Saolta
Grúpa Ollscoile Cúram Sláinte
University Health Care Group

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

Letterkenny University Hospital

HISTOPATHOLOGY USER MANUAL

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GUIDE TO USING THIS MANUAL

This User Manual has been prepared in conjunction with The Pathology Department User Manual (MP-GEN-0064) to inform the users of the Saolta University Health Care Group, Letterkenny University Hospital, Pathology Department of which services are available within the Pathology Department and how to obtain the services required.

PLEASE REFER TO DOCUMENT MP-GEN-0064, THE PATHOLOGY DEPARTMENT GENERAL USER MANUAL FOR GUIDANCE ON USING THESE DOCUMENTS.

Documents are available on Q-Pulse and also on the HSE Website **http://www.hse.ie/lulhPathology**

Change Description:

Section	Amendment
2.2	Added MLA
3.4	Updated phone numbers
3.5.3	Updated table
3.7	Added Section on Additional Testing
3.8	Added Section on Samples not examined
6.2	Added detail on completing request form when have multiple samples
7	Amalgamated into Section 8
7.5	Update Table 7 for multiple samples
7.7	Added section on FNA labelling criteria
8	Renamed 'High Risk Samples'
9	Updated
10	Reworded packaging information
11	Removed laboratory store as storage location for request forms and reports; retention time for non-gynae cytology reduced to 7 days post sign-out
12	Removed TAT for ISH Testing; updated table

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1 INTRODUCTION

1.1 Scope and purpose

This manual, in association with the Pathology Department General Information User Manual (MP-GEN-0064), is designed to provide users of the Histopathology Service at Letterkenny University Hospital with information on the proper collection and handling of primary samples destined for examination in this laboratory or referral for specialist reporting elsewhere. The Histopathology Department is concerned with the diagnosis of abnormalities within tissues and cells by microscopic examination. It is used both by hospital clinicians and General Practitioners. The Histopathology Department is an INAB-accredited laboratory (ISO15189:2012; registration number 210MT).

Please note that this manual is intended for use as a guide only; should you require any further information or clarification, please contact the relevant section of the Histopathology Department prior to submission of the sample (for contact details, please refer to the General Section of the User Manual (MP-GEN-0064, Section 6).

1.2 Services offered

The Histopathology Laboratory at Letterkenny University Hospital offers the following services to users:

- Routine histological examination of biopsy and resection specimens
- Frozen Section service if an urgent, intra-operative diagnosis is required
- Routine cytological examination of body fluids (**including urine* but excluding cervical cytology**)
- Preparation of slides (by request) and reporting of Fine Needle Aspirate (FNA) procedures (including Transbronchial FNA)
- Forwarding of muscle, nerve and renal biopsy samples for specialist histological examination and reporting
- Forwarding of samples for Molecular Testing, including Oncotype DX breast cancer assay testing

**Following discussion with the Consultant Urologist LUH, requests for urine cytology from Primary Care sources are considered inappropriate in isolation. If there is a clinical concern in relation to urological symptoms/signs, the patient should be referred for a formal urological assessment.*

1.3 Responsibility

The Chief Medical Scientist in charge of Histopathology is responsible for ensuring the implementation and maintenance of this procedure in conjunction with the Consultant Histopathologists.

2 GENERAL INFORMATION

2.1 Medical Advisory Service

Due to the difficulty in obtaining and often the unrepeatable nature of Histopathology/Cytology samples, users are strongly encouraged to contact an appropriate member of the Histopathology staff for advice and guidance, prior to taking the sample, if in any doubt as to the most appropriate sampling method to use.

2.2 Histopathology Staffing

The Histopathology Laboratory is staffed by:

Consultant Pathologists
Chief Medical Scientist
Senior Medical Scientist
Staff Grade Medical Scientists
Medical Laboratory Aides (MLA)
Departmental Secretaries

2.3 Contact Details

Please refer to General Section of the User manual (MP-GEN-0064), Section 6.

3 PATHOLOGY SERVICES AVAILABLE

3.1 Routine Histopathology

Routine histopathological examination is performed on all biopsy samples including core biopsies, endoscopic biopsies, small resection specimens and bone marrow biopsies. It is also carried out on surgical resections removed in theatre.

3.2 Frozen Section

Frozen sections should only be requested if the immediate management of the patient is likely to be altered as a result. Requests for frozen section **MUST** always be discussed with a Consultant Pathologist. Planned frozen sections should be pre-booked with a Consultant Pathologist at least 24hrs in advance, to ensure the availability of medical and technical staff. In cases where an unplanned frozen section is required, as much warning as possible should be given to the laboratory as the equipment used requires appropriate preparation.

This service is only available between the hours of 09.00 to 16.30hrs Monday to Friday.

If the operation is delayed, or if it is subsequently found that the frozen section is not required, please notify the Histopathology Department without delay.

Hazard category 3 cases, e.g. high TB risk, known HIV, Hepatitis B or C, Covid 19 are contraindications to frozen section. If such a specimen is inadvertently processed, full decontamination of the equipment used will be required, and during this time no further frozen sections can be performed for at least 24hrs. Such instances will be recorded as clinical incidents.

3.3 Routine Non-gynae Cytology

Routine cytopathological examination is performed on all fluids (inc. urine), sputum and bronchial washings/brushings/lavages/aspirates (inc. EBUS). Any fluid aspirated/drained for cytopathological examination should **NOT** be placed in 10% formalin to allow for additional tests to be performed in the laboratory.

