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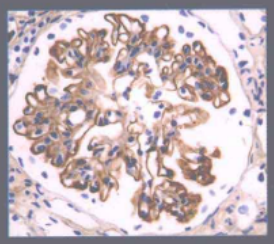
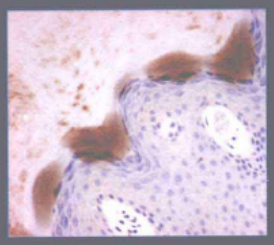
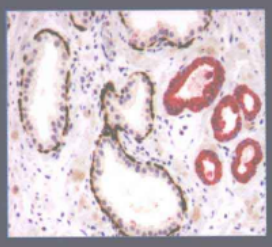

组织学技术

Theory and Practice of Histological Techniques

（第6版）

主编 John D Bancroft Marilyn Gamble

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THEORY AND PRACTICE OF HISTOLOGICAL TECHNIQUES

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（第6版）

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注 意

医学在不断进步。新的研究和临床经验正在不断拓展我们的知识，在治疗和用药方面作出某些改变也许是必需且适宜的。建议读者核对每种所开药品生产商所提供的最新信息，确认推荐计量、服用方法和时间及相关的禁忌证。决定患者服药剂量和最佳治疗方式并采取适当安全措施的责任在于实施治疗的医师，有赖于其个人经验和对各个患者的了解。对于因使用本书而引起的对人身或财产的任何损伤和（或）损失，出版商和著者不承担任何法律责任。

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Preface to the sixth edition

In the 30 years since the first edition of this book, histotechnology has continued to develop into a highly complex branch of laboratory medicine. Immunohistochemistry, in situ hybridization, molecular pathology, genetic testing and laser capture are all techniques currently in use to establish a diagnosis or to assess the changes occurring in tissues and cells in the disease process. Far more information can be obtained from these techniques than from many of the empirical methods used previously, but knowledge of the old and new is required by trained and trainee histotechnologists as well as the pathologist. The successful training of laboratory staff of all grades requires a thorough grounding in all aspects of histological techniques.

In producing this edition, we continued to be faced with the problem of achieving a balance between the new and old technology. To help achieve this some chapters from the last edition have been amalgamated to allow the introduction of the new material.

There are a number of new chapters and contributors for this edition. The new chapters include The gross room/surgical cutup by Paul Billings and William Grizzle, Tissue microarray by Wanda Grace-Jones, Genetic testing by Caroline Astbury, Laser microdissection by Diane Sterchi, and Jan Minshew has written Ergonomics.

New contributors are William Grizzle, Jerry Fredenburgh and Russell Myers who between them rewrote Fixation of tissues, Carbohydrates, and Proteins and nucleic acids. Lena Spencer updated Tissue processing and Microtomy. William Grizzle also updated the Neuroendocrine chapter, Jeanie Bartlett Microorganisms, and Scott Nestor Neuropathology and Enzyme histochemistry. Peter Jackson and David Blythe rewrote the Practical immunohistochemical chapter and Charles White its Applications in pathology. Christa Hladik with Charles White rewrote Quality control in immunohistochemistry and immunofluorescent techniques. Diane Sterchi has updated Molecular pathology. William Grizzle, Jerry Fredenburgh and Russell Myers have updated the Appendices.

As with the last edition we have had to remove some more of the less commonly used histological methods, which is unfortunate but a necessity, otherwise the book would have been impossibly large. The chapters on Cytology have not been included in this edition, as it has developed into specialized subject with numerous excellent textbooks devoted to it. Microwave methods have been assimilated into appropriate chapters.

Where relevant, we have continued the policy of outlining the uses of histological techniques in solving specific diagnostic problems.

John D. Bancroft
Nottingham, UK
2007

Marilyn Gamble
Morgantown, West Virginia, USA

Preface to the first edition

In recent years histological techniques have become increasingly sophisticated, incorporating a whole variety of specialities, and there has been a corresponding dramatic rise in the level and breadth of knowledge demanded by the examiner of trainees in histology and histopathology technology.

