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INTRODUCTION

This training logbook provides a nationally recognised framework to enable biomedical scientists to acquire the essential level of competence necessary to perform immunocytochemical techniques in a clinical context. The training syllabus covers antibodies commonly used in a district general hospital. However, an awareness of more specialised antibodies and the use of antibody panels is also essential.

Laboratories wishing to offer this training must have Institute training approval and be accredited with the Clinical Pathology Accreditation (UK) Ltd. Training is laboratory based under the overall responsibility of a named biomedical scientist or pathologist. Where other named individuals have taken responsibility for an aspect of the training this must be indicated in the training logbook. The submission of a portfolio forms part of the overall final assessment. The final examination will take the form of a written examination held in an approved examination centre. The successful completion of these requirements is recognised by the awarding of a Diploma of Expert Practice in Immunocytochemistry, which gives evidence of this particular area of expertise within cellular pathology.

TRAINING LABORATORY

Any laboratory or laboratory network wishing to offer support for a biomedical scientist preparing for the Diploma of Expert Practice in Immunocytochemistry should have a minimum annual throughput of at least 5,000 slides (this can include test and control slides). Additionally, it should undertake an antibody repertoire that includes most of the markers specified in the UK NEQAS for Immunocytochemistry General Pathology Module, (see table 1).

USE OF THE TRAINING LOGBOOK

a) Theoretical knowledge and practical skills
   Each aspect of preparation comprises the knowledge required to understand the processes that underpin the task and the practical skills and competencies to successfully execute the task. The biomedical scientist will be expected to acquire and demonstrate the knowledge that accompanies the practical skills.

b) Standard operating procedures
   All aspects of laboratory work must be covered by individually signed, indexed and dated Standard Operation Procedures (SOPs). Before commencing training it is mandatory that appropriate SOPs be in place to describe the departmental protocols for immunocytochemistry. The biomedical scientist must operate within the appropriate SOP at all times.

c) Quality control and audit
   Audit forms an integral part of the training process. The requirement for review of cases forms the basis of continuing audit of biomedical scientist competence and performance. Documentary evidence of this practice must be kept as part of the training record. Similarly, documentation of the internal quality process used for immunocytochemical tests should be included. In addition, there should be documentary evidence to show participation in external quality assessment.
<table>
<thead>
<tr>
<th><strong>Table 1</strong></th>
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<td><strong>GUIDE TO COMMONLY USED ANTIBODIES</strong></td>
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</tbody>
</table>
| **Epithelial markers**  
Pan-cytokeratins  
CAM 5.2 |
| **Lymphoid markers**  
T-cell markers e.g CD3  
B-cell markers e.g CD20, CD79a  
Leucocyte common antigen (CD45)  
CD68  
CD30  
CD15 |
| **Urological and prostatic markers**  
Prostate specific antigen  
Prostate specific acid phosphatase  
LP34 or 34 Beta E12  
Cytokeratins 5 and 5/6 |
| **Muscle markers**  
Smooth muscle actin  
Desmin  
Myogenin |
| **Neuroendocrine markers**  
Chromogranin A  
Neurone specific enolase (NSE)  
Synaptophysin  
Cytokeratin 20 |
| **Endothelial markers**  
Factor VIII related antigen  
CD31  
CD34 |
| **Mesothelial/mesothelioma markers**  
Carcinoembryonic antigen (CEA)  
AUA-1  
Ber-EP4  
Cytokeratin 5/6  
Calretinin  
Thrombomodulin (CD141) |
| **Breast markers**  
Oestrogen receptor  
Progesterone receptor  
HER 2 |
| **Miscellaneous**  
bcl-2  
Thyroglobulin  
Ki-67 or MIB 1  
CD 56  
Calcitonin  
Thyroid Transcription Factor-1 (TTF1)  
Human chorionic gonadotrophin (HCG) |
TRAINING MODULES

1. HEALTH AND SAFETY

1.1. General principles of health and safety

Understands:


1.1.2. Trust/Institution health and safety regulations

1.1.3. Departmental safety policy

1.2. Cell / tissue preparation, including section preparation

Understands the hazards associated with:

