The Royal College of Pathologists
Pathology: the science behind the cure

Standards for specialist laboratory integration and Dataset for the histopathological reporting of lymphomas

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Comments	This document replaces the 1st edition of *Dataset for histopathological reporting of lymphomas*, published in 2002. In accordance with the College's pre-publications policy, this document was on The Royal College of Pathologists' website for consultation from 4 February to 4 March 2015. Seventeen items of feedback were received. The authors considered them and amended the document as appropriate. Please email publications@rcpath.org if you wish to see the responses and comments. **Dr Lorna Williamson** **Director of Publishing and Engagement**

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Foreword

The cancer datasets published by The Royal College of Pathologists (RCPath) are a combination of textual guidance, educational information and reporting proformas. The datasets enable pathologists to grade and stage cancers in an accurate, consistent manner in compliance with international standards and provide prognostic information, thereby allowing clinicians to provide a high standard of care for patients and appropriate management for specific clinical circumstances. It may rarely be necessary or even desirable to depart from the guidelines in the interests of specific patients and special circumstances. The clinical risk of departing from the guidelines should be assessed by the relevant multidisciplinary team (MDT); just as adherence to the guidelines may not constitute defence against a claim of negligence, so deviation from them should not necessarily be deemed negligent.

Each dataset contains core data items that are mandated for inclusion in the Cancer Outcomes and Services Dataset (COSD – previously the National Cancer Data Set) in England. Core data items are items that are supported by robust published evidence and are required for cancer staging, optimal patient management and prognosis. Core data items meet the requirements of professional standards (as defined by the Information Standards Board for Health and Social Care [ISB]) and it is recommended that at least 95% of reports on cancer resections should record a full set of core data items. Other, non-core, data items are described. These may be included to provide a comprehensive report or to meet local clinical or research requirements. All data items are clearly defined to allow the unambiguous recording of data.

This document has been produced through a consultation process with the members of the British Lymphoma Pathology Group, which involves all cellular pathology professionals with a subspecialist activity in haematopathology. The recommendations made rely on published evidence, existing NICE, UK and international guidance, and the 2008 edition of the World Health Organization's (WHO) *Classification of Tumours of Haemopoietic and Lymphoid Tissue*.[1–4] Evidence evaluation was carried out as per the recommendations of The Royal College of Pathologists utilising the SIGN guidance.

Further revisions of this document will be subject to a three-yearly review cycle and national consultations amongst professionals involved in diagnosis and management of lymphomas. Amendments and changes ahead of this schedule may ensue in conjunction with important new developments in the field.

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1 Introduction

Lymphomas in the Western population represent the tenth most common cause of death, with an overall incidence in the UK of approximately 18.3 per 100,000.[5] However, from a technological point of view, diagnosis of lymphomas has historically been very complex with resource requirements disproportionately higher compared to more common cancers. Pathological diagnosis of lymphomas is also known to be problematic and difficult. The current WHO classification of lymphoid neoplasms holds more than 60 established and provisional disease entities. Over the past 30 years, through intense efforts of experts of the International Lymphoma Study Group, nomenclature and classification of lymphomas evolved through a series of radical conceptual changes. In 1994 the REAL (Revised European-American Lymphoma) classification established a new basic premise that a classification should identify disease entities using all available information: morphological, immunophenotypic, genetic and clinical.[6] The clinical and diagnostic utility of this approach was validated by the non-Hodgkin Lymphoma Classification Project in 1997.[7] The new concept has been adopted by WHO as the basis for lymphoma classification worldwide. The 2001 WHO *Classification of Tumours of Haemopoietic and Lymphoid Tissue* and the subsequent revisions categorise and illustrate the

neoplasms with details of the clinical, immunological and genetic data required to complement morphology in their assessment.[1] This classification is continuously evolving and publication of a new edition is expected in the near future. This is expected to put even more emphasis on the clinical context, age, site or organ system of involvement as entity-defining criteria. More entities will be better characterised by inclusion of molecular features. The classification requirements impact on organisation of diagnostic services. There is a need that all the relevant aspects of diagnosis including phenotype and genotype are routinely and reproducibly interrogated and this data is integrated and interpreted by pathologists in the clinical context.

Over recent decades, survival of lymphoma patients has changed dramatically. This has taken place due to better scientific understanding of lymphoma biology that is rapidly being transferred into entity specific and individualised therapies, forming the basis for the application of so-called 'precision medicine'. In many instances, the complex interrogations undertaken for pathological diagnosis provide information on valuable biomarkers that help to estimate prognosis and select most effective treatment.

The practices involved in the diagnosis of lymphoma in many respects are significantly different compared to pathological diagnosis of most solid cancers. Thus, lymphoma diagnosis does not fit comfortably into the standard model adopted for the RCPath cancer datasets encompassing a range of solid cancers and requires a considerably different approach. Furthermore, since the publication of the 2002 dataset there have been important changes in guidance regarding organisation of services in haematopathology. These issues are explained further below.

1.1 Variation from the standard dataset model for reporting solid cancers

The principal differences are in four areas.

- Haematoxylin and eosin (H&E)-stained sections are only the starting point for histological assessment of suspected lymphoma, with a panel of numerous immunostains applied routinely as an essential component of the diagnostic process.
- Many lymphoma diagnoses also rely on haematological, flow cytometric, immunological, cytogenetic and/or molecular genetic data, which are not all generated within the cellular pathology laboratory. To formulate the final diagnosis in such cases, coordination and integration of results from a variety of sources is needed, correlated with accurate clinical details to ensure an appropriate context.
- An integrated report is not simply an exercise in 'cut and paste'. Wherever possible, the facilities for providing the various inputs should be co-localised. Where this is not possible, communication links must be in place to ensure prompt data-sharing between laboratories. All the data accumulated into each report should be assessed and authorised by one or more professionals with expertise spanning all of the technologies involved, to ensure that the evidence is consistent or any discrepancies are interpreted appropriately. Safeguards need to be put in place to prevent individual components of integrated reports (e.g. PCR clonality results) being circulated as standalone information outside the context of the integrated report and pathological features of the biopsy.
- The minimum diagnostic requirements for a large number of diverse lymphoma entities are varied and different, with variable emphasis on morphology, clinical context, phenotype or genotype.

1.2 Organisational considerations – current regulation and organisational requirements

The National Institute for Health and Care Excellence (NICE) *Improving Outcomes Guidance (IOG) for Haematological Cancers* (2003) and the National Cancer Action Team's (NCAT) commissioning guidance (2012) regarding organisation of pathology services are based on studies showing greater accuracy of lymphoma diagnosis in specialist centres compared to reporting by non-specialists.[8,9] In recognition of diagnostic complexity, NICE and NCAT have

placed great emphasis on lymphoma diagnosis within the *IOG for Haematological Cancers* (2003 and 2012).[8,9] This has laid down strict parameters within which the diagnosis and reporting of lymphomas should be undertaken. In particular, the NICE IOG mandates diagnosis only by specialists in haematopathology and a systematic, algorithmic approach to acquiring data from all relevant tests and incorporating them into a final integrated report. The summary of guidance is given below.

- All patients with haematological malignancies to be managed by a networked haemato-oncology multidisciplinary team (MDT) serving a population of 500,000 or more.
- Specialists in haematopathology to review all diagnoses of possible haematological malignancy.
- Results of diagnostic tests to be integrated and interpreted by network experts linked to MDTs.
- Rapid-access diagnostic services must be available for patients with lymphadenopathy.
- There will be a maximum interval of 62 days from patient presenting to GP to start of treatment for haematological malignancies. NICE does not dictate how much of this may be absorbed by diagnostic cellular/molecular pathology but staging procedures must also all be completed within this time. In effect, the guidance requires completion of diagnostic pathology within 10–14 days of biopsy in all except the most complex lymphomas.

The specific proposals for haematopathology services are:

- a single immunophenotyping service to be established for the MDT network
- a single molecular genetic and cytogenetic laboratory service to be established for the MDT network
- histomorphology to be reported for the network only by designated specialist pathologists
- specialist pathologists must be agreed by the network, must be MDT members, must be subject to external quality assurance (EQA) and must work to agreed network guidelines
- an integrated final report to be produced, including all pathological modalities used, to be authorised singly or jointly by designated pathologists/consultant scientists.

An initial three-year period was envisaged by NICE for the implementation of this IOG, with any delay requiring justification and plans for remedial action agreed by NCAT. During 2012, with the landscape for commissioning of services within the NHS undergoing radical change, NCAT issued guidance for commissioning that encompassed an update of the original guidance, within which expansion of the population base for specialised haematopathology services is anticipated (now stated as 2 million).[8] It is clear that further reorganisation and regional centralisation of haematopathology specialist diagnostic services will be required.

