requesting doctor's name and address
  - the ward or clinic (for hospitals) the name of the laboratory
  - a telephone number for inquiries
  - the date and time of collection of the specimen a laboratory accession number for each specimen
  - the type of material (system) tested (e.g. biopsy, anatomical site, etc.)

- Are the reports scrutinized and signed before release? Is this for:
  - all reports
  - a random sample of reports
  - reports selected as meeting some predetermined criteria?

- Can multiple copies of a report be generated and sent to more than one user, if requested?

- Can a replacement report be generated if the original is lost?

- When results are amended after reporting, does the replacement report indicate this?

- If reporting is performed outside the laboratory, are the results later reported and archived?

- Are reports routinely distributed by:
  - physical distribution of paper
  - electronic transmission (e.g. fax, e-mail, remote printing)
  - direct transfer to user's computer?

- Are reports generated within the laboratory showing:
  - dangerously abnormal results to be phoned
  - biologically unlikely results to be checked
  - changes from previous results which are large and therefore require checking
  - clinically significant trends or unusual results which require consultation with clinical staff?

- Are medical comments given on the significance of the results?

- Are steps taken to ensure that laboratory reports are treated as confidential?

- Are laboratory results reported only to the referring/requesting ward or physician?

- Can laboratory results he traced back to the reporting staff member?

- Is there a documented procedure for urgent results?

- Are there documented procedures for handling of clinically critical results?

- Are all final reports provided as written reports?

- What is the estimated reporting time for each report issued by the laboratory?

- Does the report state the inadequacy in quality or quantity of a specimen obtained, if necessary?

- Are preliminary reports (e.g. in case of urgency or after microscopic examination of specimens) validated before release?

- Are results and/or reports filed in a separate register in the laboratory?

- Does the filing system allow retrieval of all results obtained for a particular patient?

- Are photographs, slides and other information kept on record?

- Is there a coding system in place for retrieval of separate disease entities?

- Are results obtained on internal quality control specimens stored for all methods?

- Are archival summaries of quality control statistics made so that long-term changes can be reviewed, as necessary?

- Are archived results retrievable by:
  - patient name
  - medical record number
  - laboratory number?

- Is archival storage
  - on paper
  - on microfiche or microfilm
  - on disk
  - on tape
  - on other machine-readable media (e.g. CD-ROM)?

- Does the laboratory report on notifiable diseases to the appropriate authority?

- Does the laboratory keep records on diagnosis of notifiable diseases?

- Are reports containing evidence for malignancy or possible malignancy kept according to national regulations?

- Are reports kept on file for a minimum period?

- Is record kept of the date and staff who have carried out laboratory investigations?

Accounting reports

- Is there provision to check that a test has been performed before an account is issued?

- Is there provision for suppressing charging for a specimen or for a test?

- Is there an audit trail to check what has occurred, if an account is questioned?

- Can the system accommodate changes in test classification or grouping, as required from time to time?

Immunohistochemistry

- Are safety policies and procedures established for:
  - preparation of antibodies
  - preparation of chromogens
  - disposal of chromogens
  - disposal of solutions containing azide?

- Is there a list of antibodies and their concentration available in the laboratory?

- Are records kept of daily immunohistochemical staining on individual cases and antibodies used?
- Are positive and negative controls used for every staining run?
- Is there a careful control as to the dilution required for the antibodies?
- Is there internal quality control of the appropriate staining and is feedback provided with each batch?
- Are there appropriate storage facilities for the antibodies?
- Are expiry dates on the antibodies checked regularly?

Tissue immunofluorescence

- Are special instructions provided for collection of specimens for immunofluorescence studies?
- Is there a special transport medium for specimens for immunofluorescence studies?
- Is there a careful control as to the dilution required for the antibodies?
- Are positive and negative controls used for every staining run?
- Is there a protocol for high-risk specimens to be cut on the frozen section cryostat?

Records

- Are filed blocks usable and retrievable?

- Are filed slides usable and retrievable?

- Is there an index or cross-reference system available to allow retrieval of information by:
  - patient name
  - diagnosis?

- Are reports, stained sections and paraffin blocks kept on file for required periods?

Autopsy service

General

- Are autopsies performed by qualified staff
  - qualified pathologist
  - registrar under supervision of pathologist?

- Are qualified technical staff available to assist in the mortuary?

Mortuary and body handling

Introduction

- Are there minimum dress standards in the mortuary?

- Are there minimum standards of personal cleanliness and hygiene?

- Are there are rules restricting access to the mortuary to unauthorized persons?