1.2.1. The handling of unfixed cells and tissues

1.2.2. The control and disposal procedures for high-risk specimens

1.2.3. Reagents used in fixation and tissue processing

1.2.4. Decalcifying agents

1.2.5. Reagents used in preparation of frozen specimens

1.2.6. Cryotomy and microtomy

1.2.7. The production of slides treated with section adhesive

1.3. Immunocytochemistry

Understands the hazards associated with:

1.3.1. Reagents used in proteolytic enzyme pre-treatment

1.3.2. Equipment and reagents used in heat mediated antigen retrieval

1.3.3. Immunological reagents

1.3.4. Potential carcinogenic chemicals

1.3.5. Dehydration, clearing and cover-slipping

1.3.6. The control and safe disposal of harmful chemicals and reagents
DECLARATION

I declare that I have satisfactorily completed the health and safety module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee) ......................................................

Date .................................................................

I declare that ......................................................... has satisfactorily completed the health and safety module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer) ......................................................

Date .................................................................
2. RISK MANAGEMENT/CLINICAL RISK

2.1. Understands the requirements for full SOP compliance, together with knowledge and understanding of risk assessment, relating to:

2.1.1. Specimen reception to include fixation, processing and specimen preparation
2.1.2. Section preparation
2.1.3. Antigen retrieval
2.1.4. Immunocytochemical method(s)
2.1.5. Automated immunostaining
2.1.6. Microscopy
2.1.7. VDU use

2.2. Is aware of the requirements of clinical governance in the following areas:

2.2.1. Clinical risk to the patient with regard to mislabelling specimens and slides
2.2.2. Clinical risk to the patient with regard to mislabelling of reagents
2.2.3. Validation of all reagents and antibodies
2.2.4. Quality control, quality assessment and quality assurance
2.2.5. Patient confidentiality and consent
2.2.6. Clinical effectiveness in the timely production of test results
2.2.7. Participation in clinical audit
2.2.8. On-going staff training and education
DECLARATION

I declare that I have satisfactorily completed the risk management/clinical risk module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee)  ...........................................

Date  .................................................

I declare that .............................................. has satisfactorily completed the risk management/clinical risk module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer)  ...........................................

Date  .................................................
3. IMMUNOCYTOCHEMISTRY METHODOLOGIES AND RELATED PROCEDURES

3.1. Fixation

3.1.1. Has an understanding of the general principles of fixation of cells and tissues, to include factors affecting the process. Understands the importance of adequate fixation for subsequent test procedures, with particular respect to immunocytochemical staining.

3.1.2. Recognises the appearance of artefacts produced by incorrect fixation.

3.1.3. Has a knowledge of specific types of commonly used fixatives and their characteristics and understands their compatibility with subsequent immunocytochemical staining procedures.

3.2. Decalcification

3.2.1. Has an understanding of the general principles of decalcification, to include factors affecting the process and practical aspects thereof.

3.2.2. Has knowledge of specific types of commonly used decalcifying agents and their characteristics and understands their suitability and compatibility with subsequent immunocytochemical staining procedures.

3.2.3. Is aware of problems associated with incomplete decalcification and the limitations of rapid surface-decalcification solutions.

3.3. Tissue and cell processing

3.3.1. Has an understanding of the general principles of tissue processing, to include factors affecting the process.

3.3.2. Understands the effects on subsequent procedures with particular regard to immunocytochemical staining and the appearance of artefacts produced by poor processing.

3.3.3. Has knowledge of specific tissue processing schedules appropriate for use with different tissue/specimen types.
3.4. Fresh tissues, cells, frozen sections

3.4.1. Has knowledge of situations in which fresh tissues or cells are required for immunocytochemical staining and understands the advantages and disadvantages of utilising fresh tissue or cells.

3.4.2. Has an understanding of the practice and problems associated with the production of frozen sections.

3.4.3. Has an understanding of the practice and problems associated with the production of samples suitable for immunocytochemical staining from cytological specimens, to include direct smears, cytocentrifuge and liquid based preparations, fine needle aspirates (FNAs), clots and cell blocks.

3.4.4. Understands the health and safety issues when making imprints from fresh tissue.

3.5. Paraffin sections and semi-thin sections

3.5.1. Is competent in the embedding and sectioning of paraffin wax embedded blocks for the purposes of immunocytochemistry, for all specimen types commonly encountered in clinical pathology.

