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***Approved By**: S. Carter, JS Charles*

Title: Primary Sample Manual - Histology

Changes made since previous version: *PAS, AB/PAS, PASD stain status changed from accredited to unaccredited. Accredited stains, antibodies and service are identified with * symbol. Updated TAT to 10 working days for routine specimens and up to 7 days for urgent specimens CMS and BUMA email address contacts updated*

***Note**: Please refer to the document record on QPulse for the revision history of this document.*

CONTENTS

INTRODUCTION

This document details the histology and cytology specimen groups processed at Eurofins Pathology, specimen requirements, turnaround times, specimen stability, and hazards.

Eurofins Pathology Address: 34 Three Rock Road, Sandyford Business Estate, D18A4C0.

Eurofins Pathology Operating Hours: Monday to Friday 0900 – 1730.

Eurofins Pathology Contact Details: **P**: 012958545 **F**: 012955399 **E**: labsecretary@ctie.eurofins.ie

For medical queries, please contact Dr. Susan Kennedy, Administrative Consultant Pathologist on 012958545

For technical and scientific queries, please contact Trinh Pham, Chief Medical Scientist on 0860841905 or 015077122 ext. 808 or ext.801 or email trinh.pham@ctie.eurofinseu.com

For business related queries, please contact Sabrina Carter, Business Unit Manager, on 0868084225 or email sabrina.carter@ctie.eurofinseu.com

Please note that this document is correct and up-to-date at the time of publishing. Eurofins Pathology is continuously updating its test repertoire, and some tests may be available even though they are not listed in this document or at the above link. If you cannot find details of a test you require, please contact the laboratory on labsecretary@ctie.eurofins.ie

While every effort is made to ensure that the turnaround times stated in this primary sample manual are adhered to at all times, they do remain a guideline as some samples may require longer fixation or extra testing.

To arrange a collection, please contact our Logistics Department (Eurofins Lablink) on free phone 1800 252 967, or e-mail lablinklogistics@ctie.eurofinseu.com

SAMPLE STABILITY

Histology specimens are stable at room temperature once immersed in 10% neutral buffered formalin in an adequately sized container.

Fresh cytology samples (without fixative) should be kept refrigerated at 4-6^{o}C and sent to the laboratory within 24hrs of collection. Cytology samples collected in CytoLyt (available on request) are stable at room temperature.

SPECIMEN COLLECTION, HANDLING, STORAGE

The collection of a histology or cytology specimen is performed by qualified medical personnel.

Specimens should be placed in an adequately sized specimen container with enough 10% neutral buffered formalin to completely immerse the specimen. This will allow for satisfactory fixation.

Only tissue or fluid should be submitted to the laboratory. The presence of surgical debris or instruments may lead to rejection of the specimen.

If a specimen requires an urgent report, please mark it urgent and where possible contact the laboratory in advance.

Eurofins Pathology does not process fresh tissue specimens

SPECIMEN TRANSPORT AND CHAIN OF CUSTODY

For information regarding the packaging requirements for histology specimens (biological substances category B, UN3373) please refer to https://www.eurofins.ie/eurofins-lablink/packaging-transportation-guidelines/

A chain of custody log must accompany any specimens sent to Eurofins Pathology. This form may be externally generated. The Eurofins Pathology Chain of Custody document HLF16 can be supplied to clients for use. The chain of custody form details each patient, the associated number of specimen containers, and the client signature.

SPECIMEN LABELLING

Preferably histology and cytology requests should be submitted using the most recent Eurofins Pathology test request form.

Test request form fields must be filled out correctly.

Hospital generated labels/stickers may be used so long as they (i) contain the same information as asked for in the test request form, (ii) are legible and easy to interpret, (iii) do not cover the sections designated for Eurofins Pathology use.

The request form and the sample container(s) must be labelled correctly with the following patient details:

Item	Minimum Required Details
Sample Container	Full name - Where possible a note should be made on the test request form if the patient has legally changed their name since their last visit. Date of Birth Medical Record Number / Hospital Number (where applicable) Specimen type / Site
Request Form	Full name - Where possible a note should be made on the test request form if the patient has legally changed their name since their last visit. Date of Birth Medical Record Number / Hospital Number (where applicable) Gender / Sex Patients address Insurer's details The name of the referring clinician Specimen type / Site Any relevant information regarding the specimen (e.g. orientation) Any relevant patient history

REJECTION OF SPECIMENS

Every effort will be made by Eurofins Pathology to process a specimen, however there may be situations where the specimen will be rejected by the laboratory.