Since 2003, extensive reconfiguration of haematopathology diagnostic services has occurred, following various models of regional centralisation and specialisation. In fact, a high level of such regional cooperation existed informally in many areas of the UK prior to that guidance and much of the reorganisation has been to formalise these arrangements. Most regional networks, however, still remain incompletely compliant with the IOG, largely reflecting geographical circumstances and lack of investment in information technology infrastructure. Further reorganisation is ongoing and greater infrastructure investment is needed to underpin this if IOG compliance is to be achieved across the NHS. It is recognised that, at present, not every region can offer a specialist referral centre for the diagnosis of all cases of lymphoma and only a smaller number can offer specialist diagnostics for all diseases within the spectrum of haematopathology. Thus, the reality of everyday pathological diagnosis of lymphoma is that different organisational set-ups, which are formally ‘non-IOG compliant’, will exist in practice for some, quite possibly long period of time. In these circumstances, there are increased risks for diagnostic delays, variable quality of investigations and diagnosis, excessive cost and suboptimal use of some specimens (particularly needle biopsy cores) due to over-investigation

of cases by non-specialists prior to referral for formal reporting at a regional specialist unit. These suboptimal aspects of practice in lymphoma diagnosis must be minimised. Until formal compliance with the IOG guidance is achieved, appropriate guidance for delivery of high-quality diagnostic service is needed, which will realistically take into consideration the currently existing organisational diversity.

With this background in mind, the dataset and guidance for the histological reporting of lymphomas that follow should be implemented for the following reasons:

- to provide the most up-to-date recommendation for diagnostic evidence-based practice, to facilitate the most effective management of lymphomas taking into account changes in classification, advances in technology, accumulated knowledge and requirements for provision of prognostic and diagnostic biomarkers, which have evolved since the first edition of this document in 2002
- to provide guidance for incorporation of histopathological aspects of lymphoma diagnosis with other data-form laboratory investigations into a final integrated report
- to provide guidance for the practice of both subspecialist and 'generalist' pathologists who may be involved in lymphoma diagnosis to a variable extent
- to support the flow of work and information between non-specialist and specialist centres in diverse geographical or administrative regions throughout the UK in the transitional period of implementation of the commissioning IOG guidance
- to provide guidance and information for clinical audit
- to facilitate accurate cancer registration.

This dataset considerably differs from the original 2002 document. Most of the text has been rewritten and the references updated to reflect scientific developments, changes in classification and service guidance. The antibody panels have been redesigned. Most notably, guidance for integrated reports is included together with recommendations for practice and service organisation in both specialist and non-specialist sites involved in lymphoma diagnosis.

This dataset does not intend to address the specific issues relating to reporting of primary skin lymphomas. A separate dataset for the reporting of primary cutaneous lymphoma is in preparation and will complement this guidance in near future. Likewise, detailed recommendations pertaining to specific issues of bone marrow biopsy assessment are not included.

To help in the use of this dataset, a list of the entities in the updated WHO classification of 2008 and their International Classification of Diseases for Oncology (ICD-O) codes is provided in Appendix A. Typical histopathological features, immunophenotyping and genetic information of the major, common entities are described in detail in a companion lymphoma diagnostic guideline published jointly by the British Committee for Standards in Haematology and The Royal College of Pathologists.[2] A list of varied pathological, immunophenotypic and molecular-genetic features for which there is appropriate scientific evidence that they impact diagnosis, prognostication or management of some of the entities is provided and, where applicable, indicated as an essential part of the integrated report. For full details of rare lymphoma entities, the WHO 'blue book' should be consulted.[1]

1.3 Target users and health benefits of this guideline

The target primary users of the dataset are trainee and consultant cellular pathologists and, on their behalf, the suppliers of IT products to laboratories. The secondary users are surgeons and oncologists, cancer registries and the National Cancer Intelligence Network. Standardised cancer reporting and multidisciplinary team (MDT) working reduce the risk of histological misdiagnosis and help to ensure that clinicians have all of the relevant pathological information required for tumour staging, management and prognosis. Collection of standardised cancer

specific data also provides information for healthcare providers and epidemiologists, and facilitates international benchmarking and research.

2 Who reports lymphoproliferative pathology

- Specialist haematopathologists have a substantial part of their job plans dedicated to haematopathology; they are recognised by their local network, must be MDT members, participate in specialist EQA schemes and be members of the British Lymphoma Pathology Group (BLPG).
- General pathologists, in the ongoing transitional period of IOG guidelines implementation play important roles in facilitating provisional diagnosis and managing referral to specialist services.

In the UK at present, haematopathology is not a formally recognised subspecialty and there is no dedicated RCPath training curriculum. Expert haematopathologists in the UK have mostly gone through informal 'apprenticeship' training and work at sites with significant diagnostic exposure, which facilitates acquisition of experience and expertise. While there is no formal regulation defining haematopathology subspecialty qualification, it would be expected that a specialist haematopathologist has a substantial part of the job plan dedicated to haematopathology. As the BLPG addresses important issues of haematopathology practice in the UK, it would also be expected that a specialist haematopathologist is a member of the group and participates in the national EQA scheme that it organises. At the local level, a haematopathologist must be recognised by the local clinical network and must be an MDT member.

Most appropriately educated general pathologists, supported by a suitably extensive range of immunostains and access to relevant FISH and PCR analyses, should be capable of making correct diagnoses of the most common forms of lymphoma on the basis of well-preserved and excised lymph node specimens. However, this involves cost inefficiency at best (particularly since specialist review is mandated by the IOG), diagnostic delay and, for numerous uncommon entities, incomplete or incorrect diagnosis at worst. It is very difficult for non-specialist pathologists to maintain the breadth and currency of skills to ensure that they have full awareness of differential diagnosis and of rare or newly emerging lymphoma entities. It is challenging for smaller laboratories to continually update their available immunohistochemical panels and for non-specialists to develop/maintain sufficient confidence to make best use of molecular genetic tests in their diagnostic pathways for suspected lymphomas. However, in an appropriate network setting, general pathologists play an important role in facilitating provisional diagnosis and, until IOG guidance is fully implemented, requirement for their close involvement will exist for some time.

3 Organisation of services – recommendations for practice during the transitional process of IOG implementation

Key points

- Each regional network will agree guidance underpinning the roles and responsibilities of non-specialist and specialist departments for lymphoma diagnosis, and apply this consistently.
- A lead pathologist will be identified in each non-specialist centre involved in specimen handling and provisional diagnosis of new and relapsed lymphoma.
- Guidance, specific to non-specialist departments, will be implemented for handling biopsy samples (see Appendices C and D).

- Appropriate provisional diagnosis must be provided to facilitate patient entry into the clinical investigative pathway and referral to appropriate MDT.
- Robust arrangements for rapid communication of reports and transfer of specimens must be put in place.
- Appropriately detailed clinical and supporting laboratory/imaging information must be provided accompanying the request for specialist diagnosis or review, including the provisional diagnosis made.
- At all stages of the specimen pathway, current lymphoma classification terminology and diagnostic coding must be used (Appendix A).

With rare exceptions, the model for regionalisation of haemato-oncology diagnostic services in England has been 'hub and spoke', with retention of a first-pass, rapid assessment locally and mechanisms for subsequent transfer of specimens to a specialist regional centre with access to a full, current immunohistochemistry service, cytogenetics and molecular genetics. Services for immunophenotyping by flow cytometry are almost exclusively provided centrally in specialist units. The latter have the benefit of ensuring range and currency, but have limited applications in lymphoma diagnosis, in particular because of logistical constraints on access to services by remote hospitals requiring transfer of unfixed cytology samples or lymph node tissue. In Wales there is a centrally funded national diagnostic service that relies on provisional diagnosis provided by non-specialist pathologists for initial patient entry into the clinical investigative pathway. Many centres in Scotland follow this model as well.

In any hub-and-spoke arrangement, each histopathology service should identify a lead consultant pathologist to take responsibility for services relating to haematopathology. That individual must ensure the operation of rapid and robust pathways for transfer of lymphoid and bone marrow specimens to an appropriate regional specialist centre, minimising duplication of testing and delay, and ensuring maximal availability of tissue for the central diagnostic process. The latter is particularly important for small samples including needle biopsy cores and endoscopic biopsy fragments. They also need to ensure that not only biopsies for suspected and previously undiagnosed lymphomas are forwarded to the specialist diagnostic services, but also those from previously diagnosed, relapsed lymphomas. Particular care is needed for investigation of lymphomas in extranodal sites, such as the gastro-intestinal tract or lung, or in a paediatric context. Histopathology generalists and subspecialist colleagues reporting in these fields must be aware of the need for specimens to follow the lymphoma pathway with minimum delay and without variation. Suspected cutaneous lymphomas are, in many instances, likely to require direct or secondary referral through a supra-regional pathway to individuals with specific expertise; the differential diagnosis, classification and treatment of cutaneous lymphomas differ substantially from those of systemic lymphoproliferative diseases.