- Are there written occupational health and safety procedures for mortuary staff?

- Do these procedures include advice on the event of
  - sharps injury
  - chemical injury
  - eye splash?

Body admission

- Is the body and accompanying documentation checked by a staff member on arrival?

- Do procedures exist for alerting senior staff in the event that the appearance of the body and the circumstances of the death do not appear to be natural?

- Are there firm procedures for securing the deceased's property and valuables?

- Are items, materials and substances brought with the body secured for later inspection by the pathologist?

- Is the body (or body part) given a unique case number at the time of admission?

- Is the body securely identified with this number?

- Are there procedures for recording the state of the body and its accompaniments at the time of admission?

- Do procedures exist for dealing with special cases differently, e.g. suspected homicide, unidentified bodies, major disasters, known infectious disease, decomposed bodies, deaths in custody, shootings, victims of hit-and-run accidents, infant death, driving fatalities, chemically hazardous bodies?

- Do arrangements exist for radiography of the deceased if required?

Identification

- Do procedures exist to document properly the visual identification of the deceased?

- If visual identification is not possible do procedures exist for:
  - dental identification
  - radiological comparisons
  - molecular biological identification techniques?

Body storage

- Do procedures exist for the proper routine refrigerated storage of bodies?

- Do procedures exist for non-routine storage:
  - long-term storage
  - infectious body storage
  - suspicious deaths and homicide storage?

Autopsies

- Do documented procedures exist for properly authorizing the autopsy and if applicable, the extent of the examination?

- Are medical and other records available prior to the conduct of the autopsy?

- Is the body weighed and is the height measured prior to autopsy?

Autopsy technique

- Is the identification of the deceased checked to ensure the autopsy is being performed on the correct body?
- Are appropriate instruments clean, prepared and available, including scales for weighing organs?
- Are there documented procedures for the technical conduct of the autopsy?
- Are there documented minimal observations to be made in particular classes of cases, e.g. in infant deaths?
- Are there special procedures for decomposed, infectious or otherwise hazardous bodies?
- Do procedures exist for the reconstruction of bodies after autopsy?
- Do procedures exist for the collection, labelling, storage and transport of specimens taken at autopsy?
- Do procedures exist for the disposal of human tissue that may have been retained at autopsy?

Body release

- As there can be no excuse for releasing the wrong body, do firm procedures exist for identifying the body for release?
- Are bodies in a clean and respectable state for release?
- Are infectious bodies identified as such on release?

- Is a body count and reconciliation performed at least once per day?

Exhibits and tissue storage

- Do procedures exist for the retention and storage of physical exhibits, tissue or fluid samples and medical or therapeutic items removed at autopsy?

- Do procedures exist for the collection and retention of large tissue samples?
  See also El-Nageh M et al. Quality systems for medical laboratories: guidelines for implementation and monitoring. *Alexandria, Egypt, World Health Organization Regional Office for the Eastern Mediterranean, 1995 (WHO Regional Publications Eastern Mediterranean Series 14).*

Photography

- Do facilities exist for the taking of autopsy and related photographs?

- Do the procedures allow for the correct identification of a photographs and its subject?

Cleaning of mortuary

- Is the autopsy area thoroughly cleaned after each session?

- Is the immediate area where the autopsy is performed cleaned after each case?

- Are all instruments cleaned and disinfected at the end of each autopsy session?

- Is protective clothing either discarded or laundered when removed each time a member of staff leaves the mortuary?

- Are overshoes or gum hoots discarded or cleaned at least on a daily basis?

- Are body trolleys washed and disinfected after the release of the body?

- Are the body storage areas emptied and thoroughly cleaned on a regular basis?

Waste disposal

- Do waste disposal procedures including biological and chemical wastes accord with relevant local laws and regulations?

- Do procedures exist for the collection and disposal of contaminated waste, including scalpel blades, needles, syringes and broken glass?

Stores and equipment

- Do procedures exist for the ordering, purchase, storage and issue of stores?

- Are all major items of equipment maintained in accordance with manufacturers' instructions?

- Are records of this maintenance kept?

Records and reports (autopsy)

- Is there a list of the types of reports issued by the organization?
- Is the authority to issue reports explicitly documented in the staff members' position specification or duty statement?
- Are there documented minimum requirements for staff authorized to issue reports under their own signature?
- Is there a list of staff with the authority to issue specific reports?
- Do all reports identify:
  - the case number and name
  - date of issue or signing
  - name and signature of person issuing report?
- Is the storage area of copies of reports specified?
- Are reports audited regularly for technical content and are the results of the audit brought to the attention of the director?
- Is this audit process documented and does it examine:
  - the appropriateness of the report
  - the style and readability
  - the presence of inconsistencies, mistakes or opinions given which are beyond the expertise of the staff member?