1. Any request form or specimen container that is not labelled as per the specimen labelling section, will be rejected until remedied by the referring institution.
    - Evidence of this remedy must be in written format and must contain the details and signature (handwritten or electronic) of the person responsible for the amendment in the referring institution.

2. All specimens must be accompanied with an adequately completed request form, preferably the latest edition of the Eurofins Pathology request form.

3. Prepared FNA slides will be rejected if they are broken on receipt.

4. Cytology samples will be rejected if there is damage to the specimen container.

It may be necessary for Eurofins Pathology to return a specimen/ specimens to the originating institution for an error to be rectified.

TECHNICAL AND PROFESSIONAL SERVICES

Clients may submit pathology material into specific histology benches for technical work-up and/or reporting services. Please contact Sabrina Carter, Business Unit Manager, on 0868084225 or email sabrina.carter@eurofins-biomnis.ie

CRITICAL DIAGNOSES

Critical diagnoses arise in histopathology and cytopathology reporting where the diagnosis has immediate clinical consequences, or the diagnosis/findings are unexpected or discrepant, or where certain infections are identified. The Consultant Pathologist contacts the clinician via phone call in these scenarios.

TURNAROUND TIMES

The turnaround times listed below refer to a typical specimen requiring minimal to no further investigations outside of routine processing.

Further testing may include special staining, immunohistochemistry, molecular testing, or referral for expert opinion. Other factors such as fixation time and the requirement for decalcification may affect turnaround times. These factors will form part of the service level agreement with the client.

Accounting for the factors listed above, Eurofins aims to report 80% of cases within 7-10 working days.

HISTOLOGY SPECIMENS

Small biopsies: encompass needle tissue cores (prostate, breast, etc) and small surgical tissue pieces measuring less than 5mm.

Endoscopic biopsies: are widely used to diagnose and manage gastro- intestinal disease. They may be taken from the upper gastrointestinal tract (e.g. oesophageal and gastric biopsies) or the lower gastrointestinal tract (e.g. colonic or rectal biopsies). The average size of a GI biopsy ranges from 1-5mm.

Non-biopsy / Other: encompasses a wide range of specimen types. Commonly encountered specimens in this category are: skin excisions, curetting's, tonsils, gallbladders, appendices.

Malignant resections: are obtained by the therapeutic surgical removal of an entire diseased area or organ. These procedures are often intended as definitive surgical treatment of a disease in which the diagnosis is already known or strongly suspected. However, pathological analysis of these specimens is critically important in confirming the previous diagnosis, staging the extent of malignant disease, establishing whether or not the entire diseased area was removed, identifying the presence of unsuspected concurrent diseases, and providing information for postoperative treatment, such as adjuvant chemotherapy in the case of cancer.

Precautions:	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen. • Details regarding orientation must be described on the test requst form. • Details regarding prior treatment (such as chemotherapy, radiotherapy, surgery) must be described on the test request form.
Accredited	No
Method	Histology Processing. Diagnosis.
Specimen Requirements	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen.
Turnaround Time	10 working days routine specimens. 15 working days dermatology specimens. Up to 7 days for urgent specimens.
Stability	Specimen(s) are stable at room temperature once fixed in 10% neutral buffered formalin.
Hazards	Biological • Fixed and Unfixed Human Tissue and Fluid. • Local precautions should be followed. Chemical • Formalin • Local precautions should be followed.
Setup Schedule	Mon ✓ | Tue ✓ | Wed ✓ | Thu ✓ | Fri ✓

SPECIAL STAINS

Unlike routine H&E staining that is either progressive or regressive, special stains require different techniques that are based on simple chemical reactions such as acid-base chemistry and oxidation-reduction.

After a tissue specimen has been examined with Haematoxylin and Eosin, a special stain is applied to a sample for a more in–depth evaluation and allow target substances and foreign elements to be identified. This includes components in tissue sections, based on their: chemical, biological and pathological character for example; lipids, calcium, carbohydrates, nerve fibers and fungi to name a small few.

The advantage of special stains is that specific stains can be applied to detect the presence of tissue structures with the addition of a more detailed evaluation of a specimen, diving in deeper into the morphological profile. The stains also act as a confirmation of changes taking place to the tissue including microorganisms and/or specific tissue molecules that cannot be picked up within routine staining.