3.5.2. Where limited material is available for analysis, is able to maximise the use of this material as appropriate.

3.5.3. Has knowledge of section adhesives and the practical importance of their use.

3.5.4. Is aware of potential problems associated with prolonged section storage and loss of tissue antigens.

3.5.5. Is aware of the problems associated with the use of immunocytochemistry on resin sections.
DECLARATION

I declare that I have satisfactorily completed the immunocytochemistry pre-analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee) ........................................

Date ......................................................

I declare that ............................................. has satisfactorily completed the immunocytochemistry pre-analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer) ........................................

Date ......................................................
ANALYSIS PHASE

3.6. Antigen retrieval

3.6.1. Knows the importance of accurate and appropriate antigen retrieval. Is familiar with all commonly used methodologies, to include proteolytic enzyme digestion, and heat mediated methods. Understands the mechanisms, as far as they are known.

3.6.2. Has knowledge and working experience of different proteolytic enzyme digestion methodologies. Is aware of the importance of optimal digestion, and can assess this in stained preparations.

3.6.3. Has knowledge and working experience of different heat mediated antigen retrieval methodologies. Has knowledge of various heat delivery systems, to include microwave ovens and pressure cookers. Has knowledge of various antigen retrieval solutions.

3.6.4. Is aware of the importance of optimal heat mediated antigen retrieval, and can assess this in stained preparations.

3.7. Primary antibodies

3.7.1. Has an understanding of the principle of primary antibody production. Is aware of the relative advantages and disadvantages of polyclonal antisera and monoclonal antibodies.

3.7.2. Has knowledge about the methods for characterisation, evaluation and validation of primary antibodies and the requirement for assessment of batch to batch variation.

3.7.3. Understands the concepts of sensitivity, specificity, avidity and affinity and understand their significance to the quality of immunocytochemical staining.

3.7.4. Has knowledge concerning the need for appropriate dilution of primary antibody reagents, and the effects on subsequent immunocytochemical staining results.

3.7.5. Has knowledge of the problems of non-specific and inappropriate staining; their causes, and methods for their reduction or elimination.

3.7.6. Has knowledge of the procedure for introducing a new primary antibody into clinical practice, including comparative costing of commercial reagents, in-house validation and evaluation, and use of appropriate control material.

3.7.7. Has knowledge of the appropriate storage requirements of antibodies and the significance of expiry dates.
3.7.8. Can demonstrate current knowledge of the diagnostic applications of primary antibodies, to include the use of panels in tumour pathology.

3.7.9. Has an appreciation of the clinical value of immunocytochemical findings, especially with regard to prognostic and predictive markers, in the treatment and management of the patient.

3.8. **Immunocytochemical staining methods**

3.8.1. Has an understanding of the general principles of immunocytochemical staining. Is familiar with, and understands the rationales behind all commonly used methodologies, to include: direct, indirect, avidin-biotin complex, labelled avidin-biotin and polymer-based.

3.8.2. Understands criteria for and is able to evaluate the appropriate use of the methods listed in 3.8.1.

3.8.3. Has knowledge and working experience of the selection of appropriate control material and understands the importance of using such material.

3.8.4. Has knowledge about the various immunocytochemical labels that are available, e.g. horse radish peroxidase, alkaline phosphatase.

3.8.5. Has knowledge about the various chromogens that are available, e.g. diaminobenzidine.

3.8.6. Understands the criteria for appropriate counter-staining and subsequent mounting.

3.8.7. Has an understanding of double-staining procedures.

3.8.8. Is aware of new developments in the field of immunocytochemistry.

3.9. **Automated immunocytochemistry**

3.9.1. Has an understanding of the general principles of automated immunocytochemistry, to include advantages and disadvantages.

3.9.2. Has specific and up-to-date knowledge of the various types of automated immunostainers available, to include flat-bed, capillary gap, closed and open systems. Is aware of potential future developments in the field.

3.9.3. Understands the use of bar coding to reduce errors.

3.9.4. Is aware of the use of image analysis systems for quantification of results.

3.9.5. Has an appreciation of the importance of choosing a cost effective automated system.
DECLARATION

I declare that I have satisfactorily completed the immunocytochemistry analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee) ........................................