Local lead consultants for haematopathology must be able to ensure that appropriate provisional or differential diagnoses are conveyed to their haemato-oncology colleagues on the basis of H&E-stained sections supplemented by, at most, minimal immunohistochemistry. Guidance for initial, limited immunohistochemistry and the contexts for its application are offered in Appendix C. The required approach to the handling of biopsy samples in haematopathology with special reference to non-specialist participation is provided in Appendix D. Diagnostic immunohistochemistry panels for both initial screening and subsequent expert assessment are provided in Appendix E.

Clinicians and pathologists locally and centrally must ensure rapid, accurate and secure return of results. In this context, it should be kept in mind that complex arrangements for specimen transfer and communication of reports may adversely affect the diagnostic turnaround time recommended by the IOG. While a diagnostic turnaround of 10–14 days, as recommended, will be sufficient for diagnosis of most common lymphomas, this will be exceeded if time for administrative handling, specimen transfer and postal communication of final reports is added. Therefore, receipt of specimens and return of results by regional specialist centres must be supported by dedicated, robust, tracked and audited processes and ideally by dedicated

secretarial support. Significant time is saved by electronic distribution of reports directly to involved haematologists or oncologists, in addition to postage of hard copies.

Where general pathologists provide a preliminary diagnosis on the basis of which a patient enters a clinical investigative pathway while specialist pathological assessment is being obtained, it is essential that feedback is provided to the referring pathologist once the central diagnosis is reached. This is of important educational value, essential to maintain the necessary skill base for robust first-pass assessments.

At all stages of the specimen pathway, current lymphoma classification terminology and diagnostic coding must be used accurately (WHO 2008 classification; Appendix A). Within regional specialist services, mechanisms must be in place to ensure that investigations remain current and comprehensive, are applied systematically and appropriately, and that high levels of diagnostic and technical skills are maintained by the medical consultants, clinical scientists and biomedical scientists providing the service.

4 Clinical information required on the specimen request form

This should include:

- full name
- date of birth
- sex
- referring organisation
- reporting organisation
- NHS number
- hospital number
- specimen number at referring organisation
- specimen number at reporting organisation
- date of biopsy/surgery
- biopsy taker (surgeon, interventional radiologist or other physician)
- date of dispatch from referring organisation
- date received at reporting organisation
- date of final report
- caring physician (haematologist, oncologist or other, if known)
- indications for investigation (primary diagnosis; staging; relapse/progression; re-staging; review; clinical trial; post mortem)
- specimen type (excision biopsy; needle core biopsy; endocsopic biopsy; extranodal resection (including splenectomy); bone marrow trephine; other biopsy (specify).

In addition, the final integrated report should also include records of all clinical, imaging and laboratory findings relayed via the request form, provided by the clinician or acquired actively by the haematopathologist (from hospital information systems, MDT discussion, personal enquiry, etc.), which are relevant for diagnosis or staging.

5 Preparation of specimens before dissection

Key points

- Procedures for sampling and utilising fresh tissue for microbiology, cytogenetics, flow cytometry, preparation of imprints and snap-freezing should be considered.
- Robust protocols for communication and transfer of fresh tissue between different laboratories must be developed.
- Neutral buffered formalin should be utilised as standard fixative. EDTA is recommended for decalcification of bone marrow trephine specimens because of its currently superior performance for PCR assays such as *IGH* and *TCR* rearrangement studies. Formic acid and zinc/acetic acid combinations may provide faster throughput and are equally satisfactory for morphological and antigenic preservation. Resin embedding without decalcification can offer excellent morphological preservation in laboratories with relevant expertise; however, staining methods require adaptation and antigenicity as well as nucleic acid preservation are adversely affected.

In ideal circumstances, where facilities exist for handling fresh specimens, tissue with suspected lymphoma should be received unfixed as soon as possible after surgery. Consideration should always be given to sampling fresh tissue for microbiology, cytogenetics, flow cytometry, preparation of imprints and snap-freezing. This needs to be part of robust protocols facilitating communication and transfer of materials between different laboratories (see section 9). Integrity of the fresh tissue samples is maximised if it is placed into cytogenetic transfer medium. Unfixed solid tissue of appropriate size to allow for good perfusion (<5 mm) in this medium remains viable with good retention of morphological features and integrity of DNA/RNA over a period of 24 hours at room temperature. With good communication arrangements, this facilitates transport of fresh material over considerable distances to reference laboratories. Centres that have arrangements for use of a proportion of trucut needle cores taken as fresh specimens should have protocols in place to make this medium readily available to the biopsy takers.

When tissue is received unfixed, this should be recorded in the report or a permanent laboratory record, including an estimate of the time in transit following removal from the patient (‘cold ischaemic time’) and details of initial procedures undertaken with unfixed material (e.g. imprints for cytological assessment, dispersal of cells for flow cytometry, number and size of pieces frozen). Storage of frozen tissue should comply to standard laboratory protocols enabling easy identification of materials for potential use in future investigations (Appendices G and H).

If no protocol for handling of fresh specimens is available, biopsies should be placed in formalin in the operating theatre.

Large lymph nodes (>10 mm maximum diameter) and spleens should be sliced on receipt for optimal fixation.

Material from needle cores and other small biopsy specimens should be prioritised for routine histology processing. If this material is received fresh, imprints can be prepared and a proportion allocated for flow cytometry, but it is seldom warranted to divide such samples for frozen storage. Useful information can be obtained by flow cytometry utilising only the transfer fluid in which the fresh cores or other specimens have been transported. A substantial amount of cellular material is released spontaneously from the specimens into this fluid and can be increased by gentle agitation without detriment to subsequent histological quality.

Large resection specimens should be handled according to respective subspecialty protocols. They provide recommendation for optimal specimen handling so that tissue is best preserved and presented for appropriate sampling. In many instances, macroscopic examination alone would not necessarily provide definite clues as to the nature of the malignancy being

investigated. If lymphoma is suspected at macroscopic handling, particular attention should be given to careful slicing of tumour masses to achieve best fixation throughout the tissue. However, it would be prudent to follow the general recommendations for sampling of non-haematological tumours so that their diagnosis is not compromised with non-standardised cut-up and sampling. It should be pointed out that proforma datasets for reporting of non-haematological malignancies in large resection specimens do not apply to lymphoma (e.g. reporting of resection margins in case of haematological malignancies is irrelevant).

Further guidance on best practice in handling lymphoid tissues for diagnosis are included in a companion College tissue pathways document, which also includes specific detail regarding diagnosis of non-neoplastic diseases in such tissues.[2]

The standard fixative enabling high-quality immunohistochemistry and genetic investigation is neutral buffered formalin. A consistent fixation time of approximately 24 hours aids uniform tissue preservation and reproducibility in immunostaining.

6 Specimen handling, block selection and laboratory processing

A brief description of the lymph node size, consistency, circumscription and appearance of cut surfaces is generally adequate.

For splenectomy specimens, the spleen weight and presence of macroscopic nodules, with an indication of their size and appearance, should be recorded; hilar lymph nodes should be sought, described and sampled.

For extranodal lymphomas, the standard protocol for the appropriate organ (stomach, bowel, etc.) should be followed and given as a free-text report.

When more than one core has been obtained and the clinical indication includes an explicit statement of suspicion of lymphoma, it is recommended that each core is embedded in a separate block, to maximise tissue availability for immunostaining and molecular genetic studies. Duplicate H&E-stained sections should be prepared at a single level from each block, bearing in mind that 15–20 subsequent unstained sections may be required to complete the diagnosis. In cases with one block only, spare sections may be cut proactively; when two or more blocks can be created, initial screening of H&E appearances is advised to select the best block for immunohistochemistry. It is essential that stepped levels are not cut from tissue cores with suspected lymphoma without assessment of H&E-stained appearances after initial laboratory processing; in most instances these will be inappropriate and significantly wasteful of tissue. Processing protocols are included in Appendix D.

When lymphoma is not suspected initially (needle cores for suspected lymph node metastasis or cores from non-lymphoid tissues such as breast, liver and kidney), consideration should be given to varying local protocols for levels etc., appropriate for non-lymphoid diagnoses, to ensure that at least 10 unstained sections are prepared and retained between levels to anticipate possible immunohistochemical requirements (see section 3, above). This has little cost and is efficient for both speed of diagnosis and sparing of tissue by avoiding later requirement for re-cutting.

Good-quality thin sections (3 µm) are essential for accurate diagnosis. Initial diagnosis can be reached using standard H&E-stained sections, aided if required by reticulin staining to assess follicular architecture and a PAS stain to highlight vascular structure. Giemsa is useful to demonstrate cytoplasmic basophilia, blast cells and mast cells but many histopathology laboratories find it difficult to achieve reproducible results in fixed histological specimens. Other stains to identify mast cells such as toluidine blue or chloroacetate esterase should be available.

7 Core data items

- WHO lymphoma entity and grade
- Clinical context
- Morphology
- Immunophenotype
- Clonality
- Genotype.