Computer services

Introduction

The use of this checklist will vary greatly between laboratories because of the wide range of complexity of computer systems. Where only

small or partial computer-based systems are in use it is still appropriate to ensure that the principles behind the detailed questions are appreciated and considered.

Documentation

- Have the objectives of the system been defined and documented?
- Have the data, information and processing requirements been defined and documented?
- Have the equipment performance requirements been defined and documented?
- Have management requirements in terms of data integrity, confidentiality, privacy, security and accountability been defined, documented and implemented?
- Have management requirements on back-up, long-term storage and archiving been defined, documented and implemented?
- Have the hardware and software compatibility requirements been defined and documented for data storage, the central processing unit, local area networks, terminal emulation, backups, audit trails and security?
- Have external data exchange systems and their compatibility been defined and documented?

Hardware

- Is there a brief description of all laboratory computer systems, including:
  - date of manufacture and purchase

- serial number
- the servicing agent and arrangements
- memory capacity
- secondary storage (floppy disk, optical disk, tape) capacity and access principle number of terminals
- number of on-line connections
- location of terminals or other parts of the network (if applicable)
- manufacturer, type and model
- operating manuals and troubleshooting procedures?

- Can the computer system handle a rate of data input as great as the maximum rate of data output of the transducer and AC/DC converter?

- Have the ergonomic requirements of keyboard. visual display, software, furniture and accessories been implemented?

- Is the main computer area air-conditioned with a dedicated system, if required?

- Is the main computer area secured against fire, theft and water damage?

- Is the main computer area or important workstation protected against power surge, spikes, low or high voltage?

Software

- Is there a brief description of all laboratory computer systems software, including:
  - name and version number
  - supplier's name
  - a succinct description of the function of the software

- a description of any modifications made to it, where applicable, with dates?

- Is there a complete record of all software installation, testing and/or modification, including:
  - dates of tests
  - purpose of tests
  - names of personnel performing the tests and/or making modifications
  - list of input test data used
  - output of tests
  - conclusions drawn from tests?

- Is there a dedicated data set and area allocated for tests of amended or new software?

- Are dates recorded when software is taken into routine use?

- Does the laboratory have sufficient in-house competence for testing and installation of acquired software?

- Is there an updated back-up version of all programmes and system instructions?

- Is there a list of all software titles residing on the system, with dates and version numbers?

- Does each software application which exists on the system have a user manual?

- Are software manuals amended when the software operations are changed?

Operations

- Have operating procedures been documented for the care of magnetic media (hard disks, floppy disks, tapes) when labelling, handling or storing the media?

- Have operating procedures been defined for the management of data libraries including floppy disk libraries, tape libraries, backup procedures, off-site backups, authorization?

System management

- Has a system manager been assigned to each system throughout the laboratory?

- Does the system manager play an active role in the administration and surveillance of the day-to-day use and requirements of the system?

- Has the system manager defined training procedures for staff for each software package?

- Is there a defined procedure to disseminate information on system changes?

- Are complete operations and servicing records kept?

- Have operating procedures been documented for the care and servicing of computer hardware, peripherals, read/write heads and servicing requirements?

- Do documented backup procedures exist for each separate computer configuration?

- If there is more than one backup in existence at any one time, is there a system of multiple or progressive backups, with adequate documentation?

- Is a logbook used for each system which records the time, date, operator and particulars of each backup?

- Are the backups stored in a secure place which minimizes the effect of fire, water damage, laboratory fumes, strong electromagnetic fields and dust?

Recovery

- Are the recovery procedures documented in a systems recovery manual?

- Are one or more persons trained in the correct recovery procedures as documented? •Have these people practised the recovery procedures?

- When a recovery is performed, are the time and date and other particulars entered into an appropriate log-book. e.g. backup log-hook or recovery log-book?

Risk management

- Has a risk management plan been implemented and is it reviewed periodically?

- Have threats been documented
  - in terms of actual and perceived threats
  - in terms of environmental factors, authorized users and unauthorized users?

- Has the vulnerability of the system been assessed in terms of natural disasters, environmental factors, the housing facility, access, and personnel (both trusted and unknown)?

- Are there sufficient security measures to prevent any involuntary or unauthorized access to or manipulation of the system?