All fresh (unfixed) fluids **MUST** be delivered to the laboratory within the hours of 08:00 to 16:30 Monday to Friday, **AND** as promptly as possible after sample collection, to ensure cell viablity. If immediate transfer is not possible, samples should be refrigerated at 4-8^{o}C to preserve cells. Unrefrigerated cases >24hrs old, or refrigerated samples >72hrs, will not be processed.
Please note that all requests for gynaecological cytology are now handled through the National Cervical Screening Program.

3.4 Fine Needle Aspiration (FNA)

Consultant Pathologists will perform Fine Needle Aspiration (FNA) procedures **ONLY** by prior arrangement. (Please contact the department on 074 91 23579 or 074 91 24468 to book an FNA appointment).

3.5 Histological/Cytological samples requiring specialist reporting

Some histological/cytological samples received by the Histopathology Department LUH are forwarded to specialist centres for examination and reporting.

3.5.1 Renal Biopsy

Renal biopsies performed for medical diagnosis are examined and reported by the Pathology Department, Beaumont Hospital, Dublin. **The Histopathology Department LUH must be notified 5-7 days in advance of a renal biopsy for medical diagnosis being taken**. (This is to allow time to source the necessary transport medium). Biopsies from renal tumours are processed at Letterkenny University Hospital.

3.5.2 Muscle/Nerve Biopsy

Muscle/nerve biopsies for diagnosis of Neurodegenerative disorders and metabolic studies are examined and reported by the Neuropathology Department at Beaumont Hospital, Dublin.

3.5.3 Molecular Testing

Molecular Testing of archived Histopathology blocks are prepared in the Histopathology Department and sent to the relevant facility for analysis. Requests for the following molecular tests can be facilitated:

Molecular Test			**Testing Location**
BRCA *(for PARP Inhibitor Selection)*			Beaumont/SJH Hospital
c-MYC			Tallaght Hospital
Colorectal Mutation Panel *(KRAS, NRAS, BRAF, NTRK-1/2/3)*			Beaumont Hospital
EGFR *(T790M Mutation)*			Cancer Molecular Diagnostics, SJH
FoundationOne CDx *(324 genetic alterations)*			Foundation Medicine Inc. (part of the Roche group), Germany
GIST Mutation Panel *(KIT, PDGFRA, BRAF V600)*			Cancer Molecular Diagnostics, SJH
Her2 ISH			Source Bioscience, UK Poundbury Cancer Institute, UK
Lung Mutation Panel *(EGFR, BRAF, KRAS, ERBB2, PIK3CA, ALK, ROS1, RET, MET, NTRK-1/2/3)*			Beaumont Hospital
Melanoma Mutation Panel *(BRAF, NRAS, KIT, NTRK-1/2/3)*			Beaumont Hospital
Mis-match Repair Analysis *(by IHC)*			On-site in LUH
Microsatellite Instability *(by PCR)*			Beaumont Hospital
MLH1 Methylation			Beaumont Hospital
Oncoytpe DX			Genomic Health Inc., USA
PD-L1 *(tumour and treatment dependent)*			See below
Tumour	***Proposed Treatment***	***PD-L1 Clone***	
Breast (TNBC)	*Atezolizumab*	*SP142*	Galway University Hospital
	Pembrolizumab	*22C3*	Poundbury Cancer Institute
Bladder	*Atezolizumab*	*SP142*	Poundbury Cancer Institute
	Pembrolizumab	*22C3*	St Vincent's University Hospital
	Nivolumab	*28.8*	Poundbury Cancer Institute
Lymphoma	*Atezolizumab*	*SP142*	Poundbury Cancer Institute
Lung	*Atezolizumab*	*SP263*	Beaumont Hospital
Melanoma	*Atezolizumab*	*SP263*	Beaumont Hospital
	Nivolumab	*28.8*	Poundbury Cancer Institute
Upper GI	*Pembrolizumab*	*22C3*	Poundbury Cancer Institute
	Nivolumab	*28.8*	Poundbury Cancer Institute
Gynae	*Pembrolizumab*	*22C3*	St Vincent's University Hospital
Head and Neck	*Pembrolizumab*	*22C3*	Poundbury Cancer Institute
TCR Gene Rearrangement			Cancer Molecular Diagnostics, SJH

(Table 1)

3.6 TSE patients

Tissue samples from patients with known or suspected new variant Creutzfeldt–Jakob disease (nvCJD), or any other Transmissible Spongiform Encephalopathy (TSE), **MUST NOT BE SENT TO THE HISTOPATHOLOGY LABORATORY AT LETTERKENNY UNIVERSITY HOSPITAL UNDER ANY CIRCUMSTANCES**. The clinical team **MUST** liaise directly with the Neuropathology Department at Beaumont Hospital, Dublin, who have the necessary facilities and expertise for dealing with such cases. They can be contacted directly on 01-8092633.

3.7 Additional Testing

Requests for specific/additional testing of already-submitted samples must be made within a certain time-limit (see Table 2 below). A Consultant Pathologist must approve all such requests. Contact the department to arrange.

Sample Type	Time Limit
Histopathology	• Tissue (all embedded): no limit • Tissue (not all embedded): < 28 days after report signed out
Non-Gynae Cytology	• Unrefrigerated: must be < 24hrs old Refrigerated (unfixed): must be < 72hrs old • Refrigerated (fixed): < 7 days after report signed out
Renal Biopsy	• Contact the Pathology Department, Beaumont Hospital for advice Direct Dial: 01 809 2353 or 01 809 2634 Beaumont Ext. No.: 2353 or 2634
Muscle/Nerve Biopsy	• Contact the Neuropathology Department, Beaumont Hospital for advice Direct Dial: 01 8092633
Molecular Testing	• No limit

(Table 2)

3.8 Samples not examined

In the following incidences, a report will be issued with the prescibed wording:

Sample submitted	Report wording
Cytology request on correct form BUT with no separate sample	No cytology sample received.