Each lymphoma entity as specified by the WHO classification is defined by a combination of features including clinical context, morphology, immunophenotype and genotype (including clonality where appropriate). In addition to definitive diagnosis, these represent in principle a core of items that must be included in pathology reports. Depending on the lymphoma entity, emphasis varies. Diagnosis of all entities requires careful assessment of morphology and immunophenotyping. For some, the clinical setting, appearance and distribution of lesions are crucially important. In other entities, presence of specific genetic markers and assessment of clonality are essential for diagnosis and/or prognostication. The table in Appendix A includes a list of specific essential investigations to be undertaken for diagnosis of individual lymphoma entities. The results of these essential investigations for the individual entities represent the mandatory core information to be included in the report.

7.1 WHO lymphoma entity and grade

Lymphomas are categorised according to the WHO classification of haematological malignancies.[1] This encompasses Hodgkin and non-Hodgkin lymphomas, myeloid and histiocytic neoplasms. The diagnosis of myeloid proliferations is beyond the scope of this dataset. Each entity represents a distinctive disease with a typical clinical course, prognosis and specific therapeutic approach (Appendix A). The WHO recognises established entities indicated in the classification table by non-italicised text. So-called 'provisional entities', for which additional evidence is being gathered for their establishment as distinctive diseases are shown as italicised text. However, for the purpose of diagnosis and management, there is no difference between the two in the requirement to provide core item data. Formal grading is applied only for specific entities (follicular lymphoma and lymphomatoid granulomatosis) as specified by WHO classification. Accurate grading of these entities requires representative tissue sampling. In this context, grading of follicular lymphoma in trucut needle biopsies could be problematic. In generous trucut biopsies, grade could be assessed with reasonable certainty. In instances where tissue is particularly scanty, pathologists should use their discretion to state the degree of confidence or their ability to assess grade.

Definitive pathological diagnosis cannot be reached in all instances and in these cases diagnostic ambiguity/uncertainty should be clearly indicated. The MDT can be used in such circumstances as the appropriate forum for dialogue to achieve a pragmatic consensus to guide clinical management, acknowledging and taking mutual responsibility for any risk involved.

Where only an incomplete diagnosis or differential diagnosis can be achieved, the basis for this should be stated, since it has implications for follow-up actions. The basis of uncertainty should be specified as due to:

- limited sample quantity (e.g. depleted biopsy core)
- limited sample quality (e.g. poor fixation)

- operational, and presumed temporary, limitation (e.g. poor performance of a particular immunostain, not improved on repeating, or non-availability of a stain or method on a particular occasion)
- complexity of histological interpretation (e.g. 'grey zone' pathology with overlapping features between entities)
- contradiction between results of component tests contributing to diagnosis (e.g. unexpected combinations of immunohistochemistry results; flow cytometry differing from immunohistochemistry)
- difference of opinion between appropriately skilled individuals assessing the case (e.g. failure to achieve consensus during double reporting).

[Level of evidence: Clinical context is an essential component contributing to specific diagnosis of a WHO lymphoma entity – level A.]

7.2 Clinical context

Clinical context is an essential component contributing to the diagnosis of a specific lymphoma entity as defined by the WHO lymphoma classification. Its diagnostic contribution is variable, depending on lymphoma type. For some entities pathological investigations alone cannot provide definitive diagnosis outside the appropriate clinical context (Appendix A).

Specific clinical settings that impact on diagnosis of some entities are age, organ or system restriction of lesions, clinical appearance of lesions and immunosuppression. While information on age is always available, the additional relevant clinical details may not be obvious from pathology request forms. It is essential that multidisciplinary teams and biopsy takers are focused on the need to provide this information and of its relevance for diagnosis. Pathologists are encouraged proactively to seek information about clinical context. This particularly refers to iatrogenic immunosuppression, which is often omitted by requesting clinicians.

For specific diagnoses where clinical setting represents the key diagnostic feature, pathology reports should positively state that this has been taken into account in formulating the diagnosis (Appendix G).

[Level of evidence: Clinical context is an essential component contributing to specific diagnosis of a WHO lymphoma entity – level A.]

7.3 Morphology

Histomorphological appearances are the cornerstone for diagnosis of lymphoma; a starting point for differential diagnosis, choice of corroborative investigations and interpretation of results. A description of histological appearances is required, using widely understood terminology and clearly stating, where appropriate, points of agreement or discrepancy with expectations for the diagnoses under consideration.

[Level of evidence: Lymphoma entities are characterised by typical histomorphological features – level A.]

7.4 Immunophenotype

In addition to morphology, lymphoma entities are defined by specific immunophenotypes obtained by immunohistochemistry and, when fresh tissue and access to suitable facilities are available, by flow cytometry.
Characteristic diagnostic phenotypes are detailed in the WHO 'blue book' and the guideline published jointly by the British Committee for Standards in Haematology and The Royal College of Pathologists.[1,2] Immunohistochemical assessment provides not only the diagnostic

phenotypes but also information essential for prognostication and choice of treatment. In many instances, immunohistochemical markers also serve as surrogates for underlying genetic abnormalities. Immunophenotypic assessment is an obligatory component of the diagnosis of all lymphoma entities, with diagnosis usually reliant on a pattern of positive and negative marker expression. Some entities are characterised by expression of distinctive and unique markers that must be specifically interrogated. These are listed in Appendix A.

Immunohistochemistry requires good technique and careful interpretation, with the use of appropriate controls and participation in an accredited EQA scheme. Laboratory staff must be fully aware of the staining characteristics of all antibodies employed (e.g. nuclear *versus* cytoplasmic) and the technical requirements for each antibody. A list of the most commonly used markers for immunohistochemistry with descriptions of the typical staining patterns and diagnostic significance is provided in Appendix F. Cooperation between laboratories should be considered to ensure cost-effective use of antibodies that are needed only infrequently in an individual centre.

In practice, there is considerable variation in the way immunohistochemical markers are selected. Some pathologists prefer choosing individual antibodies, while others utilise pre-selected antibody panels. Each of these approaches has potential benefits and disadvantages. Selecting individual antibodies is beneficial in circumstances of limited tissue availability and is more economical as there is no redundant use of immunological markers. A potential disadvantage is requirement for multiple sequential rounds of immunostaining, causing diagnostic delay as well as lack of investigative uniformity and standardisation. Antibody panels are commonly used in large laboratories with high workloads, in which a certain amount of redundant use of some markers is counterbalanced by standardisation, diagnostic uniformity, less need for repeated immunohistochemistry rounds and time saved on selecting immunostains. These two approaches are not mutually exclusive and a flexible, common-sense approach should be applied, particularly in the work-up of small specimens.

The range of antibodies available is vast, ever-increasing and beyond the scope of most non-specialist laboratories. A basic panel of antibodies should be available in every laboratory in which haematopathology reporting occurs. These are listed in Appendix E, together with extended antibody panels suitable for specialist laboratories. The suggested panels should be used as a guide and may be adopted locally to fit best with established practices and diagnostic team preferences. However, it must be noted that the NICE IOG places responsibility for diagnostic immunophenotyping of haematological cancers solely on the regional specialist centre.

The immunophenotype can be provided as part of a descriptive textual report or laid out as a string or table of markers with positivity indicated by +, lack of expression by -, or partial expression as +/- or -/+. Where relevant, spatial distribution, intensity and cellular patterns of expression should be indicated and immunostaining patterns interpreted in conjunction with morphological findings.

The phenotypes obtained by immunohistochemistry or flow cytometry are meaningful only in the context of the totality of pathological findings and must be part of the integrated pathology report (see below).

[Level of evidence: Immunophenotype is an essential component contributing to specific diagnosis of a WHO lymphoma entity – level A.]

7.5 Clonality

Clonality studies of the immunoglobulin and T-cell receptor genes by polymerase chain reaction (PCR) can be very useful in some cases of diagnostic difficulty. While not necessary for the diagnosis of majority of lymphoma entities, clonality must be interrogated for the selected few (Appendix A). The main contexts are equivocal morphological and/or immunophenotypic features between reactive and neoplastic entities, or a low level of potential disease involvement precluding confident interpretation of morphology and immunophenotype alone.

All laboratories in the United Kingdom undertaking lymphoid clonality studies by PCR use the Biomed-2 sets of PCR primers. Guidance on interpretation is available in the most recent Euroclonality report.[10]

PCR analysis requires dedicated specialist staff, careful interpretation and participation in an accredited EQA scheme.

Antigen receptor gene PCR results are meaningful only in the context of the totality of pathological findings and must be part of the integrated pathology report (see below).
[Level of evidence: Evidence of clonality is by PCR is evidence of lymphoid neoplasia and is essential for diagnosis of some of the WHO lymphoma entities – level B.]

7.6 Genotype

Many lymphoma entities are characterised by various types of stable genetic markers.[1] For contentious and difficult cases in which morphology and immunophenotype are not sufficiently diagnostic, there may be a requirement to look for specific genetic abnormalities. These can be interrogated by metaphase karyotyping. While this method provides valuable information, it is labour intensive, time consuming and often not feasible logistically due to its requirement for fresh, unfixed tissue. In recent years, interphase FISH has become the 'gold standard' for assessment of genetic abnormalities in diagnosis of lymphomas.