Precautions:	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen. • Details regarding orientation must be described on the test requst form. • Details regarding prior treatment (such as chemotherapy, radiotherapy, surgery) must be described on the test request form.
Accredited	No
Method	Manual
Special Stains	• Gram Stain • Ziehl Neelsen • PAS and PAS-D • Congo Red • Reticulin Stain • Perl's Prussian Blue • Alician Blue • Elastic Van Gieson • Masson Trichrome • Alician Blue/PAS
Specimen Requirements	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen.
Turnaround Time	+5 working days
Stability	Specimen(s) are stable at room temperature once fixed in 10% neutral buffered formalin.
Hazards	Biological • Fixed and Unfixed Human Tissue and Fluid. • Local precautions should be followed. Chemical • Formalin • Local precautions should be followed.
Setup Schedule	Mon ✓ Tue ✓ Wed ✓ Thu ✓ Fri ✓

IMMUNOHISTOCHEMISTRY (IHC) STAINS

IHC is used in histology to detect the presence of specific protein markers that can assist with accurate tumour classification and diagnosis. IHC has evolved to complement the Haematoxylin & Eosin (H&E) and Special Stain techniques that typically show tissue morphology (structure). Where H&E and Special Stains are non-specific, IHC is directed to a specific protein marker or markers. IHC is used as a diagnostic tool to assist in the diagnosis of solid tumours and cytological specimens and has been used as a mainstream diagnostic tool for almost half a century.

The Platform used in Eurofins Pathology is the BenchMark ULTRA Advanced Staining System which is intended to automatically stain histological or cytological specimens on microscope slides with specific immunohistochemistry or in situ hybridization reagents for in vitro diagnostic use. Evolved from the BenchMark series of instruments, the BenchMark ULTRA instrument fully automates the processes of baking, deparaffinization, and staining.

Precautions:	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen. • Details regarding orientation must be described on the test requst form. • Details regarding prior treatment (such as chemotherapy, radiotherapy, surgery) must be described on the test request form.
Accredited	No
Method	Automated
In-house IHCStains	• Anti-Pan Keratin (AE1/AE3/PCK26) • Basal Cell Cocktail • Pcl-2 (SP66) • BCL-6 • BRAF • Calretinin • CAM5.2 • CD10 (SP67) • CD117 (EP10) • CD-138 (B-A38) • CD15 (MMA) • CD20 (L26) • CD21 (EP3093) • CD23 (SP230 • CD3 (2VG6) • CD30 (Ber-H2) • CD31 (JC70) • CD34 (QBEnd/10) • CD4 (SP35) • CD45 (LCA) (RP2/18) • CD5 (SP19) • CD56 (MRQ-42) • CD68 (KP-1) • CD79a (SP18) • CD8 (SP57) • CDX-2 (EPR2764Y) • Chromogranin (LK2H10) • Cyclin D1 (SP4-R) • Cytokeratin 20 (SP33)

	• Cytokeratin 5/6 (D5/16B4) • Cytokeratin 7 (SP52) • Desmin (DE-R-11) • E-cadherin (36) • EMA (E29) • Ep-CAM (Ber-EP4) • ERG (EPR3864) • Oestrogen Receptor (SP1) • GATA3 • Helicobacter pylori (SP1) • Kappa • Ki-67 (30-9) • Lambda • MART-1 / Melan A (A103) • Melanosome (HMB45) • MUM1 (EP190) • Muscle Specific Actin • Myeloperoxidase • NKX3.1 • p16 (CINTECH) • P40 (BC28) • p504s/AMACR • p53 (DO-7) • p63 (4A4) • PAX8 (EP331) • PRAME • Progesterone Receptor (1E2) • PSA (polyclonal) • S100 (4C4.9) • SM Actin • Smooth Muscle Myosin • SOX-10 (SP267) • Synaptophysin (MRQ-40) • TTF-1 (SP141) • Vimentin • WT1 (6F-H2)
Specimen Requirements	• The specimen container(s) must be adequatedly labelled. • An adequately completed test request form must accompany the specimen.
Turnaround Time	+5 working days
Stability	Specimen(s) are stable at room temperature once fixed in 10% neutral buffered formalin.
Hazards	Biological • Fixed and Unfixed Human Tissue and Fluid. • Local precautions should be followed. Chemical • Formalin • Local precautions should be followed.
Setup Schedule	<table><tr><td>Mon</td><td>Tue</td><td>Wed</td><td>Thu</td><td>Fri</td></tr><tr><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td></tr></table>

CYTOLOGY SPECIMENS

A range of fluid types can be collected from the body including urine, cyst fluid, pleural fluid, and ascetic fluid. Cytology specimens can be sent to the laboratory in conjunction with a histopathology specimen or in isolation. Collecting a cytology specimen can be less invasive than sampling for a histopathology specimen, but there are associated limits in the diagnostic value of a cytology specimen.

