TRAINING LOGBOOK

FOR THE

DIPLOMA OF EXPERT PRACTICE IN IMMUNOCYTOCHEMISTRY

Institute of Biomedical Science
12 Coldbath Square
London
EC1R 5HL

Tel: 020 7713 0214 ext 142
Email: examinations@ibms.org

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INTRODUCTION

All biomedical scientists undergoing training in preparation for sitting the IBMS Diploma of Expert Practice in Immunocytochemistry must use this training logbook. It 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 hospital however an awareness of more specialised antibodies and the use of antibody panels is also essential.

Laboratories wishing to offer this training must be approved by the Institute for training and the laboratory manager must support the training of biomedical scientists in immunocytochemistry. Where a laboratory belongs to a single organisation, with laboratories on multiple sites, or is a member of a network, if there is a single training policy and procedure in place that has been submitted for training status approval, the overarching approval is acceptable for the individual member laboratories.

All laboratories wishing to participate in this training process must be United Kingdom Accreditation Service (UKAS) registered and have full accreditation or be actively seeking accreditation.

The final assessment of competence is based upon the submission of an evidence-based portfolio and the subsequent written examination. The successful completion of these requirements will be 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 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.

GUIDANCE TO CANDIDATES AND SUPERVISORS

Details about this qualification, such as eligibility criteria, aims and learning outcomes, portfolio of evidence, final examination as well as sample questions and an indicative reading list are available in discipline specific guidance to candidates. These documents can be obtained by contacting the Institute office or downloaded from the Institute's website, www.ibms.org.

USE OF THE TRAINING LOGBOOK

Theoretical knowledge and practical skills

Each aspect of preparation comprises of 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.

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.

Audit

Audit forms an integral part of the training process and on-going practice. The requirement for review of cases forms the basis of the continuing audit of biomedical scientist competence and performance and must be clearly demonstrated within the portfolio of evidence presented for assessment. Similarly, documentation of the internal quality process used for immunocytochemical tests should be included and there should be evidence to show participation in external quality assessment.

GUIDE TO COMMONLY USED ANTIBODIES

Epithelial markers
Broad Spectrum Cytokeratins
CAM 5.2

Urological and prostatic markers
Prostate Specific Antigen (PSA)
Prostatic Acid Phosphatase (PAP)
LP34
34 Beta E12
Cytokeratins 5 and 5/6
Cytokeratin 7
P504S
P63

Neuroendocrine markers
Chromogranin
Neurone Specific Enolase (NSE)
Synaptophysin
PGP9.5
CD56

Mesothelial/mesothelioma markers
Carcinoembryonic Antigen (CEA)
AUA-1
Ber-EP4
Cytokeratin 5/6
Calretinin
Thrombomodulin (CD141)

Melanoma markers
HMB45
Melan-A
SOX-10

Predictive Biomarkers
Oestrogen receptor (breast)
HER2 (breast and gastric)
ALK (lung and lymphoid)
PD-L1 (several cancers)
C-KIT (GIST cancers)
BRAF (several cancers)
Mismatch repair (Colorectal)

Lymphoid markers
T-cell markers e.g. CD3
B-cell markers e.g. CD20, CD79a
Leucocyte Common Antigen (CD45)
CD68
Kappa/lambda light chain Ig

Muscle markers
Smooth Muscle Actin (SMA)
Desmin
Myogenin

Endothelial markers
Von Willebrand (Factor VIII related antigen)
CD31
CD34
D2-40

Breast markers
Oestrogen receptor
Progesterone receptor
HER 2
Ki67 or equivalent

Miscellaneous
Thyroglobulin
Ki-67 or MIB 1
Human Chorionic Gonadotrophin (HCG)
Calcitonin
CD 56
Thyroid Transcription Factor-1 (TTF1)
WT1
CDX2
S-100
bcl-2
bcl-6

TRAINING MODULES

1. HEALTH AND SAFETY

1.1. General principles of health and safety

Knows and understands:

1.1.1. The safety responsibilities of employees under the Health and Safety at Work Act 1974, Control of Substances Hazardous to Health (COSHH), Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (RIDDOR) and any other relevant current safety legislation