Routine genetic investigations are currently reserved for a limited number of circumstances (Appendix A). Notably, diagnosis of 'B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma' should always be corroborated by investigation of the most common genetic abnormalities, in this context particularly rearrangements of the MYC, BCL2 and BCL6 genes.

FISH and conventional karyotyping require good technique, dedicated specialist staff, careful interpretation and participation in accredited EQA schemes.

The results of genetic investigations performed using tissue specimens are meaningful only in the context of the totality of pathological findings and must be part of the integrated pathology report (see below).

[Level of evidence: Lymphoma entities are characterised by specific genetic markers – level B.]

8 Non-core data items

A range of immunophenotypic and genetic features that may aid diagnosis and provide additional prognostic information may be assessed in individual circumstances. More information is becoming available through new techniques such as next-generation sequencing and some is being considered for routine clinical use but has not yet been adopted as routine for investigation of lymphomas. Additional investigations, useful but not essential for diagnosis, are highlighted in column B of Appendix A.

9 Integration of laboratory services and reporting

Key points

- All results of investigations obtained from tissue in the course of investigation of suspected lymphoma should be integrated into a single pathology report (integrated report).
- Centralised molecular and other diagnostic laboratory services need to take into consideration specific requirements for diagnosis of lymphoma and ensure a suitable profile of staff and management to meet the requirements of users of their services. 'Users' in this context include other staff in departments of cellular pathology and haematopathology specialists.
- Robust and dedicated lines of communication and information exchange must be established between the different laboratory services contributing to integrated reporting.
- All participants in the laboratory diagnostic process, including key laboratory scientists and consultant pathologists, should be members of their local and/or regional laboratory multidisciplinary teams (LMDTs).
- Within cellular pathology, protocols for tissue handling, test requesting and multidisciplinary interpretation and reporting should be in place, organised collaboratively with centralised molecular (PCR and FISH) and other specialist laboratory services.
- Final responsibility for the integrated report and overall interpretation of results lies with haematopathologists.
- Individual results of the various investigations obtained from one tissue sample (e.g. flow cytometry, FISH and PCR) should not be circulated or made available via hospital information system to managing physicians as stand-alone reports. Their contribution is always as a component of an integrated report, interpreted alongside morphological and immunophenotypic information.

The contribution of laboratory investigations traditionally not housed in cellular pathology departments to the diagnosis of cancer is increasing. Pathologists must be proactive in embracing new technologies and incorporating them into their tissue reports. In doing this, they streamline the diagnostic process, facilitate accurate interpretation of results for treating clinicians and prevent errors arising from the fragmentation of complex diagnostic data. The College's Specialty Advisory Committee on Cellular Pathology has recently recognised the need for general guidance on integration of molecular and other tests into pathology reports in order to maintain the primacy of cellular pathology in cancer diagnosis. The ultimate aim of interrogation of tumour tissue samples by multiple techniques is a meaningful, clinically relevant report which amalgamates all diagnostic and prognostic parameters and interprets them in conjunction with each other. This is best achieved by integration of all of the components of the tissue analysis obtained by a variety of investigative techniques (including molecular genetic tests) into a single, integrated report (IR).

Regardless of regional specificities and organisational differences, the principles of integration of laboratory results in the process of diagnosis of cancer, including lymphoma, should be the same. For this process to be successful, it is necessary that policies and protocols at different levels of involvement (from generalist to extreme specialist) are clearly defined and coordinated with local practices.

Configuration of centralised molecular and other laboratory services contributing to lymphoma diagnosis should be exercised with the full cooperation of, and in consultation with, all key service users on behalf of patients. Pathology departments and haematopathologists are essential members of this service-user community. Formal arrangements should be made to facilitate cooperative daily working and strategy development, through management structures affiliated to centralised molecular laboratories, such as 'user interface groups'. Such structures

will provide the best and overarching professional input for planning services, quality assurance and innovation.

Within centralised molecular services and other laboratories (e.g. flow cytometry), dedicated teams with experience in providing and interpreting tests relevant and specific to lymphoma diagnosis should be available. All the participants in the laboratory diagnostic process relevant to lymphoma should be members of a LMDT to facilitate discussion of all aspects of results between pathologists and laboratory scientists, before incorporation into definitive pathology reports for interpretation.

Dedicated and robust lines of communication must be in place to enable transfer of specimens, cross-referencing of different (internal and external) laboratory reference numbers, electronic data communication and integration. With this in mind, commissioning and organisation of services should consider requirements for dedicated clerical support.

Protocols for requesting of tests, tissue transfer between laboratories and diagnostic algorithms must be developed to fit best with local geographical arrangements and availability of skills. This approach will facilitate the undertaking of appropriate investigations on tissue specimens, without excess and achieving the greatest synergy between the individual tests (Figure 1).

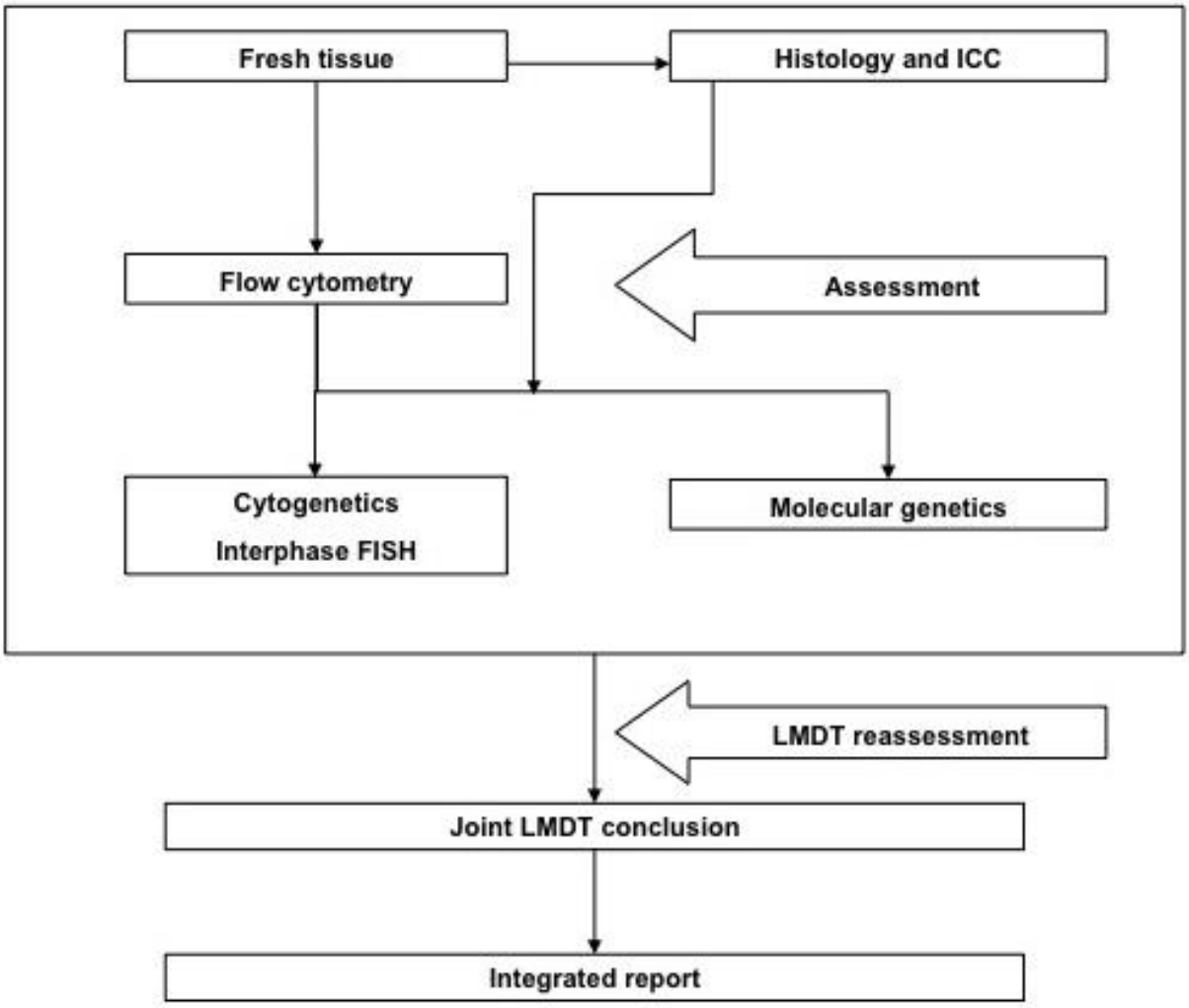

Figure 1 Multidisciplinary laboratory diagnosis of lymphoma:
tissue sample flow chart, assessment algorithm and integrated reporting

Figure 1 provides an example of integrated tissue flow through a number of laboratories in the process of integrated laboratory reporting. Upon receipt, fresh tissue is assessed, placed for routine processing and, if a sufficient amount is deemed available, one part is forwarded to flow cytometry laboratory where cells are disaggregated. An aliquot is forwarded to central molecular and genetic services for DNA/RNA isolation, cell culture and storage. These materials are available for further testing, which is indicated by pathologists based on the H&E appearances, immunohistochemistry and/or flow cytometry. If formalin-fixed material only is available, paraffin sections are forwarded for molecular investigations after initial assessment of HE sections +/- immunohistochemistry. Once all investigations have been completed, the members of the LMDT discuss the results and agree on their interpretation. All the results are amalgamated into a single pathology report and final interpretation and conclusion is provided by the pathologist.