We believe that the time has arrived when no single author can produce a comprehensive book on histology technique sufficiently authoritative in the many differing fields of knowledge with which the technologist must be familiar. Many books exist which are solely devoted to one particular facet such as electron microscopy or autoradiography, and the dedicated technologist will, of course, read these in the process of self-education. Nevertheless the need has arisen for a book which covers the entire spectrum of histology technology, from the principles of tissue fixation and the production of paraffin sections, the more esoteric level of the principles of scanning electron microscopy. It has been our aim then, to produce a book which the trainee technologist can purchase at the beginning of his career and which will remain valuable to him as he rises on the ladder of experience and seniority.

The book has been designed as a comprehensive reference work for those preparing for examinations in histopathology, both in Britain and elsewhere. Although the content is particularly suitable for students working towards the Special Examination in Histopathology of the Institute of Medical Laboratory Sciences, the level is such that more advanced students, along with research workers, histologists, and pathologists, will find the book beneficial. To achieve this we have gathered a team of expert contributors, many of whom have written specialised books or articles on their own subject; most are intimately involved in the teaching of histology and some are examiners in the HNC and Special Examination in Histopathology. The medically qualified contributors are also involved in technician education.

All contributors have taken care to give, where applicable, the theoretical basis of the techniques, for we believe that the standard of their education has risen so remarkably in recent years that the time is surely coming when medical laboratory technicians will be renamed 'medical laboratory scientists'; we hope that the increase in 'scientific' content in parts of this book will assist in this essential transformation.

John D. Bancroft
Alan Stevens
Nottingham, 1977

General acknowledgments

Many Laboratory Scientists and Pathologists have contributed in different ways to the six editions of this text and to acknowledge their individual advice and assistance is impossible. We express our thanks to everyone who has contributed since 1977. We owe Harry Cook special thanks for his advice and contributions to the earlier editions. Our thanks are also due to the colleagues we worked with in Nottingham and Los Angeles during the lifetime of this book.

We would like to thank all of our current authors, and those contributors whose previous work remains in some of the chapters in this new edition. Special thanks go to Richard Horobin who has contributed to all of the editions, and Bob Francis and David Hopwood who contributed to the first five.

Our thanks go to those who assisted in the preparation of the manuscripts and the production of the illustrations. We are grateful to Carol Bancroft for her considerable help with the editing and proof-reading.

Finally, we wish to thank the staff of our publishers for their unfailing help and courtesy.

John D. Bancroft
Nottingham, UK

Marilyn Gamble
Morgantown, West Virginia, USA

2007

Acknowledgment to Alan Stevens

I have known Alan since he joined the Pathology Department at the University of Nottingham some thirty years ago. We had many discussions in those early years over whether the time had arrived for a multi-authored text on histological technique. It was apparent at that time that the subject was becoming too diverse for any single or two authors to cover in the depth that was required in the laboratories or the colleges where histotechnologists received their academic education.

In 1977 the first edition of this text was published and was due in no small part to Alan's vision and diligent work in editing and even rewriting some of the chapters. His contributions to the succeeding editions were just as important and his medical knowledge was a significant factor in the development of the book. It has been a great pleasure working with him and I have greatly missed his contribution to the editing of this new edition, although much of his writing in the various chapters remains. The success over the years of Bancroft and Stevens owes a great deal to Alan Stevens. I wish to thank him and wish him well in his current and future medical education publications.

John D. Bancroft
Nottingham, UK
2001

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1

Managing the Laboratory

Marilyn Gamble, Iain Banks and John D. Bancroft

INTRODUCTION

Management is an important aspect of the day-to-day life of the histopathology laboratory particularly since the emergence of accreditation. The accreditation standards include management as part of the evaluation and it is necessary that the laboratory worker is familiar with the processes involved. There are excellent books available which cover management issues in depth, and it is not the objective of this chapter to be a comprehensive guide to the subject. It discusses and concentrates on specific areas which have an impact on the operation of the laboratory; these are:

- Risk management
- Quality management and establishing a quality system.