- Have contingency plans been documented for actions to be taken in the event of loss of power, loss of air-conditioning, loss of data, loss of storage device, loss of printer, loss of personal computer, loss of mini or mainframe, loss of processing site extended systems down-time?

- Has management reviewed the above assessments and then selected appropriate countermeasures, e.g. locked doors, audit trails, software access systems, passwords?

- Do users keep their passwords secure?

- Does documentation exist for action to be taken in case of system failure, physical damage to system (fire, flood, lightning, strike, etc.), death or absence of key personnel?

- Are waste paper, disks and tapes disposed of properly considering their degree of confidentiality?

- Can the organization function without the main computer facility?

- Are all wires, cables, etc. adequately located and protected from traffic, spills and gases?

- Are software and data files stored to be protected against theft or intentional misuse?

Data management

- If requests for one patient have erroneously been entered into the computer, can these requests be corrected leaving an audit trail?

- If requests have been linked to the wrong patient, can this be corrected (leaving an audit trail)?

- Are data entered into the computer:
  - via automatic hardware input
  - by hand?

- If by hand, is the input checked via a documented standard operating procedure?

- Can the system generate a paper copy of data entered?

Testing process

- Does the computer provide lists of work for particular work areas?

- Do the work lists provide loading information for analytical instruments?

- Does the computer system allow quality control results to be entered along with patient results?

- Does the computer use the results of quality control samples to assess if a batch of results is out of range, using a formal acceptance algorithm (control rule)?

Instrument interfaces

- Is interface software properly documented?
- Is interface hardware exchangeable and spares available?
- Are instructions for interfaces handling and maintenance easily accessible?
- Who is responsible for the updating of interfaces?

Calculations

- Is every algorithm for calculation of results from raw data (e.g. radio immunoassay calibration curves) soundly based and appropriate to the assay?
- Are calibration curves viewed and validated by laboratory staff before acceptance of the calculated results?
- Can the computer automatically calculate results for tests which are derived from other tests, e.g. creatinine clearance?

Results

- Are the results entered online and/or offline into the computer system?
- Are all results protected from being reported until validated?
- Are there automated methods of entering results other than online ones (e.g. document reader)?

- Does the computer highlight dangerous (life-threatening) numeric results?

- If abbreviated comments are being entered, does the computer display the full comment for verification during result entry or subsequently?

Modification of reports and results

- Is modification of the data limited to those who need this facility?

- Are these people permitted only to modify those data files which are relevant to them?

- Does the system provide an audit trail to keep historical records of modified files?

- Are there documented procedures to follow if data are altered after reports have been issued?

- If data are altered after reports have been issued, does the system have the capability of sending amended reports?

- Does the audit trail allow recovery of the originally reported result?

- Are all reports and amended results signed?

Patient's reports

- Does the system generate printed reports?

- Are reports available through terminals in wards or other non-laboratory areas such as:

  hard copy

- visible on screen only?

- How would reports be produced if the system was down for 24 hours:
  - urgent results only, reported manually
  - full reversion to manual system?

- Does the system make provision for storage and reporting of comments on:
  - the unsuitability of a specimen for one or more of the requested analyses
  - the relationship of a result to a reference interval
  - the relationship of a result with reference to the preceding result (D-check)?

- Does the system make provision for storage and reporting of free-text comments on the results, or descriptions of qualitative results, such as:
  - predetermined comments
  - any text required?

- Are any results or combinations of results commented on by the system without human intervention:
  - by comparison with simple criteria
  - by an expert system?

- If an expert system is used, has its accuracy been verified?

Transferring/copying data

- When transferring or copying data, is there an error checking process performed?

- When transferring or communicating files across dedicated or normal phone lines, is the confidentiality of the data ensured?

- What process ensures that the confidentiality is maintained?

Quality control reports

- Does the system produce graphical or numerical summaries of quality control results or patient means and calculate appropriate statistics?

Accounting reports

- Does the system transfer information from the request entry process to a billing system?

Management reports

- Does the system provide reports on:
  - all specimens received (a log)
  - tests not performed within a predetermined time
  - monthly workload by number of specimens
  - monthly workload by total number of tissue blocks
  - monthly workload by numbers of each case
  - monthly workload by source?