Investigations including immunohistochemistry, cytogenetic and molecular genetic analyses are requested by the reporting haematopathologist, who is responsible for discussion of the results with the members of the LMDT, incorporating the results in the integrated report and providing final interpretation (Figure 1).

10 Diagnostic coding and staging

ICD-O-3 codes with the corresponding lymphoma entities as listed in the WHO lymphoma classification are detailed in Appendix A. These codes largely overlap with the SNOMED-RT codes (Appendix B). These should accompany the pathological diagnosis. The corresponding SNOMED-CT morphology (M) codes are provided in Appendix B. Most laboratory reporting systems now use SNOMED-CT and these codes should be used consistently for definitive diagnostic coding of lymphomas in reports. SNOMED topography (T) codes in standard use should be used in conjunction.

11 Double reporting

Key points

- Double reporting is not considered a mandatory diagnostic practice in haematopathology.
- There is no consensus on the ideal mechanism or the benefits of double reporting of lymphomas; it creates its own diagnostic risks while reducing some others.
- Haematopathologists should consider a rational approach to formalising double reporting on a risk-stratified basis, depending on local conditions (such as staffing levels and experience, geographical location, etc).

A recent survey of BLPG members indicated that there is currently marked variation in practice with regard to formal and informal arrangements for double reporting of haematological cancers. In practice, formal systems exist only in a minority of departments. Consistent evidence on the unequivocal value of double reporting for safety and accuracy of diagnosis is lacking. While this practice potentially provides benefits, it is not without risks. A blanket or naïve approach to double reporting cannot overcome the genuine difficulty in reaching a final diagnosis in highly complex cases. It will not protect against potential ‘rubber stamping’ of an incorrect diagnosis following rapid assessment by the second pathologist, or unintentional ‘leading’ of a consensus discussion by the initial pathologist or a dominant individual. Double reporting will not address circumstances in which a consensus cannot be reached. There is no place for a ‘one size fits all’ solution, either within haematopathology itself or more broadly across all histopathology subspecialties. The strong view of a majority of BLPG members in this survey was that double reporting by two inadequately trained haematopathologists cannot substitute for a report from a single, well-trained haematopathologist. Therefore, mandatory

double reporting is not considered appropriate. Haematopathologists should consider whether a rational approach to formalising double reporting on a risk-stratified basis can be achieved in their local practice. For example, the differential diagnosis between non-neoplastic and neoplastic can be one of the most fundamental errors. Under- or over-diagnosis of Burkitt lymphoma is another critical area.

12 Reporting of small biopsy specimens

Key points

- Reporting of small biopsy specimens follows the same principles for other biopsy material taken for lymphoma diagnosis.
- Pathologists should be mindful of diagnostic limitations of small biopsies, particularly of trucut needle cores.
- Appropriate local arrangements are needed with biopsy takers to facilitate acquisition of diagnostic material of sufficient quality. Likelihood of positive diagnostic utility of a trucut needle biopsy depends on:
  - the needle gauge (at least 1 mm core, needle gauge 17 or lower)
  - number of cores taken (at least two cores, separately embedded).

In recent years, use of trucut needle biopsy for diagnosis of lymphoma has increased massively. A recent audit undertaken by the BLPG indicates that over the past 10 years representation of trucut needle biopsies in the diagnostic workload increased from 9% to 33%. In some centres in the UK it is as high as 95%. While this diagnostic approach reduces cost and patient discomfort and provides rapid access to tissue of potential diagnostic value, there are a number of limitations.

- There is a significant rate of achieving non-diagnostic samples. Specimen adequacy varies between different centres, indicating operator dependence, ranging from 57% to 90%.
- Diagnosis is not always possible due to the quality of the sample itself or as a result of interpretational difficulties. The latter mainly reflect lack of architectural features required for diagnosis of certain entities and inability to apply the full spectrum of investigative techniques due to paucity of material.
- Following needle biopsy, diagnosis of a lymphoma entity with full characterisation, as per the requirements of the WHO classification, is possible in approximately 70% of cases. Only 30–40% of patients who have unsuccessful biopsies undergo a subsequent excision biopsy, with a considerable diagnostic delay of 4–8 weeks.
- The average core diameter recorded in the UK, based on the BLPG national audit data, is 0.75 (needle gauge between 18 and 19). This core diameter results in 80% and 90% likelihood of definitive diagnosis from deep and superficial sites respectively. A core diameter of 1 mm (needle gauge 17) increases the likelihood of successful diagnosis in deep and superficial sites to 90% and 95% respectively.
- In 4% of cases, regardless of needle gauge and with adequate tissue available, diagnosis is not possible due to lack of architectural features needed for diagnosis.
- Differential diagnoses typically difficult to resolve in these circumstances include those between T-cell/histiocyte-rich large B-cell lymphoma and both nodular lymphocyte predominant and classical Hodgkin lymphomas, follicular lymphoma and diffuse large B-cell lymphoma, and classical Hodgkin lymphoma and diffuse large B-cell lymphoma.

Use of fine-needle aspiration for lymphoma diagnosis has also increased in recent years, particularly as endobronchial ultrasound (EBUS) sampling has expanded as a modality for investigating mediastinal lymphadenopathy. This is to be welcomed in centres with access to flow cytometry for immunophenotyping. The relative non-invasiveness, rapidity of turnaround and ability to achieve aspects of immunophenotyping that can be difficult (e.g. immunoglobulin light chain expression) or not currently possible (e.g. FMC7) with fixed tissue are all valuable benefits. It should be noted that the value of cytomorphological assessment without additional immunophenotyping is limited in this context. As EBUS techniques are refined, the procedure increasingly yields mini-biopsy fragments that can be fixed and processed into cell blocks which subsequently offer equivalent utility to needle biopsy cores. With the proviso that these small fragments are generally best processed as a single block rather than separately, comments applicable to the handling and reporting of needle biopsy cores are equally appropriate for these samples.

13 Reporting of frozen sections

Diagnosis of lymphoma in the UK rarely involves frozen section assessment. In unusual circumstances, if lymphoma is considered as the diagnosis on frozen section analysis, pathologists should be aware that establishing a definitive diagnosis is seldom possible without additional studies. In rare circumstances, if suspicion exists, additional useful phenotypic information might be obtained expediently by flow cytometry immunophenotyping while the patient is still on the operating table.

14 Specific aspects of tumours not covered elsewhere

Important diagnostic issues regarding most common lymphomas and contentious issues that require clarification and guidance are explained below.

14.1 CLL/SLL

The largest proportion of patients with CLL/SLL are diagnosed by haematologists based on the investigations undertaken on peripheral blood and bone marrow, yet a proportion of cases are diagnosed by histological examination of lymph node specimens or represent an incidental finding in lymph nodes removed for unrelated reasons. Management of CLL/SLL is rapidly changing; risk stratification and choice of therapy is highly dependent on identification of genetic abnormalities in tumour cells.[11,12] Description of the genetic complexity of CLL/SLL is beyond the scope of this text. However, for the purpose of routine diagnosis, it needs to be emphasised that assessment of important genetic hallmarks of CLL/SLL is best made using blood or a bone marrow aspirate, not fixed, paraffin-embedded lymph node tissue.

14.2 Follicular lymphoma (FL)

Distinction between subtypes of follicular lymphomas (FL) in pathology reports must be clearly made in conjunction with the clinical context. Systemic FL must be distinguished from primary paediatric, primary intestinal, FL *in situ* and primary cutaneous follicle centre cell lymphoma.[1,13-15]

For systemic FL, grade remains the only prognostic histological parameter impacting on management, with a key distinction to be made between grade 3A and grade 3B.[1] Other forms of FL are not graded.

Diagnosis of paediatric FL must be supported by FISH analysis for BCL2, BCL6 and IGH clonality by PCR, since critical distinction from reactive hyperplasia cannot be made reliably by morphology and immunophenotype alone. Diagnosis of FL of the Waldeyer's ring in young individuals should also include immunohistochemistry for MUM1.