1.1.2. Trust/Institution health and safety regulations

1.1.3. The departmental safety policy

1.1.4. The need to wear appropriate personal protective equipment (PPE)

1.1.5. The hazards associated with the use of equipment

1.1.6. Methods of dealing with spillage

1.1.7. Operation and use of ventilated work areas

1.2. Cell / tissue preparation, including section preparation

Knows and understands:

1.2.1. The universal precautions for handling specimens

1.2.2. The handling of unfixed cells and tissues

1.2.3. The need to disinfect and sterilise equipment after use

1.2.4. The requirements for clinical waste disposal

1.2.5. The local procedures for the disposal of high-risk specimens

1.2.6. Reagents used in fixation and tissue processing for paraffin embedding

1.2.7. Decalcifying agents

1.2.8. Reagents used in preparation of frozen specimens

1.2.9. Cryotomy and microtomy

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 DEP in Immunocytochemistry as required by the IBMS.

Signed (trainee) ……

Date ……

I declare that …… has satisfactorily completed the health and safety module for the DEP in Immunocytochemistry as required by the IBMS.

Name (trainer) ……

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 and processing for paraffin wax embedding

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. In relation to clinical governance knows and understands:

2.2.1. The clinical risk to the patient with regard to the mislabelling of specimens, slides and reagents

2.2.2. The importance of correctly dictated patient details and how transposition errors can impact on the suitability of the patient for ICC assessment and subsequent treatment

2.2.3. How to deal with inadequately or incorrectly labelled specimens and incomplete requests

2.2.4. Validation and verification of all reagents and antibodies

2.2.5. Acceptance testing of reagents used in immunocytochemical procedures

2.2.6. The principles and importance of maintaining patient confidentiality and consent

2.2.7. Clinical effectiveness in the timely production of test results

2.2.8. Clinical risk to patient with regard to equipment malfunction issues, sub-optimal reagent performance and appropriate reporting mechanisms and corrective actions

2.2.9. When specimens need referral to a more experienced biomedical scientist or other colleagues

2.2.10. The importance of on-going staff training and education and how this can be delivered

DECLARATION

I declare that I have satisfactorily completed the risk management/clinical risk module for the DEP in Immunocytochemistry as required by the IBMS.

Signed (trainee) ……

Date ……

I declare that …… has satisfactorily completed the risk management/clinical risk module for the DEP in Immunocytochemistry as required by the IBMS.

Name (trainer) ……

Signed (trainer) ……

Date ……

PRE-ANALYTICAL PHASE

3. IMMUNOCYTOCHEMISTRY METHODOLOGIES AND RELATED PROCEDURES

3.1. Fixation

3.1.1. Has an understanding of the general principles and underlying mechanisms of fixation of cells and tissues including the variables that can affect the optimisation of the fixing process

3.1.2. Understands the importance of adequate fixation for subsequent test procedures

3.1.3. Recognises the appearance of artefacts produced by incorrect fixation

3.1.4. Has 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 a knowledge and understanding of the variety of decalcification agents available, their characteristics, and the general principles of decalcification including factors affecting the process and practical aspects thereof

3.2.2. 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 and methods

3.3. Tissue and cell processing

Knows and understands:

3.3.1. The principles and methods of tissue processing for paraffin wax embedding and the factors affecting the process

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

3.3.3. Specific tissue processing schedules appropriate for use with different tissue/specimen types

3.3.4. The principles and methods of xylene free tissue processing and the effect on immunocytochemistry protocols

3.4. Fresh tissues, cells, frozen sections

3.4.1. Knows the 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. Understands the practice and problems associated with the production of frozen sections in relation to immunocytochemical preparations

3.4.3. Understands the practice and problems associated with the production of samples suitable for immunocytochemical staining from cytological specimens, including direct smears, cyto-centrifuge and liquid based preparations, fine needle aspirates (FNAs), clots and cell block

3.4.4. Understands the advantages and disadvantages of making imprints from fresh tissue

3.5. Paraffin sections and resin 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 and prioritise 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 pre-analytical phase module for the DEP in Immunocytochemistry as required by the IBMS.