Cytology request on correct form BUT with inappropriate sample type	An inappropriate sample type was submitted for cytological examination. Consequently, it has not been processed and no result is available.
Urine from Primary Care	Following discussion with the Consultant Urologist LUH, requests for urine cytology from Primary Care sources are considered inappropriate in isolation. If there is a clinical concern in relation to urological symptoms/signs, the patient should be referred for a formal urological assessment.
Urine in inappropriate container	As per previous communication, urine samples for cytolgy must be collected into universal containers. Samples received in urine tubes have insufficient volume and therefore cannot be processed.
Placenta	In line with established Histopathology Specimen Acceptance Criteria, as detailed in LP-Histo-0088, this specimen has been deemed inappropriate for histopathological examination.
CSF	This specimen has been deemed inappropriate for cytological examination based on the clinical information provided.
Breast aspirates NOT performed by the Breast Care team	As per Consultant Pathologist.
Urine for fat emboli	As per Consultant Pathologist.
Sputa for PCP	As per Consultant Pathologist.

(*Table 3*)

4 TURNAROUND TIMES

Turnaround time is defined as the number of working days from the date that the specimen is receipted in the Histopathology laboratory until the time an authorised report is available. The time taken for a result to be available depends on the type, size and complexity of the specimen.

4.1.1 Small biopsy specimens (e.g. endoscopic biopsy, core biopsy)

The majority (>80%) will be reported within 5 working days of receipt.

4.1.2 Large and unfixed specimens (e.g. surgical resections)

These will require additional fixation. The majority (>80%) will be reported within 7 working days of receipt.

4.1.3 Non-gynae Cytology specimens

The majority (>80%) will be reported within 5 working days of receipt.

4.1.4 Urgent Samples

Specimens requiring urgent reporting must be discussed with the Consultant Pathologist covering "cut-up", ideally **PRIOR** to submitting the specimen to the lab. Urgent requests **must** be made by the Consultant in charge of the patient. A case will not be dealt with as urgent if direct contact with a Consultant Pathologist has not been made.

The request for urgent analysis must be used appropriately. Abuse of the urgent request facility will have an adverse effect on the turnaround times of genuinely urgent requests.

4.1.5 Exceptions

In the following situations the final report may be delayed. In such cases, an interim report **may** be issued, or the Consultant Pathologist may discuss the case with the referring doctor:

- Specimens where the tissue requires decalcification e.g. bone
- Specimens requiring lengthy and involved procedures, particularly those requiring immunohistochemistry or special stains
- Specimens where the pathologist feels a second opinion may be of benefit to the patient
- High risk specimens (Category 3)

5 ISSUING OF RESULTS

The Department of Histopathology, Letterkenny University Hospital adheres with ISO 15189 to give assurance to users and to safeguard the quality of the results obtained. The Histopathology Department is an INAB-accredited laboratory (ISO15189:2012; registration number 210MT).

Please refer to the **General Information User Guide, MP-GEN-0064, Section 14.4 which describes the procedure in place for the reporting of Histopathology results.** This manual is available on Q-Pulse and the HSE website **http://www.hse.ie/luhPathology**

For all enquiries relating to the reporting of Histopathology/Cytology requests, please contact the Pathology Secretaries (Please refer to General User Manual MP-GEN-0064, Section 6 for contact details).

6 LABORATORY REQUEST FORM

6.1 General Information

All requests for Histological/Cytological examination must be made using the correct version of the blue, controlled Histopathology/Cytology request card (MF-0370). Supplies of these forms are available from Pathology Reception. The General Laboratory request form **MUST NOT** be used.

All requests for Urine Cytology MUST be accompanied by a fully-completed Urine Cytology Accompanying Request Form (available from the Urology Department).

Requests made on unapproved forms will not be processed.

Histopathology do NOT accept verbal requests for examinations.

The specimen type and anatomical site are particularly important in Histopathology where specimens may be multipart or left or right etc. Failure to submit essential information will result in a delay in specimen processing pending amendments being made to request forms or specimens. This may cause unnecessary delays in issuing reports.

6.2 Completing the Request Form

Note: Failure to provide the minimum data required on the Histopathology/Cytology Request form will result in a delay in processing the sample.

The following ESSENTIAL information MUST be documented, in a legible manner, on the request form. An addressograph label if available, should ideally be attached. The request form SHOULD also contain the following DESIRABLE information.

When more than one sample/specimen pot is being submitted for the same patient at the same time, the details (inc. side) of each one must be clearly stated under 'Specimen Nature/Site' i.e. A. denotes first specimen pot, B denotes second specimen pot, etc.

Request Form Essential Criteria	**Histopathology** **Essential Labelling Requirement:**
	Blue request form (RF) (active revision)
	Hospital patients Three unique identifiers: **1. Patient's PCN** **2. Forename & Surname** **3. Date of Birth** Addressographs preferred (hand-written: must be legible) Match sample labelling Match details held on LIS exactly
	GP patients Three unique identifiers: 1. **Forename & Surname** **2. Date of Birth** plus one of the following: **3. Patient's PCN** **4. Patient's Address** Addressographs preferred (hand-written: must be legible) Match sample labelling Match details held on LIS exactly
	Ward/Practice Location
	Patient Consultant/GP
	MCRN or name of requesting doctor/nurse
	Signature of Requester
	Date of Request
	Specimen Nature/Site inc. side for each sample
Desirable	**Clinical Details** *Relevant clinical details should be provided; absence/inadequacy of clinical information may be noted in final report*
	Patient Gender
	If in doubt - ask!