14.3 Mantle cell lymphoma (MCL)

MCL represents a spectrum of disease, ranging from very indolent to highly aggressive. This must be assessed in conjunction with the clinical features in each patient. Additional immunohistochemical investigations are helpful. A relatively common problem is absence of demonstrable cyclin D1 expression, in which case demonstration of SOX11 expression and FISH to show t(11;14) are helpful. Most conventional forms of MCL are positive for SOX11 while the clinically indolent variants and MCL *in situ* lack expression of this marker. Proliferation fraction assessed by Ki67 immunostaining is a valuable prognostic parameter. While assessment of proliferation is part of the minimum immunohistochemical panel for the assessment of lymphoma, interrogation of SOX11 is not a core data item and should be investigated upon clinicopathological correlation and at the discretion the pathologist.[13,16-18]

14.4 Diffuse large B-cell lymphoma (DLBCL), 'B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Burkitt lymphoma' (BCLU) and Burkitt lymphoma (BL)

Management and prognosis of aggressive B-cell lymphomas depends on lymphoma type; different therapies for DLBCL and BL have for a long time been justified by the experience of clinical trials. Regarding diagnosis of DLBCL and BL, distinction between the two and support of this distinction by evidence of expression of clinically meaningful biomarkers is currently an area of evolving diagnostic practice.

- **B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLU):** Considerable pathological overlaps exist between DLBCL and BL. In the most recent attempt to clarify diagnostic ambiguity which arises in some cases, the 2008 WHO classification introduced the entity of BCLU.[1] While this provides help in clearing the BL diagnostic category of genetically and biologically unrelated aggressive lymphomas, the new category has attracted significant criticism in that it remains relatively vaguely defined and its use provides little to help assess clinical significance or guide management. Patients with lymphomas classified into this group based on morphology and immunophenotype alone show no survival or treatment response differences compared with DLBCL patients.[19] A significant proportion of lymphomas categorised as BCLU are 'double-hit' or 'triple-hit' lymphomas with rearrangements of MYC most often alongside rearrangements of BCL2 and/or BCL6. These have been shown to pursue a particularly aggressive clinical course but, as yet, no specific effective therapy is available. It has also been shown that DLBCL is significantly heterogeneous with respect to rearrangements of MYC, BCL2 and BCL6.[19–23] In particular, the presence of MYC rearrangement has been shown to affect prognosis and response to therapy adversely; in this regard there is not much difference between DLBCL and BCLU.[19] The forthcoming revision of the WHO classification will reiterate the importance of these genetic alterations and the recognition of "intermediate" morphology. This group of lymphomas will be renamed as "high-grade B-cell lymphoma, NOS", with the requirement to specify the double/triple hit genotype or document absence of rearrangements of BCL2, BCL6 or MYC (personal communication, Dr Leticia Quintanilla-Martinez de Fend).

- **MYC and BCL2 gene rearrangements and expression in DLBCL:** The International Prognostic Index (IPI) defines five factors that independently predict outcome and remains valid for DLBCL in the R-CHOP era.[24] However, clinical factors are still unable to predict patients with progression-free survival of less than 40% over 5 years. Recent studies have demonstrated that there are means of up-regulation and over-expression of MYC in DLBCL other than gene rearrangement and translocation. Thus, while 8–10% of DLBCL harbour MYC gene rearrangement, considerably more (up to 30%) show evidence of significant MYC gene expression (>40%) by immunohistochemistry in the absence of the gene rearrangement. While MYC appears up-regulated as a result of translocation in lymphomas of germinal centre phenotype, over-expression unrelated to

translocation is a feature of DLBCL of non-germinal centre phenotype.[25] An array of recent studies has shown that over-expression of MYC, assessed by immunohistochemistry, in conjunction with the expression of BCL2, even without rearrangement of either gene, provides prognostication independent of the IPI. These cases are referred to as 'double expressors' (DE) and are almost exclusively of activated B-cell type (ABC). DLBCLs with concurrent MYC and BCL2 gene rearrangements confer the worst outcomes in comparison with DEs and cases without either co-rearrangements or co-expression of the two genes. These cases are referred to as 'double hit' (DH) DLBCLs and are almost exclusively of GCB type. The DH DLBCLs are different from those described under the BCLU category. The distinction is purely morphological (intermediate cell size is required for diagnosis of BCLU, excluding lymphomas with large cell morphology). Especially in elderly patients, DE and DH DLBCLs exert particularly aggressive clinical behaviour with a high risk for treatment failure. In young patients, diagnosis of DE or DH DLBCL may aid the decision to consider allogeneic bone marrow transplantation earlier in the course of disease. Novel therapeutic protocols more successful for the management of these lymphomas are emerging.[27,28]

- Different groups of researchers have proposed a variety of approaches to use this information and provide best prognostication, ranging from the application of immunohistochemistry alone to combining protein expression with the assessment of MYC rearrangement by FISH.[19-23, 26, 29-34]

In addition, testing for MYC facilitates accurate distinction between BL and DLBCL in cases in which morphology and immunophenotype deviate from the typical. Reliance on selective testing only in instances where BL is suspected on morphological grounds has been shown not to define a cohort of patients with reproducible, consistent treatment responses. The MRC LY10 trial in BL showed that 50% of patients entered did not have BL when investigated further by assessing MYC status; 50% of the patients in the trial therefore received inappropriate treatment. Without routine and standardised testing for MYC, management in everyday practice would not differ from the experience of that trial. The MRC LY10 trial results indicate the likelihood that many patients with BL in the UK are currently not recognised. This condition is very successfully treated with intensive chemotherapy but not with CHOP-R, the standard treatment for DLBCL.[35] For utility, therefore, testing needs to be considered as per recommendations for the diagnosis of BCLU.

- **Assessment of cell of origin – Germinal centre B-cell (GCB) *versus* activated B-cell type (ABC):** Phenotyping of DLBCL by immunohistochemistry to determine GCB or ABC types as a surrogate for gene expression profiling has been in routine use for some years. The two main immunophenotyping algorithms have been proposed by Hans *et al* and Choi *et al*.[36,37] The value of discriminating between GCB and ABC by IHC has been disputed. The assessment process is criticised for being associated with a significant degree of subjectivity and lack of reproducibility. Clinical utility has been reported as variable in published literature. At present there is no evidence to justify choice of management based on this phenotype. However, new therapies based on the assessment of cell of origin in DLBCL are emerging. In addition, identification of some of the special subtypes of DLBCL does depend on the identification GCB *versus* ABC phenotypic features. Finally, more recent studies comparing the IHC-based algorithms with gene expression analysis do show a high level of concordance between the two, in the range of 95%, and provide prognostic stratification in the R-CHOP era. The practice has become routine and the upcoming update of the WHO lymphoma classification (due in 2016) includes this subtyping by means of IHC as a mandatory requirement.[36-43]

The issues listed above have been variably addressed in practices around the world and within the UK. Regarding genetic testing and immunophenotyping for MYC, BCL2 and BCL6, and categorisation of DLBCL as GCB *versus* ABC, clinicians in the UK vary in their requirement and use of this information. At present, there is no evidence-based guidance on these topics

derived from clinical trials. Clinical utility of GCB *versus* ABC profiles of DLBCL is currently being investigated as the basis for different management approaches within the REMoDL-B trial. This trial incorporates diagnostic, real-time gene expression profiling for randomisation. Despite lack of clinical trial data, substantial literature evidence supports the benefit of this information. A recommendation for diagnostic practice in assessing DLBCL, BL and BCLU regarding determination of phenotype (GCB *versus* ABC), application of IHC for expression of MYC, BCL2 and BCL6 and application of FISH is provided below and indicated in Appendix A.

- Phenotyping for determination of GCB *versus* ABC DLBCL should be undertaken utilising the Hans or Choi algorithm and represents a core data item in all cases of DLBCL.[36,37]

- All DLBCL benefit from immunophenotyping and should be tested for MYC and BCL2. The antibodies for these tests have proved easy to introduce into routine use; reproducibility has been shown in a number of studies.[19-23,26-34] Testing is inexpensive and represents part of the core dataset for DLBCL.

- Testing for MYC and BCL2 rearrangements in DLBCL could be conducted in different ways depending on local preference and levels of financial support. Each of the testing approaches provides certain advantages but also some downsides:

    i) Using selection criteria set out by the WHO classification update (due for publication in 2016). These have been agreed by the Clinical Advisory Board of the WHO classification [personal communication with Dr Leticia Quintanilla-Martinez de Fend, ahead of publication]. The criteria indicate clinical value from MYC and BCL2 FISH testing in DLBCL in the following circumstances: DLBCL of GCB type which is BCL2 positive and contains >40% MYC-positive cells as assessed by immunostaining. The rationale for this recommendation is that MYC gene rearrangement in the absence of BCL2 overexpression does not result in inferior outcomes. However, this approach will not identify approximately 5% of MYC rearranged cases which may show only low levels of MYC expression by immunostaining (<40%). Depending on local preference, FISH for MYC gene rearrangement could be applied as the initial test and only the MYC rearranged cases might be further tested for rearrangements of the BCL2 gene. Alternatively, FISH for all three genes could be conducted at one time. This would result in an amount of redundant tests but would facilitate faster turnaround times.

    ii) All cases of GCB type DLBCL could be tested for MYC rearrangement by FISH, alongside immunostaining, with or without concurrent FISH for BCL2 gene rearrangement. If FISH for BCL2 rearrangement is not conducted alongside testing for MYC, there is value in testing separately only the MYC rearranged cases which are BCL2-positive by immunostaining. A majority of cases harbouring rearrangements of the MYC gene are identified by split-apart probes.