Annex 3

External quality assessment organizations

College of American Pathologists

http://www.cap.org/html/lip/surveys.html#EXCEL

HTEQA Services, UK

http://www.hteqa.demon.co.uk/hteqa/hteqa.html

United Kingdom National External Quality Assessment Service (UK NEQAS)

http://www.ukneqas.org.uk/Directory/serv05.htm

Royal College of Pathologists of Australia

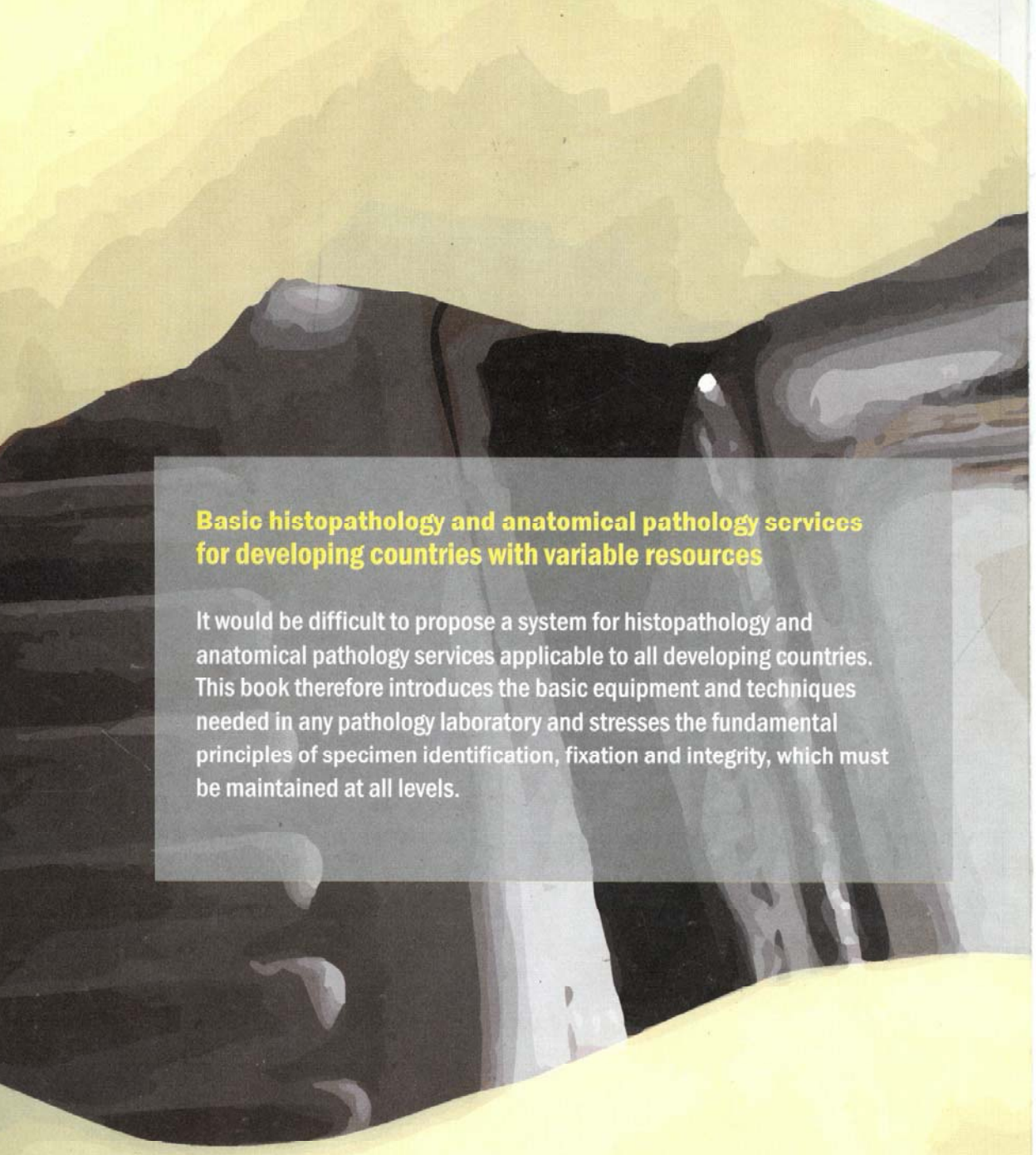
http://www.rcpaqapa.netcore.com.au
http://www.rcpaqap.com.au/benchmarking
http://www.rcpaqap.com.au/cytopathology/index.html

Labquality, Finland

http://www.labquality.fi/english/index.htm

EQUALIS, Sweden

http://www.equalis.se



Basic histopathology and anatomical pathology services for developing countries with variable resources

It would be difficult to propose a system for histopathology and anatomical pathology services applicable to all developing countries. This book therefore introduces the basic equipment and techniques needed in any pathology laboratory and stresses the fundamental principles of specimen identification, fixation and integrity, which must be maintained at all levels.

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