(Table 4)

6.3 Responsibility

Responsibility for full and correct completion of the Histopathology/Cytology request form lies solely with the requesting clinician (Hospital or GP based). Incomplete, or incorrectly completed, request forms will not be accepted under any circumstances and will not be processed until the discrepancy is amended. This, in turn, will cause a delay in sample reporting. In the event that the eligibility status of the patient is not clearly identifiable from

the data available/provided, it may be necessary to seek additional information from the referring Clinician prior to accepting the specimen for processing/reporting.

7 SAMPLE COLLECTION

7.1 Health & safety advice

7.1.1 General

- Treat all biological samples as potentially-infectious and wear proper protective equipment
- Ensure that all lids are securely attached to specimen containers to minimise spillages
- Dispose of excess cytological fluid in a safe and responsible manner
- Clean up all spillages immediately and disinfect the area

7.1.2 Formalin/Formaldehyde

- Avoid skin contact as sensitisation may occur
- Always wear proper protective equipment when handling as there is a possible risk of irreversible effects
- Avoid breathing in fumes
- Ensure container is always properly labelled with formalin hazard labels

7.2 Medical indications and appropriate selection of available procedures

Certain clinical indicators will dictate the most appropriate sampling method to use when submitting tissue/fluid for histological/cytological examination. Where any doubt exists, it is advisable to contact one of the Consultant Pathologists prior to performing the procedure, who will be pleased to offer help and advice.

7.2.1 Histopathology

Sample	Clinical indicator	Appropriate action/recommendation	Reason
Skin lesion	Pigmented	Punch biopsy NOT recommended; excise in full	Risk of sampling error
Lymph Node	? lymphoma	Core biopsy/FNA of lymph node NOT recommended	Excision-biopsy of an entire lymph node is investigation of choice

Any	? malignant	Biopsy specimen recommended over cytology sample	Immunocytochemistry difficult to perform on cytology samples and often gives unsatisfactory results

(Table 5)

7.2.2 Cytology

Sample	Clinical indicator	Appropriate action/recommendation	Reason
CSF		Routine cytological examination of CSF is inappropriate. Discuss individual case with Consultant Pathologist before taking sample	Minimal cellular yield
Breast Cyst Fluid aspirate		Do NOT submit fluid aspirated from breast cysts for cytology; refer to Breast Care North West	As per National Cancer Control Programme (NCCP) guidelines
Urine		SECOND urine sample of the day (from the beginning of the stream) collected into a sterile, white-top bottle; sample reaches Pathology Department without delay	First morning sample less suitable than other times as cells in the low pH and hypertonic environment undergo degenerative changes making cytologic assessment difficult; forward without delay to preserve cells

(Table 6)

7.3 General information

All samples sent for histological/cytological examination **MUST** be submitted to the laboratory in the appropriate manner. Inappropriately fixed/submitted specimens have an effect on downstream patient testing and treatment decisions, therefore it is imperative that the importance of prompt and adequate tissue fixation and submission to the laboratory are not overlooked.

7.3.1 Routine Histopathology

All specimens for routine histological examination **MUST** be collected into containers of 10% neutral-buffered formalin solution. There **MUST** be sufficient formalin in the container to completely cover the specimen. The ideal ratio is 5:1 (formalin:specimen) for large samples (e.g. breast tissue, large portion of GI tract, hysterectomy, ovarian cyst, placenta) and 10:1 for small (biopsy) samples (generally any tissue smaller than a 'normal' gallbladder).

No container should be filled more than three-quarters full on health and safety grounds; select a container large enough to accommodate the tissue specimen and fixative.

Large surgical resection specimens should **NOT** be sliced or opened by the surgeon, but sent directly to the laboratory without delay.

Specimens **MUST NOT** be placed into any other solution, or into a dry container (except frozen section requests), as irreversible deterioration of the specimen will take place, making accurate microscopic interpretation impossible.

7.3.2 Frozen section

Specimens for frozen section **MUST** be placed in a suitably-sized **DRY** specimen container and transferred immediately to the Histopathology Department by hand.

Tissue for frozen section must be handed directly to a Medical Scientist or Consultant Pathologist.

7.3.3 Routine Non-Gynae Cytology

A separate sample, specifically for cytological analysis, **MUST** be submitted with the approved request form.

Requests for Cytology made on the General Laboratory request form, or without a separate sample for cytology, will not be processed.

The method of submission of non-gynae cytology samples depends on the sample type. These samples **must be forwarded to the laboratory without delay (less than 24 hours); if a delay cannot be avoided, the sample may still be accepted if kept refrigerated, up to a maximum of 72 hours.**

The main sample types are detailed below:

- **Pleural, Peritoneal and Cyst Fluids:** Up to a maximum of 25ml of fresh fluid should be sent to the laboratory in a white topped, sterile, universal container.

Drainage bags of fluid MUST NOT be sent to the laboratory. Transport of such bags poses a serious infection risk to staff and public within the hospital.