Signed (trainee) ……

Date ……

I declare that …… has satisfactorily completed the pre-analytical phase module for the DEP in Immunocytochemistry as required by the IBMS.

Name (trainer) ……

Signed (trainer) ……

Date ……

ANALYTICAL PHASE

3.6. Antigen retrieval

3.6.1. Knows the importance of accurate and appropriate antigen retrieval. Is familiar with all commonly used methodologies including proteolytic enzyme digestion and heat mediated methods

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 and heat delivery systems including microwave ovens, pressure cookers and on-board, automated antigen retrieval techniques. Has knowledge of various antigen retrieval solutions

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

3.7. Primary antibodies

Knows and Understands:

3.7.1. Principles of primary antibody production and is aware of the relative advantages and disadvantages of polyclonal and monoclonal antibodies

3.7.2. Methods for characterisation, evaluation, validation and verification of primary antibodies and the requirement for assessment of batch to batch variation

3.7.3. The concepts of sensitivity, specificity, avidity and affinity and their impact on the quality of immunocytochemical staining

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

3.7.5. Problems of non-specific and inappropriate staining; their causes, and methods for their reduction or elimination

3.7.6. Procedure for introducing a new primary antibody into clinical practice, including comparative costing of commercial reagents, validation or verification of anti-sera and evaluation, and use of appropriate control material

3.7.7. Appropriate storage requirements of antibodies and the significance of expiry dates

3.7.8. Diagnostic applications of primary antibodies including the use of antibody panels in tumour pathology

3.7.9. Clinical value of immunocytochemical findings, especially with regard to prognostic and predictive markers, in the treatment and management of the patient and the value of immunocytochemical staining in companion diagnostics

3.8. Immunocytochemical staining methods

3.8.1. Has an understanding of the general principles of immunocytochemical staining and the rationale behind the common methodologies including: direct, indirect, avidin-biotin complex, labelled avidin-biotin, polymer-based and multiplex technologies

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. Understands the importance of incorporating negative controls into immunocytochemical runs and how this is practically undertaken

3.8.5. Knows and understands the various immunocytochemical labels that are available and their incorporation into immunocytochemical methods

3.8.6. Has knowledge about the various chromogens and substrate systems that are available, e.g. Diaminobenzidene (DAB), Fast Red

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

3.8.8. Has an understanding of double and multiplex staining procedures

3.8.9. Is aware of new developments in the field of immunocytochemistry including biomarkers, companion tests and the links to molecular testing

3.9. Automated immunocytochemistry

Knows and Understands:

3.9.1. The general principles, advantages and disadvantages of automated immunocytochemistry

3.9.2. The various types of automated immunostainers available including flat-bed, capillary gap, closed and open systems and has an appreciation of

liquid overlay and dynamic gap staining systems. Is aware of potential future developments in the field

3.9.3. The use and importance of accurate bar coding of slides and reagents to reduce errors

3.9.4. The importance of choosing an appropriate and cost effective automated system

DECLARATION

I declare that I have satisfactorily completed the analytical phase module for the DEP in Immunocytochemistry as required by the IBMS.

Signed (trainee) ……

Date ……

I declare that …… has satisfactorily completed the analytical phase module for the DEP in Immunocytochemistry as required by the IBMS.

Name (trainer) ……

Signed (trainer) ……

Date ……

POST ANALYTICAL 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

3.10.5. Understands the general principles behind and is aware of the use of image analysis systems for the quantification of results

3.10.6. Is aware of the tests that can benefit from image analysis systems and can explain the potential benefits of such systems

Quality and audit

3.10.7. Knows and understands the principles and procedures of clinical audit, quality control, quality assessment, quality assurance, incident reporting, risk assessment and route cause analysis

3.10.8. Can demonstrate expertise in the identification of appropriate and inappropriate staining; its causes and remedies

3.10.9. Understands the principles of how to validate a new immunocytochemistry test

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

3.10.11. Participates in the auditing of their own and others work against an agreed set of criteria

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

3.10.13. The mechanisms and methods of demonstrating reflection on the learning outcomes within own practice

DECLARATION

I declare that I have satisfactorily completed the post-analytical phase module for the DEP in Immunocytochemistry as required by the IBMS.