Date ....................................................

I declare that ......................................... has satisfactorily completed the immunocytochemistry analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer) ........................................

Date ....................................................
POST ANALYSIS PHASE

3.10. Morphology

3.10.1. Has a knowledge of, and can identify the different types of specimen e.g. needle biopsy, resection, curettings and chippings, cell blocks, direct smears.

3.10.2. Understands the potential adverse effects of sub-optimal fixation and tissue processing on tissue and cell morphology.

3.10.3. Has knowledge of and can identify the microscopic appearance of normal and abnormal cells and tissue types.

3.10.4. Has an understanding of the patterns and localisation in normal and abnormal cells and tissue types.

DECLARATION

I declare that I have satisfactorily completed the morphology post-analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee) ...........................................

Date ...................................................

I declare that ............................................. has satisfactorily completed the morphology post-analysis phase module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer) ...........................................

Date .....................................................
3.11. Quality and audit

3.11.1. Has an understanding of the general principles of quality control, quality assessment and quality assurance.

3.11.2. Has knowledge of the procedures for day-to-day quality control, and can demonstrate expertise in the identification of appropriate and inappropriate staining; its causes and remedies.

3.11.3. Understands the importance of participation in external quality assessment schemes and their value in quality management.

3.11.4. Is able to audit their own and others work.

3.11.5. Understands the importance to clinical care of the timely provision of immunocytochemical findings.

DECLARATION

I declare that I have satisfactorily completed the quality, audit and management module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainee) .................................................

Date .............................................................

I declare that .................................................. has satisfactorily completed the quality, audit and management module for the Diploma of Expert Practice in Immunocytochemistry as required by the Institute of Biomedical Science

Signed (trainer) .................................................

Date .............................................................
Glossary of Principle Immunocytochemical Terms

**Affinity**: Measure of the binding strength between an antigenic epitope and its specific antibody combining site

**Antibody label**: The enzyme (usually peroxidase or alkaline phosphatase) or other marker moiety attached to the final layer that allows for visualisation of the antibody binding when viewed down the microscope.

**Antibody specificity**: specificity refers to the characteristics of an antibody to bind selectively to a single epitope on an antigen.

**Antigen retrieval**: in the context of the logbook this refers to all techniques that result in greater exposure of antigenic epitopes in formalin fixed tissues and cells. It most commonly comprises heat induced epitope retrieval (HIER) and proteolytic enzyme digestion.

**Audit**: A systematic documented review of an aspect of working practice with the objective of demonstrating any deficiencies or examples of good practice. Corrective action must be reviewed in a similar way.

**Automated immunostainers**:  
*Flat bed*: an automated system, in which the reagents are delivered with the slides in a horizontal position,  
*Capillary gap*: an automated system in which the reagents are delivered with the slides in a vertical position. A minute capillary gap is created between the test slide and a plastic clip or an additional glass slide, which when immersed in a shallow tray of reagent results in the reagents rising up the gap by capillary motion, thus bringing the reagent in contact with the test section on the slide.  
*A closed system*: each step in the automated staining method is pre-defined and cannot be modified.  
*An open system*: some or all of the steps in the automated staining can be modified.

**Avidin Biotin Techniques**: Typically three step procedures consisting of unlabelled primary antibody (first layer), a second layer consisting of affinity purified biotin labelled anti-Ig specific for the first layer and a third layer consisting of a preformed avidin-biotin labelled complex (the label usually being peroxidase or alkaline phosphatase).

**Avidity**: The functional combining strength of an antibody with its antigen which is related to both the affinity of the reaction between the epitopes and antibody combining sites and the valencies of the antibody and antigen.

**Chromogen**: The visible reaction product viewed under the microscope at the sight of antibody binding. For example the substrate of the peroxidase enzyme label (hyrodgen peroxide) oxidatively polymerises the colourless diaminobenzidine (DAB) chromogen to a brown precipitate at the site of the antibody binding.
Cluster of Differentiation (CD): The systematic characterisation and classification of human leucocyte antigens by a series of workshops originating from the 1980’s and continuing to this day (Bernard et al., 1984. Leucoyte typing I. Berlin: Springer-Verlag). The numbering system was extended in 1996 to include endothelial antigens. The cluster relates to a group of independently produced antibodies to have the same specificity as indicated by two or more antibody based techniques e.g. western blotting, immunocytochemistry, fluorescent activated cell sorting (FACS). This can be a guide to the potential use of antibodies in a diagnostic setting.