RISK MANAGEMENT

Every laboratory has to have an effective risk management policy, because, as in most aspects of life, 'if it can go wrong, it will go wrong'. In the laboratory, it is important that the chance of something going wrong is either negated or minimized. The risk management process involves:

- Identifying all risks that exist within your environment
- Assessing risks for likelihood and severity
- Eliminating those risks that can be removed
- Reducing the effect of risks that cannot be eliminated.

Medicine itself is a risky business, which requires careful clinical management. Histopathology is a significant aspect of the medical risk management process. The surgical biopsy is sent for histopathological assessment to corroborate or dispute the clinical diagnosis by providing confirmation of data provided from other diagnostic tests. It gives the clinician valuable information on how to proceed with the treatment of the disease. Major resections are referred to the laboratory to confirm the diagnosis, and in the case of malignant tumors to ensure that there are adequate resection margins, to determine the extent of lymphatic involvement and/or direct spread, and to stage and classify the disease. Autopsies provide definitive data for medical audit, and can be used to determine where medical procedures have been ineffective and also to give additional data for the future treatment of other patients with similar medical conditions. Cervical cytological smears are used as a screening process to assist in the early diagnosis of disease prior to the development of symptoms and thereby enable effective treatment. This is accomplished by using non-invasive or minimally invasive techniques, which have a low risk of complications to the patient.

These clinical diagnostic aspects are not the only types of risk that apply to the laboratory. To function effectively and safely all of its procedures and activities are subjected to the risk management process. The risks in the laboratory are similar worldwide with a variation due to local circumstances. Health and safety and quality assurance incorporate a major aspect of risk management. All aspects of our working life incorporate a degree of risk and the risk management process allows us to prioritize, evaluate, and handle the risk appropriately. It is not possible to avoid or eliminate all risks, and in reality this may not be practical or possible. It is important to identify and understand the risks that are involved in your working practices. An individual's perception of

risk is dependent upon that individual's role within the organization. The chief executive, for example, will be concerned mainly with risks associated with strategic issues affecting the organization as a whole and would only include histopathology within the risk assessment if it had a direct impact on these issues. Matters concerning the day-to-day running of the laboratory would not be of direct interest unless, of course, there was a significant reason for involvement such as political or major financial concerns.

The laboratory manager or supervisor deals with the risks associated with ensuring that adequate resources are available to deliver the service and guaranteeing that the laboratory provides a service that is safe. Staffing levels and competence, budgetary management, consumable and equipment supplies, and maintenance are some of the areas of concern, but also included would be ensuring that risk management procedures are in place for the laboratory.

The laboratory manager must ensure that day-to-day errors do not arise as a result of inadequacies in laboratory procedures and that quality control checks are in place to eradicate human errors such as transcription or misreading. Standard operating procedures (SOPs) should be detailed to include Control of Substances Hazardous to Health (COSHH) risk assessments and also to include other health and safety information relevant to the procedure.

The histotechnologists and biomedical scientists at the bench face risks that involve equipment malfunction due to poor maintenance or design; poor-quality reagents produce poor processing of tissues or inaccurate staining results. The routine use of laboratory equipment, e.g. in microtomy, can result in one of the most common accidents that occur in the laboratory, cutting a finger or hand on microtome knives or blades. It is the responsibility of each laboratory worker to reduce the risks associated with their day-to-day work by using safety guards where available, checking the quality of reagents, and carrying out checks with diligence.

The risk management process

Risk management is a continual process and not a single step evaluation. Figure 1.1 shows the complete process.

Risk identification

A group of individuals with different roles in the laboratory best identifies risks. This ensures that the broadest possible spectrum of viewpoints is considered. During this process it is also useful to divide the risks into different categories such as 'political', 'organizational', 'financial', 'clinical', 'physical', 'chemical', 'infectious', etc. This helps to ensure that all aspects of the laboratory's operation are included. Different professions and grades of staff will be best placed to ensure the identification of all risks in the above categories.