Signed (trainee) ……

Date ……

I declare that …… has satisfactorily completed the post-analytical module for the DEP in Immunocytochemistry as required by the IBMS.

Name (trainer) ……

Signed (trainer) ……

Date ……

Glossary of Common Immunocytochemical Terms

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

Antibody Label: A compound, usually an enzyme (fluorescent, peroxidase or alkaline phosphatase) attached to the final linking complex which reacts to allows for the visualisation of a final reaction product at the site of the antigen-antibody reaction.

Antibody Specificity: Characteristics of an antibody to bind selectively to a given antigen epitope.

Antigen Retrieval: Refers to the techniques that result in the controlled greater exposure of antigenic epitopes in formalin fixed tissues and cells. It can be achieved by heating for various periods of time (Heat Induced Epitope Retrieval (HIER)) or using enzyme digestion (Proteolytic Induced Epitope Retrieval (PIER)).

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.
Dynamic gap: Uses capillary forces to secure homogeneous spreading fo reagent throughout the staining area during reagent application.
A closed system: Each step in the automated staining method is pre-defined (locked) and cannot be modified. Such a system offers a higher degree of standardisation and flexibility in use of staff with varying skills sets.
A semi or open system: Some (semi system), or all (open system), of the steps in the automated staining can be modified by the user. An advantage of such systems is that they offer a high degree of flexibility.

Avidin Biotin Techniques: Typically, a three-step procedure 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.

Avidity: The functional combining strength of an antibody with its antigen which is directly related to both the affinity of the antibody-antigen interaction and the valency of the antibody and antigen.

Background: the presence of non-specific reaction observed at the conclusion of an immunocytochemical run; see also signal/noise ratio.

Biomarker: A diagnostic test which can be both predictive (see below) and/or prognostic (see below).

Chromogen: A compound that interacts with the antibody-antigen complex allowing the visualization of the final reaction product under the microscope at the site of the antibody-antigen interaction. For example, the substrate of the peroxidase enzyme label (hydrogen 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. The cluster number relates to different antibodies that have been found to have the same specificity as defined by at least two or more antibody-based techniques.

Companion Diagnostic: An in vitro diagnostic device that provides information for the selection and effective use of a corresponding therapy.

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

External Quality Assessment (EQA): The principal purpose 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 universally 'acceptable'. EQA provides an assessment, by means of an external agency, of the accuracy of investigations with respect to other test sites. This is done periodically and retrospectively; hence, the term 'assessment' rather than 'control'.

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. These detection methods may be multistep e.g., avidin biotin or labelled polymer methods.

Internal Quality Control (IQC): 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 controls and compare the result with predetermined limits of acceptability or tolerances.

Monoclonal Antibodies: This is the product of a single clone of chimaeric cells formed from an antibody-producing B-cells and a myeloma cell line. Consequently it is expected to be uniform in its molecular properties including specificity, affinity and avidity and can be produced in as large an amount as necessary.

Polyclonal Antibodies: These are produced by different antibody producing cells and therefore will vary in terms of how they recognise the various epitopes to which they are raised.

Predictive Markers: Pathological tests that indicate the likely response of the disease to a specific type of therapy (see also companion diagnostics).

Prognostic Markers: Pathological tests that indicate the likely short and long-term outcome for a patient with a particular disease (see also companion diagnostics).

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.

Sensitivity: The relative amount of antigen that an immunocytochemical technique is able to detect. A technique with high sensitivity will be able to detect lower amounts of antigen than a technique with low sensitivity. As a result, if used to detect the same amount of antigen, the technique with high sensitivity would produce a higher signal than a comparable method with lower sensitivity. A drop or loss in sensitivity can result in a false-negative immunocytochemistry results.

Signal/Noise Ratio: this defines the strength of the specific demonstration of an antigen against any non-specific background reaction present at the conclusion of an immunocytochemical method. Optimal results have a high signal/noise ratio.

Specificity: Ability of an immunohistochemical test to correctly identify a true negative result. Poor specificity can lead to false-positive immunocytochemistry results.