COSHH: Central of Substances Hazardous to Health. This is cross-referenced in laboratory SOPs.

Diagnosis: Identification of a specific disease process.

Direct technique: The label is conjugated directly to the primary antibody.

External Quality Assessment (EQA): The usefulness of IQC is dependent upon the limits of acceptability set by the laboratory concerned. The principal advantage of EQA is that it is able to detect differences of quality between laboratories and provide guidance on how to achieve the standards deemed to be more universally ‘acceptable’. External quality assessment is defined by the World Health Organisation and the European Committee for Clinical Laboratory Standards as a system of retrospectively and objectively comparing results from different laboratories by means of an external agency (WHO, 1981; Leblanc et al., 1985). Whilst IQC controls the precision of investigations, EQA provides an assessment of their accuracy with respect to other test sites. This is done periodically and retrospectively; hence, the term ‘assessment’ rather than ‘control’.

Heat induced epitope retrieval (HIER): The use of heating of slides immersed in a buffer (typically sodium citrate), usually at high temperatures in a microwave oven or a pressure cooker to expose antigenic epitopes prior to immunostaining.

Indirect technique: The primary antibody e.g. a mouse monoclonal, is unlabelled and detected by a secondary labelled antibody that is raised against the species of the primary antibody e.g. rabbit anti-mouse.

Internal Quality Control (ICQ): Defined as the set of procedures undertaken by the staff of a laboratory for the continual evaluation of the reliability of the work of the laboratory and its emergent results, in order to decide whether they are reliable enough to be released on a day-to-day basis. Most IQC procedures employ analysis of a control material and compare the result with predetermined limits of acceptability or tolerances.

Monoclonal antibodies: A monoclonal antibody is in principle the product of a single clone of immortalised B-cells. Consequently it is expected to be uniform in its molecular properties including specificity, affinity and avidity and can be produced in potentially as large an amount as necessary.

Peroxidase-anti-Peroxidase (PAP) technique: A three layer bridge method, in which the primary unlabelled antibody is applied, followed by a bridge antibody specific for the species of the primary antibody. The bridge or link antibody is applied in excess in order that only one arm binds to the primary unlabelled antibody. This then leaves its free arm
to bind to the Fc terminal of the third layer; a soluble preformed Peroxidase anti Peroxidase complex (PAP) comprised of antibodies of the same species as the primary antibody. The PAP complex consists of antibodies with their antibody binding sites bound to horseradish peroxidase, to which they have been raised.

**Polyclonal antisera:** Antibodies produced by immunizing an animal with purified antigen (immunogen). As these are the product of numerous different plasma cells (polyclonal) they will react to several epitopes on the antigen of interest and also may contain antibodies that recognise other antigens that will require removal by absorption or adsorption procedures.

**Polymer based techniques:** To date these have been available commercially as direct and indirect systems but with sensitivity reputedly to be equivalent to that of 3-layer ABC methods. The reagent consists of a large number of primary antibody molecules and peroxidase enzymes attached to a dextran polymer in the direct technique (DakoCytomation Enhanced Polymer One Step Staining [EPOS]). With the indirect method, the dextran polymer is attached to the second layer antibodies (DakoCytomation Envision system).

**Predictive markers:** Pathological tests that indicate the likely response of the disease to a specific type of therapy.

**Prognostic markers:** Pathological tests that indicate the likely short and long-term outcome for a patient with a particular disease.

**Quality assurance (QA):** All measures taken to ensure the reliability of investigations, starting from satisfactory test sample selection, analysing it appropriately, to recording the result accurately and reporting it to the clinician for appropriate action, with all procedures being documented for reference. Two of the main features of QA are Internal Quality Control and External Quality Assessment.

**Sensitivity:** The relative amount of antigen that an immunocytochemical technique is able to detect. A technique with high sensitivity will be able to detect smaller amounts of antigen than a technique with low sensitivity. Consequently, if used to detect the same amount of antigen, the technique with high sensitivity would produce a larger signal than a comparable method with lower sensitivity.