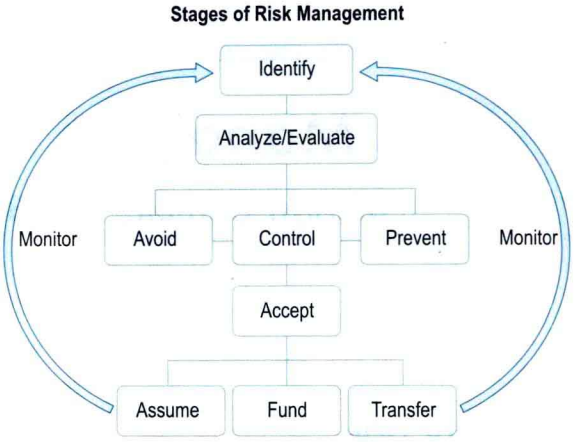

Fig. 1.1 The risk management process.

Risk analysis/evaluation

Analysis and evaluation of potential risks is an essential part of the process, and one that is used to identify both the likelihood and severity of these risks. By scoring the risks for likelihood and severity, it is then possible to use the matrix (Figure 1.2) as a tool that will put a value on specific risks, which helps in prioritizing them for further action.

The risk manager should put a system in place whereby all incidents and accidents are reported no matter how small. It is only by recording data that the full picture can be obtained and analyzed.

Severity and likelihood values

Incidents may be scored on a scale of 1–5 for severity.

1. *No injury*, potential for individual claim up to $10,000:
   - Breach of guidance
   - Minimal loss of reputation
   - No/minimal disruption to normal services.
2. *Minor injury*, potential for multiple or individual claims $10,000–50,000:

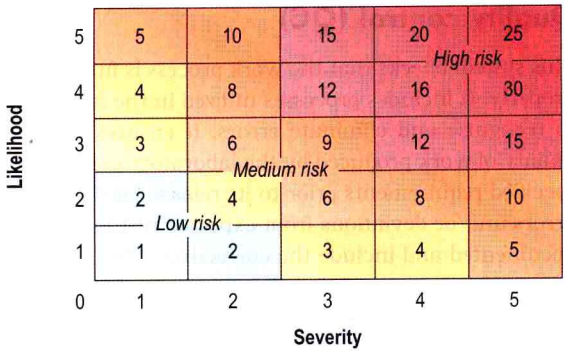

Fig. 1.2 The likelihood and severity matrix can be used as a tool to put a value on specific risks, prioritizing them for further action.

- Breach of legal requirement or authoritative guidance
- Loss of reputation
- Disruption to normal services.

3 *Moderate* potential for multiple or individual claims $50,000–200,000:
- Breach of significant legal requirement or authoritative guidance
- Significant loss of reputation
- Significant disruption to normal services.

4 *Moderately severe injury*, potential for multiple or individual claims $200,000–2.5 million:
- Breach of significant legal requirement with likely enforcement action
- Serious loss of reputation
- Serious disruption to normal services.

5 *Serious injury*, potential for multiple claims or individual claim exceeding $2.5 million:
- Breach of significant legal requirement with imminent action
- Serious loss of reputation
- Serious disruption to normal services.

Incidents may also be scored 1–5 for likelihood:

1. Incident unlikely to occur.
2. Incident likely to occur once in a 5-year period.
3. Incident likely to occur yearly.
4. Incident likely to occur once in a 6-month period.
5. Incident likely to occur once every 4 weeks or more frequently.

Risks should also be evaluated against the standards of one's peers to ascertain whether or not the risk is acceptable, as there could be more than one reason for obtaining poor results. A surgeon who has a record of poor results could either be a bad surgeon, or could be operating mainly on high-risk cases. The results may be poor in general comparison with other surgeons, but may be exceptionally good when compared to other surgeons operating on similar cases. It is for these reasons that laboratories should participate in benchmarking, and as laboratories deal with variable types of work the benchmarking should be compared to other similar operations, i.e. teaching hospital laboratories should only be compared to other teaching hospital laboratories. Benchmarking results are an effective aid to risk management and let you know how you are performing compared to your peers.