- **Sputum:** Use a white topped, sterile, universal container to collect the first deep cough specimen produced in the morning, or a sample taken after physiotherapy. The sample should be collected before breakfast or teeth cleaning. Samples comprising predominantly saliva are inadequate for cytological investigation of the lower respiratory tract. Sputum cytology should not be used as a screening investigation. It should be limited to individuals in whom a histological or cytological diagnosis is desired but in whom bronchoscopy is inappropriate or unsuccessful. Ideally, the test should be carried out on three consecutive days, with each specimen being delivered to the lab as soon as it is taken. For other suspected pathology, discuss with a Consultant Pathologist in advance.

- **Bronchial Brushings**: Collect brushings and brush tip into a 30ml Cytolyt® centrifuge tube (available in the Endoscopy Unit).

- **Bronchial Washings:** Collect into a 30ml Cytolyt® collection cup (available in the Endoscopy Unit).

- **Bronchoalveolar Lavage (BAL):** Collect into sterile, screw-top container. Do NOT add any fixative/preservative.

- **TBNA (inc. EBUS):** Collect into a 30ml Cytolyt® centrifuge tube (available in the Endoscopy Unit).

- **FNA (excluding TBNA):** Consultant Pathologists prepare FNA slides at the clinic or in the radiology department by prior arrangement, and return the sample(s) to the Histopathology laboratory for processing. Wash needle into sterile

white-topped universal containing Cytolyt® solution (obtain from Histopathology laboratory).

- **Urine:** 20ml of urine in a white topped, sterile, universal container will be sufficient. For best cell yields the sample should be collected at the beginning or end of voiding urine – SECOND sample of day. It is extremely important that no alcohol or any other fixative is added to the specimen.
Note: Sample and request form MUST be accompained by a fully-completed Urine Cytology Accompanying Request Form (available from the Urology Department).

7.3.4 Muscle Biopsy

Muscle biopsy procedures **MUST** be performed as early in the day as possible to ensure that the sample is delivered to the Histopathology Laboratory LGH by 11am sharp the same day. This is to facilitate the specialist reporting centre in Dublin, who require that the biopsy arrives with them no later than 4pm on the same day. The specimen should ideally measure 15x10x10mm and be wrapped in saline dampened gauze before being placed into an empty white topped Universal container**. Do not over soak the gauze as this causes problems with ice crystal artefact formation during subsequent processing.** Make sure the specimen is reasonably loose within the container. Take care not to squash the specimen**. Do not send the sample on ice**.

Please refer to Appendix A for ward instructions on the collection of muscle biopsies.

7.3.5 Renal Biopsy (for medical diagnosis)

Two specimens should be taken: one into 10% buffered formalin and the other into Zeus fluid (transport medium). Both fixatives are available for collection by prior arrangement (5-7 days notice required) from the Histopathology Department at Letterkenny University Hospital. Renal biopsies should be received in the Histopathology Department before 11am to ensure next-day delivery to the specialist reporting centre via the laboratory courier service. The Histopathology Laboratory **MUST** be informed if a sample is clinically urgent so that it can be sent by taxi the same day, and the specialist centre informed.

7.4 Summary of Appropriate Sample Collection Methods

All requests, and the appropriate submission method for each, are summarised in Table 7 below:

Request	Specimen example	Appropriate submission state
Routine Histopathology	Endoscopic/core biopsy Surgical resection Bone marrow biopsy	10% Formalin (4% Formaldehyde)*
Frozen Section	Breast Colon	Dry: hand-delivered direct to lab immediately
Routine Non-gynae Cytology	Pleural fluid Sputum	Max. 25ml fresh (i.e. unfixed) in white-topped, sterile, universal container: delivered to lab without delay
	Bronchial Brushing/Washings/TBNA (inc. EBUS)	Collect into centrifuge tube/collection cup containing 30ml Cytolyt® **
	Bronchoalveolar Lavage	Collect into sterile, screw-top container. Do NOT add fixative/preservative.
	Urine	20ml Fresh (beginning or end of **second** morning voiding) in white-topped, sterile, universal container: delivered to lab without delay with Urine Cytology Accompanying Request Form
	FNA: Thyroid Lymph Node	Prepared by Consultant Pathologist in clinic/radiology department; wash needle into 20ml Cytolyt® ***
Muscle biopsy	Muscle biopsy Nerve biopsy	Fresh: wrapped in saline-moistened gauze (Please refer to Appendix A for ward instructions on the collection of muscle biopsies)
Renal biopsy	Renal biopsy	One sample in 10% Formalin plus one sample in Zeus Fluid***

(Table 7)

*40ml pre-filled, red-topped formalin pots, suitable for small biopsy samples, are available to all wards from the Pharmacy Department, Letterkenny University Hospital. Theatres acquire their own supply of formalin and specimen containers for use in the submission of large surgical resections. It is the responsibility of individual GP Surgeries to supply their own formalin and specimen containers.

** Sourced direct by Bronchoscopy Unit.

*** Available direct from the Histopathology Laboratory by prior arrangement.

Please ensure that all specimen container lids are securely fastened before forwarding to the Histopathology Laboratory.

7.5 Labelling the sample container

Specimen containers MUST be labelled in such a way as to provide an unequivocal link to the patient. Best practice dictates that labelling should take place in the presence of the patient, immediately after sample collection.

The following essential information MUST be documented, in a legible manner, on the specimen container(s). An addressograph label, if available, may be used. Large sample containers MUST be labelled on the pot AND on the lid.