Fine needle aspiration of mass lesions is commonly utilized in the detection and characterization of a variety of malignant diseases. Obtaining an adequate specimen requires attention to good aspiration technique as well as processing of material obtained. It is highly desirable that several direct smears are prepared (preferably air-dried) for all fine needle aspiration specimens submitted to the laboratory.

Precautions:	• The cytology specimen should be transported to the lab as soon as possible after collection, unless placed in CytoRich Red Collection Fluid or ThinPrep CytoLyt.
Accredited	No
Method	Cytology Processing. Diagnosis.

Specimen Requirements	• The specimen container(s) must be adequatedly labelled.
	• An adequately completed test request form must accompany the specimen.
	• Fluid should be freshly collected and placed into:
	  ○ a container with an equal volume of CytoRich Red Collection Fluid, or
	  ○ an empty sterile container and kept refrigerated
	• Up to 20mls of the fluid is an adequate volume for testing.
	• The aspirated material should be spread onto a glass slides and air dried or fixed (fixative available from the lab)
	• If a needle rinse is available, it should be collected into a container with CytoRich Red Collection Fluid.
	• All slides must be labelled with patient name and date of birth written using pencil on the frosted end of the slide.

Turnaround Time	10 working days
Stability	If transport of the specimen will be delayed for more than 24 hours, the cytofix should be added at collection.The specimen is stable for 48 hours if stored at 4 degrees .
Hazards	Biological • Fixed and Unfixed Human Fluid. • Local precautions should be followed. Chemical • CytoRich Red Collection Fluid • ThinPrep CytoLyt • Local precautions should be followed.
Setup Schedule	<table><tr><td>Mon</td><td>Tue</td><td>Wed</td><td>Thu</td><td>Fri</td></tr><tr><td>✓</td><td>✓</td><td>✓</td><td>✓</td><td>✓</td></tr></table>

TECHNICAL AND PROFESSIONAL HISTOLOGY SERVICES	
Clients may submit tissue blocks for microtomy and/or staining only to Eurofins Pathology. Clients may submit unstained slides for staining only. Clients may submit pathology material for Consultant Services. In some instances, routine histology technical work up may also be carried out in Eurofins Pathology prior to submission to a Consultant Pathologist.	

Precautions:	**Unstained Slides:**
	• Sections must be mounted on a glass slide suitable for the tissue type and test required.
	• Sections must be mounted on the slide such that there is sufficient space for a control section at the foot of said slide if needed.
	• Slides must be clearly labelled in pencil.
	• Slides must **not** be heated / baked prior to sending
	• Where possible an extra unstained slide should be submitted on each case.
	
	Stained Slides:
	• Slides should be clearly labelled, stained, and coverslipped.
	• Slides should be accompanied by:
	  ○ the case report when submitting slides for second opinion
	  ○ the gross report and test request form when primary reporting is required.
	
	Paraffin Blocks (FFPE)
	• An adequate amount of tissue should be present within the block to account for alignment and cutting on Eurofins Pathology microtomes.

Accredited	No
Method	Histology Processing. Diagnosis.
Specimen Requirements	*Any unstained slide cases for staining **only** must be accompanied by • HLF64 Blocks and Slides Chain of Custody Log or, an approved local version • Clear identification of case number on slide *Any block cases for microtomy and staining **only** must be accompanied by • HLF64 Blocks and Slides Chain of Custody Log or, an approved local version • Clear identification of case number on block *Any block cases for microtomy, staining and reporting should be accompanied by • HLF64 Blocks and Slides Chain of Custody Log or, an approved local version, and a Test Request Form with gross description • Previous history where available Any slide cases for reporting only should be accompanied by the following: • HLF16 Chain of Custody Log or an approved local version • A copy of gross report and test request form • Previous history where available • Clear identification of case number on slide
Turnaround Time	As per service level agreement with client.

TECHNICAL AND PROFESSIONAL HISTOLOGY SERVICES	
Stability	Slides, and specimen tissue blocks are both stable at room temperature. They should be kept out of direct sunlight. Slides should be securely placed and sealed in a slide mailer or slide tray. Specimen tissue blocks should be wrapped in a piece of tissue.
Hazards	Biological • Fixed Human Tissue. • Local precautions should be followed. Chemical • Formalin fixed tissue • Local precautions should be followed.
Setup Schedule	Mon ✓ Tue ✓ Wed ✓ Thu ✓ Fri ✓