Risk control

The objective of the whole risk management process is to control risks. It may be possible in certain circumstances to avoid a risk or even prevent it completely. This would be possible, for example, by looking for alternatives to high-risk, harmful chemicals used in the laboratory. Prior to the 1970's, it was common practice to use mercuric chloride as a constituent of fixatives and, although this gave excellent quality fixation, it was extremely harmful to the environment and also to laboratory staff. Its use was subsequently stopped and alternative fixatives replaced it.

Despite efforts to eliminate risks it is not possible to remove them totally. Efforts should be made to reduce the effect or the possibility of the risk happening, but some risk may remain. Ways of controlling risk are numerous, but frequently there will be expert guidance or regulations issued by professional bodies or government that the risk manager should ensure are implemented. Where there is residual risk it must be funded as part of the control mechanism.

Risk funding

Risk management is not only about insurance, although this is an important option. All medical staff carry medical liability insurance, which covers them in the event of any negligence claims. Similarly, professional indemnity insurance is commonly available today for non-medical laboratory staff who are much more at risk in today's litigation-conscious society. The decision whether or not to insure should be based on the risk

assessment and the severity and likelihood of the risk. Some risks will not be appropriate for insurance cover for whatever reason, and in these instances the risk must be accepted by the organization.

Risk monitoring

Monitoring risk is an ongoing process and diligent documentation of all incidents and accidents is necessary for the process to be successful. The records are analyzed to identify whether or not the control measures implemented are effective. Each incident should be investigated, and where possible additional measures taken to ensure a repeat is avoided. It is important to realize that when an effective monitoring system is utilized the likelihood is that the number of reported incidents will increase due to the higher profile that risk management has with the staff. If not handled correctly this can have a detrimental effect on staff morale, as the initial perception will be that incidents have increased in number.

Risk management and quality control

Powers (2005) maintained that the laboratory quality control requirements should be based on risk management principles and answer the real question that the laboratory must answer, 'What are the appropriate laboratory controls to minimize patient risk?' He refers to the ISO 14971 risk management process as a systematic way to answer that question and recommends that standards be developed for clinical laboratories. In the interest of patient safety, determining the appropriate amount of quality control (QC) may require going beyond the current regulatory and accreditation requirements.

QUALITY MANAGEMENT

Accreditation/certification/regulatory agencies' standards, with some variation, utilize the components listed in the ISO section (see page 6). In conjunction with 'quality assurance' and 'continuing quality improvement', 'quality control' is an integral component of a required 'quality system'. A good QC system provides information for quality assurance activities.

Quality control (QC)

This system checks that the work process is functioning properly. It includes processes utilized in the laboratory to recognize and eliminate errors. It ensures that the quality of work produced by the laboratory conforms to specified requirements prior to its release for diagnoses. Errors and/or deviations from expected results must be documented and include the corrective action taken. It is not the intention of this chapter to give step-by-step guidelines for performing quality control. In the laboratory, quality control has long been a component of accreditation requirements and is ingrained in histotechnologists as a daily practice. Most laboratories have experienced technologists who have the responsibility of performing routine quality control checks prior to the release of slides for diagnoses. This QC evaluation will include, but is not limited to: accurate patient identification, fixation, adequate processing, appropriate embedding techniques, acceptable microtomy, unacceptable artefacts, and inspection of controls to determine correctness of special staining and immunohistochemistry methods. Criteria should be established that would trigger a repeat if the QC findings were qualitatively or quantitatively discordant with expected findings. Despite having a conscientious QC system in the laboratory, pathologists with a higher level of expertise perform the final QC examination as they 'read' the slide. It is their responsibility to determine that the section/slide is adequate for diagnostic interpretation. Errors/problems reported by pathologists and others should be included as part of the laboratory QC data collection.

Quality assurance (QA)

This is a shift from a focus on the end product or service to a focus on the process. Subsequent statistical analysis of good QC documentation provides the data for quality assurance activities where correlation of errors, complaints, failures or other unexpected results are evaluated against the laboratory expectations. This monitoring program evaluates errors and problems and addresses resolution. Reviewing the data allows identification of declining quality in specific areas and should trigger appropriate corrective action. A high number of 'repeats', for example, would indicate that a system check be done