Specimen Essential Criteria	Histopathology Sample Labelling Requirement:
	Hospital and GP patients Two unique identifiers: **1. Forename & Surname** plus one of the following: **2. Patient's PCN** **3. Date of Birth** Addressographs preferred (hand-written: must be legible) Match request form labelling Match details held on LIS exactly
	Specimen Nature/Site inc. side for each sample - when more than one sample/specimen pot is being submitted for the same patient at the same time, pots should be labelled as A. denotes first specimen pot, B denotes second specimen pot, etc. **Exceptions:** • Non-gynae Cytology received in sealed bag with accompanying request form • GP skin samples received in sealed bag with accompanying request form

	• Cases with a single pot where nature of tissue is clearly evident e.g. placenta, appendix, gallbladder etc.
	If in doubt - ask!

(Table 8)

Note: Failure to provide the minimum data required on the Histopathology/Cytology sample container(s) will result in a delay in processing the sample.

7.6 Responsibility

Responsibility for full and correct labelling of any Histopathology/Cytology sample submitted to the laboratory for testing lies solely with the requesting clinician (Hospital or GP based). Unlabelled, or incorrectly labelled, samples will not be accepted under any circumstances and will not be processed until the discrepancy is amended. This, in turn, will cause a delay in sample reporting.

7.7 Labelling slides (applicable only to FNA requests performed by Consultant Pathologists)

Each slide prepared, and any needle washings being submitted for examination, MUST be labelled in such a way as to provide an unequivocal link to the patient. Best practice dictates that labelling should take place in the presence of the patient, immediately after sample collection and preparation.

The following essential information MUST be documented, in a legible manner, on the slide (s) and needle washings (if applicable).

FNA Essential Criteria	**Histopathology Slide and/or Needle Washings Labelling Requirement:**
	Hospital and GP patients Two unique identifiers: **1. Forename & Surname** plus one of the following: **2. Patient's PCN** **3. Date of Birth** Match request form labelling Match details held on LIS exactly
	Number slides consecutively i.e. first slide prepared is labelled A1, second slide labelled A2, etc. If more than one area is being sampled, slides from the second area should be labelled B1, B2,

	etc. Needle washings should be labelled with the corresponding letter only i.e. A, B, etc.
	If in doubt - ask!

(Table 9)

8 HIGH RISK SAMPLES

A "BIOHAZARD" sticker:

MUST be placed on Histopathology / Cytology sample containers **and** request forms of any specimen from a patient who is known to have, or is being investigated for, any of the following:

- Jaundice of unexplained origin
- HIV
- Hepatitis B or C
- Tuberculosis
- Viral Haemorrhagic Fever
- History of I.V. drug abuse
- Severe Acute Respiratory Syndrome (SARS)

The sample **MUST** undergo a minimum of 24 hours fixation before cut-up and processing.

It is desirable that the nature of the infection be stated in the clinical information section of the blue Histopathology/Cytology request form.

High risk samples, without appropriate biohazard labelling, pose a serious health and safety risk to laboratory staff; consequently, they will not be processed until a member of the clinical team attends and appropriately labels the case.

9 SAMPLE QUALITY

9.1 Submission State

Laboratory personnel inspect all samples received into the laboratory for adequate fixation (if appropriate) and/or appropriate submission state (see Table 6, Section 7.4).

9.2 Sample Transport Time

Tissue for examination by frozen section must be hand-delivered to the laboratory without delay to ensure cell viability and reportability of the sample.

Large resection specimens should be transferred to the laboratory without delay (< 60 minutes), to allow preparation of the tissue to facilitate optimal fixation.

All fresh (unfixed) fluids **MUST** be delivered to the laboratory within the hours of 08:00 to 16:30 Monday to Friday, **AND** as promptly as possible after sample collection, to ensure cell viablity. If immediate transfer is not possible, samples should be refrigerated at 4-8^{o}C to preserve cells. Unrefrigerated cases >24hrs old, or refrigerated samples >72hrs, will not be processed.

9.3 Unlabelled and inadequately completed samples and request forms

Acknowledging the unrepeatable nature of many Histopathology samples, the clinical team/GP will be given the opportunity to amend any unlabelled or inadequately completed samples or request forms.

Failure to comply with any of the submission and/or labelling requirements of the Histopathology Laboratory will result in a delay in sample processing. Details of the discrepancy may be added to the final report.

10 PACKAGING, DELIVERY AND TRANSPORT OF DIAGNOSTIC AND INFECTIOUS SAMPLES TO THE LABORATORY

10.1 General Information

The instructions for the transport of specimens to the Laboratory are described in a separate procedure for Sample Transportation: **MP-GEN-0060**, which is available on Q-Pulse and also available on http://www.hse.ie/luhPathology

It is the responsibility of the person dispatching the specimen to the laboratory to ensure that it is packaged correctly, and does not pose a risk to anyone coming in contact with it during transport or on receipt in the laboratory.

10.2 Sample Packaging

Sample packaging is dependent on a number of factors as summarised in Table 8:

Sample being sent	Requestor Location	Method of delivery	Packaging Instruction

Biopsy (inc. frozen section) Non-gynae cytology	Within hospital	By hand	Biohazard bag, with completed request form in separate pocket
Biopsy Non-gynae cytology	Within hospital	Pneumatic chute	Bagged (individually) as for hand-delivery above and placed into pod; pad out pod if necessary
Surgical resection +/- non-gynae cytology	Within hospital	By hand	Directly into steel trolley, with completed request form
Biopsy	Out-patients (off-site)	Routine taxi transport	Bagged (individually) as for hand-delivery above, placed into dedicated transport barrel and barrel sealed (barrel returned to out-patients after samples removed)
Biopsy Non-gynae cytology	GP Surgery	Routine GP Transport	Biohazard bag, with completed request form in separate pocket

(*Table 10*)

11 STORAGE OF SAMPLES, REQUEST FORMS & REPORTS

The Histopathology department retains all specimens, blocks, slides and associated documentation in accordance with Royal College of Pathologists guidelines. Dedicated space is available for archiving tissue blocks, slides, request forms and reports. All unprocessed tissue is retained in formalin fixative in the dissection room. Non-gynae cytological samples are stored at 2-4^{o}C in a dedicated Histopathology laboratory fridge. After the storage period, specimens are disposed of according to HSE procedure for the disposal of clinical waste.

The period of time that all histological and cytological material must be retained for is detailed in Table 9:

Item	Retention time	Storage Location
Histological Tissue Block	(Permanently) Min 30 years	Histopathology laboratory & store
Histological Slide	(Permanently) Min 10 years	Histopathology laboratory & store
Unprocessed Tissue	28 days after report issued	Histopathology laboratory
Cytological sample	7 days after report issued	Histopathology laboratory
Cytological slide	(Permanently) Min 10 years	Histopathology laboratory & store
Cytological block	(Permanently) Min 30 years	Histopathology laboratory & store
Histo/Cyto request form	Min 30 years	Histopathology office and electronically
Histo/Cyto report	Min 30 years	Histopathology office and electronically

(Table 11)

12 EXTERNAL LABORATORY TESTING

12.1 Referral for Quantitative Analysis

Tissue from patients with equivocal Her2 results on immunocytochemistry are forwarded for quantitative analysis by *in-situ* Hybridisation (ISH) to one of two external laboratories:

Source Bioscience Healthcare Ltd.
Nottingham
UK

OR

Poundbury Cancer Institute (PCI)
Dorchester
UK

An addendum report will be issued by the Histopathology Department to the requesting clinician when the result of ISH testing becomes available. A copy will also be made available to the Oncology Department via a shared drive.

12.2 Molecular Testing

12.2.1 Requesting

Requests for Molecular Testing of archived Histopathology blocks must be submitted by the requesting clinician via email to Histopathology.LUH@hse.ie, and using the appropriate external request format:

Molecular Test	Appropriate request form/format
BRCA *(for PARP Inhibitor Selection)*	Beaumont or SJH CMD BRCA Test Request & Consent Form for PARP Inhibitor Selection
c-MYC	Cover letter to Dr Michael Jeffers, Tallaght
Colorectal Mutation Panel *(KRAS, NRAS, BRAF, NTRK-1/2/3)*	Beaumont Hospital Molecular Histopathology Test Request Form
EGFR *(T790M Mutation)*	Cancer Molecular Diagnostics, SJH Request Form
FoundationOne CDx *(324 genetic alterations)*	FoundationOne CDx online portal
GIST Mutation Panel *(KIT, PDGFRA, BRAF V600)*	Cancer Molecular Diagnostics, SJH Request Form
Lung Mutation Panel *(EGFR, BRAF, KRAS, ERBB2, PIK3CA, ALK, ROS1, RET, MET, NTRK-1/2/3)*	Beaumont Hospital Molecular Histopathology Test Request Form

Melanoma Mutation Panel *(BRAF, NRAS, KIT, NTRK-1/2/3)*	Beaumont Hospital Molecular Histopathology Test Request Form
Mis-match Repair Analysis *(by IHC)*	Beaumont Hospital Molecular Histopathology Test Request Form
Microsatellite Instability *(by PCR)*	Beaumont Hospital Molecular Histopathology Test Request Form
MLH1 Methylation	Beaumont Hospital Molecular Histopathology Test Request Form
Oncoytpe DX	Exact Sciences online portal
PD-L1 *(tumour and treatment dependent)*	See below

Tumour	***Proposed Treatment***	***PD-L1 Clone***	
Breast (TNBC)	*Atezolizumab*	*SP142*	Cover letter to Dr Ann-Marie Quinn, GUH
	Pembrolizumab	*22C3*	Poundbury Cancer Institute Request Form
Bladder	*Atezolizumab*	*SP142*	Poundbury Cancer Institute Request Form
	Pembrolizumab	*22C3*	SVUH PD-L1 Request Form
	Nivolumab	*28.8*	Poundbury Cancer Institute Request Form
Lymphoma	*Atezolizumab*	*SP142*	Poundbury Cancer Institute Request Form
Lung	*Atezolizumab*	*SP263*	Beaumont Hospital Molecular Histopathology Test Request Form
Melanoma	*Atezolizumab*	*SP263*	Beaumont Hospital Molecular Histopathology Test Request Form
	Nivolumab	*28.8*	Poundbury Cancer Institute Request Form
Upper GI	*Pembrolizumab*	*22C3*	Poundbury Cancer Institute Request Form
	Nivolumab	*28.8*	Poundbury Cancer Institute Request Form
Gynae	*Pembrolizumab*	*22C3*	SVUH PD-L1 Request Form
Head and Neck	*Pembrolizumab*	*22C3*	Poundbury Cancer Institute Request Form
TCR Gene Rearrangement			Cancer Molecular Diagnostics, SJH Request Form

(Table 12)

12.2.2 Resulting

The Histopathology and Oncology Departments have a shared drive, available to selected staff only, to facilitate the return of molecular test results. When a new report is added to the shared drive, a notification email is sent out to inform users. All results are communicated back as shown in Table 10. Turnaround times are dependent on test(s) requested and on case complexity.

Molecular Test	Results
BRCA *(for PARP Inhibitor Selection)*	Direct to requesting clinician; copy to Histopathology Department; added to shared drive
c-MYC	Direct to requesting clinician; copy to Histopathology Department; scanned to iCM
Colorectal Mutation Panel *(KRAS, NRAS, BRAF, NTRK-1/2/3)*	Direct to Histopathology Department via secure email; added to shared drive
EGFR *(T790M Mutation)*	Direct to requesting clinician, copy to Histopathology Department; added to shared drive
FoundationOne CDx *(324 genetic alterations)*	Available on FoundationOne CDx online portal
GIST Mutation Panel *(KIT, PDGFRA, BRAF V600)*	Direct to requesting clinician; copy to Histopathology Department; added to shared drive
Lung Mutation Panel *(EGFR, BRAF, KRAS, ERBB2, PIK3CA, ALK, ROS1, RET, MET, NTRK-1/2/3)*	Direct to Histopathology Department via secure email; added to shared drive
Melanoma Mutation Panel *(BRAF, NRAS, KIT, NTRK-1/2/3)*	Direct to Histopathology Department via secure email; added to shared drive
Mis-match Repair Analysis *(by IHC)*	Addendum report issued to requesting clinician by Histopathology Department; viewable on iCM
Microsatellite Instability *(by PCR)*	Direct to Histopathology Department via secure email; added to shared drive
MLH1 Methylation	Direct to Histopathology Department via secure email; added to shared drive
Oncoytpe DX	Available on Genomic Health online portal; copy added to shared drive
PD-L1 *(tumour and treatment dependent)*	See below

Tumour	***Proposed Treatment***	***PD-L1 Clone***	
Breast (TNBC)	*Atezolizumab*	*SP142*	Direct to requesting clinician; copy to Histopathology Department; added to shared drive
	Pembrolizumab	*22C3*	Direct to Histopathology Department via secure email; added to shared drive
Bladder	*Atezolizumab*	*SP142*	Direct to Histopathology Department via secure email; added to shared drive
	Pembrolizumab	*22C3*	Direct to requesting clinician; copy to Histopathology Department; added to shared drive
	Nivolumab	*28.8*	Direct to Histopathology Department via secure email; added to shared drive
Lymphoma	*Atezolizumab*	*SP142*	Direct to Histopathology Department via secure email; added to shared drive
Lung	*Atezolizumab*	*SP263*	Direct to Histopathology Department via secure email; added to shared drive
Melanoma	*Atezolizumab*	*SP263*	Direct to Histopathology Department via secure email; added to shared drive
	Nivolumab	*28.8*	Direct to Histopathology Department via secure email; added to shared drive
Upper GI	*Pembrolizumab*	*22C3*	Direct to Histopathology Department via secure email; added to shared drive
	Nivolumab	*28.8*	Direct to Histopathology Department via secure email; added to shared drive
Gynae	*Pembrolizumab*	*22C3*	Direct to requesting clinician; copy to Histopathology Department; added to shared drive
Head and Neck	*Pembrolizumab*	*22C3*	Direct to Histopathology Department via secure email; added to shared drive
TCR Gene Rearrangement			Direct to requesting clinician; copy to Histopathology Department; scanned to iCM

(Table 13)

13 MULTI-DISCIPLINARY MEETINGS (MDM)

Consultant Pathologists from the Histopathology Department attend regular multi-disciplinary meetings (MDMs) to discuss individual cases. Also present at these meetings are members of surgical, radiological, oncological and nursing staff from within Letterkenny University Hospital, as well as staff from specialist centres via video-conferencing. The schedule is as follows:

MDM	Frequency
Gynae	Weekly (Tuesday)
Breast	Weekly (Thursday)
GI	Weekly (Friday)
Endocrine	Fortnightly (Monday)
Colposcopy	Monthly (Tuesday)
Inflammatory GI	Monthly (Friday)

(*Table 14*)

Slides will be referred to outside institutions for MDM discussion at the request of the clinical team, and upon completion of MF-0399 Request for Pathology Material Review (Histopathology/Cytology).

14 FURTHER EXAMINATION OF SAMPLE

14.1 By Reporting Pathologist

Consultant Pathology staff may refer cases to colleagues, both on-site and elsewhere, when they feel that a second opinion or further stains may be of benefit to the patient. All cases of equivocal Her2 results will also be referred for quantitative Her2 analysis by *in-situ* Hybridization (ISH). In any such instance, a final report will be issued, with an addendum report following once all investigations are complete.

14.2 By Requesting Clinician

All requests for further examination of the primary sample, by the requesting clinician, **MUST** be made directly to the reporting Primary Pathologist by the Consultant in charge of the patient and on completion of the appropriate request form (MF-0399 Request for Pathology Material Review (Histopathology/Cytology)).

15 APPENDIX A

Ward Information for collection of Muscle Biopsies

1. Muscle biopsies should be performed as early in the morning as possible as they must arrive at the Beaumont Hospital in Dublin before 4.00pm.

2. Check that both specimen containers and request form are correctly labelled.

3. The specimen should ideally measure 15x10x10mm.

4. Wrap the specimen in saline dampened gauze and place into an empty white topped Universal container. **Do not over soak the gauze as this causes problems with ice crystal artefact formation during subsequent processing.**

5. Make sure the wrapped specimen is reasonably loose within the container. **Take care not to squash the specimen.**

6. **Do not send the samples on ice.**

7. Send the sample to the Histopathology Department at Letterkenny University Hospital, to arrive by 11:00am at the latest.