- All cases being considered for a diagnosis of BCLU should be assessed by FISH for MYC, BCL2 and BCL6.

- Morphologically typical cases of BL with a typical CD10+/BCL2- immunophenotype and ~100% Ki67 expression do not require additional genetic investigations. In instances where diagnosis of BL is suspected but the morphological features and/or phenotype are atypical (particularly with BCL2 and MUM1 expression), FISH for MYC, BCL2 and BCL6 should be undertaken as per diagnosis of BCLU.

14.5 EBV

A number of WHO lymphoma entities are characterised by the presence of EBV, as listed in Appendix A. For these entities, testing for EBV is a diagnostic prerequisite and represents a core data item. Pathologists should be mindful that clinical information regarding known or potential causes of immunosuppression is frequently omitted from pathology request forms. This information should be sought proactively and testing for EBV is mandatory in all instances where underlying immunosuppression is suspected. It provides diagnostic and prognostic information.

The majority of EBV-positive non-Hodgkin lymphomas are aggressive malignancies with poor outcomes. In lymphoproliferations arising in immunosuppressed patients, EBV status impacts on the choice and timing of management. A proportion of large B-cell lymphomas in elderly patients are EBV positive and are characterised by much poorer prognosis compared to EBV-negative DLBCL in the same population. This has recently been recognised as a WHO entity (EBV-positive large B-cell lymphoma of the elderly; EBV+LBLE) associated with the immunosenescence that develops in old age. Its prevalence is variably quoted and ranges between 2% in the Western population and 10–15% in Asian and South American countries.[44,45] This entity is probably under-diagnosed, due to overlapping morphological and immunophenotypic features with classical Hodgkin lymphoma and lack of routine testing for EBV.[46,47] While the majority of cases with systemic involvement are aggressive, variants presenting with localised mucosal and cutaneous disease (EBV positive mucocutaneous ulcer [EBV+ MCU]) are important to identify as they have a good prognosis and require little treatment. EBV+ LBLE is frequently misdiagnosed as classical Hodgkin lymphoma.[46]

The current WHO classification sets an arbitrary age of 50+ years for the diagnosis of this lymphoma type. It must be kept in mind that this is only a guide, intended to emphasise the natural but variable occurrence of immunosenescence. This lymphoma can be encountered in young individuals.[46-53] Consequently, in the WHO classification update due in 2016 the entity of EBV+ LBLE will be renamed as EBV+ DLBCL, NOS. EBV+ MCU will comprise a separate entity to highlight its indolent clinical behaviour.

Routine testing for EBV as a core data item in the investigation of a lymphoma that might be EBV+LBLE should be undertaken in the following circumstances:

- polymorphous (PTLD-like) morphology
- Hodgkin-like morphology in an extranodal site (particularly skin and mucosa)
- copious necrosis and angioinvasion.

As EBER is uniformly present in all types of latency in EBV-related tumours, its assessment by *in situ* hybridisation is the ‘gold standard’ for detection of this virus and cannot be substituted by immunohistochemistry for LMP1. The latter is expressed only in restricted patterns of viral latency.

14.6 Future technological developments – next-generation sequencing (NGS)

Evolving high-throughput and genome-wide NGS platforms for interrogation of genetic abnormalities of tumours are providing a wealth of information about the genetic heterogeneity of haematological malignancies, enabling better insight into cellular pathway activities driving tumour development, progression and response to therapy.[38] This information is at present predominantly of academic interest and NGS approaches remain beyond practical, routine and widespread diagnostic use. While any type of genetic abnormality could potentially be identified by this new technology – including single nucleotide changes, insertions, deletions and large structural alterations such as translocations – standardisation is lacking currently. Platforms for some of the most common genetic abnormalities currently interrogated by interphase FISH are not available.

However, platforms for systematic interrogation by NGS of genes of interest in lymphoid malignancies are being developed.[38-42] In addition, the Euroclonality project (formerly Biomed-2) is currently developing an NGS-based platform for antigen receptor gene analysis for clonality assessment, minimal residual disease evaluation and tumour repertoire analysis.[43]

It is vitally important for pathologists to keep these new developments in view and plan for funding their integration into routine diagnostic assessment in the near future.

15 Criteria for audit of the dataset

The Royal College of Pathologists has issued guidance on monitoring of key performance indicators (KPIs) – see www.rcpath.org/clinical-effectiveness/kpi/KPI. In accordance with this guidance, the following items should be subject to audit.

Audit item	Standard
The inclusion of SNOMED codes	95% reports should have T and M coding.
Availability of pathology reports and data at MDT meetings	90% of cases discussed at MDT meetings where biopsies or resections have been taken should have pathology reports/core data available for discussion. 90% of cases where pathology has been reviewed for the MDT meeting should have the process of review recorded.
Use of electronic structured reports or locally agreed proformas	95% data items presented in a structured format.
Core data items	100% of reports should record a full set of core data items, including use of laboratory investigations essential for diagnosis of specific entities.
Integrated reports	100% of reports, where a spectrum of investigations is carried out, are presented in a fully integrated format.
Turnaround times for biopsies and resection specimens	80% of diagnostic biopsy specimens processed initially through non-specialist sites will be provisionally reported within four days of the specimen being received. 80% of diagnostic biopsy specimens in specialist centres, with standard histology, immunohistochemistry and/or flow cytometry will be reported within 10 calendar days of the specimen being received. 80% of diagnostic specimens in specialist centres, requiring additional investigations, including genetic and molecular studies, will be reported within 14 calendar days of the specimen being received.

16 References

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Appendix A WHO classification of lymphoid neoplasms with ICD-O-3 codes, core and non-core diagnostic requirements

Description of histomorphology and interrogation of immunophenotype are essential core data items for the diagnosis of all WHO lymphoma classification entities. Many of the entities, in addition to broad phenotypic characterisation, require interrogation of small numbers of immunohistochemical markers that are entity specific or provide important prognostic information. Some of the entities are characterised by additional genetic features and/or characteristic clinical setting and their interrogation is essential as well. The column headed 'Core data' indicates the mandatory components for diagnosis in addition to morphological description and general phenotypic characterisation. Immunophenotypic and genetic/molecular features that must be interrogated, together with the mandatory requirement to examine clinical context, are highlighted. The 'non-core data' column highlights immunohistochemical and genetic markers that provide additional useful diagnostic, or prognostic information that is not considered mandatory or is not essential for management decisions.

(*) Items usually not interrogated on tissue sections.

WHO 2008 classification of lymphoid neoplasms	ICD-O3	Core data	Non-core data
Precursor lymphoid neoplasms			
B-lymphoblastic leukaemia/lymphoma NOS	9811/3	Genotype (*)	
B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities			
B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); BCR-ABL1	9812/3		
B-lymphoblastic leukaemia/lymphoma with t(v;11q23); MLL rearranged	9813/3		
B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13;q22); TEL-AML1(ETV6-RUNX1)	9814/3		
B-lymphoblastic leukaemia/lymphoma with hyperdiploidy	9815/3		
B-lymphoblastic leukaemia/lymphoma with hypodiploidy	9816/3		
B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31;q32); IL3-IGH	9817/3		
B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); E2P-PBX1 (TCF3-PBX1)	9818/3		
T-lymphoblastic lymphoma/leukaemia	9837/3		
Mature B-cell neoplasms			
Chronic lymphocytic leukaemia/small lymphocytic lymphoma	9823/3	Genotype (*) -17p13 (*P53*) -13q14.3 +12 -11q22-13 (*ATM*)	Phenotype ZAP70, CD38 Genotype(mut)(*) *TP53* *NOTCH1* *MYD88* *SF3B1* *BRC3*
B-cell prolymphocytic leukaemia	9833/3		
Splenic B-cell marginal zone lymphoma	9689/3		
Hairy cell leukaemia	9940/3	Phenotype Annexin A1 Genotype (mut)(*) *BRAF V600E*	
Splenic B-cell lymphoma/leukaemia, unclassifiable *Splenic diffuse red pulp small B-cell lymphoma* *Hairy cell leukaemia-variant*	9591/3	Phenotype Annexin A1	Phenotype *P53*

WHO 2008 classification of lymphoid neoplasms	ICD-O3	Core data	Non-core data
Lymphoplasmacytic lymphoma Waldenstrom macroglobulinaemia	9671/3 9761/3	Clinical context Genotype (mut)(*) *MYD88 L256P*	
Heavy chain disease Alpha heavy chain disease Gamma heavy chain disease Mu heavy chain disease	9762/3	Clinical context	
Plasma cell myeloma	9732/3	Clinical context	
Solitary plasmacytoma of bone	9731/3	Clinical context	
Extraosseous plasmacytoma	9734/3	Clinical context	
Extra nodal marginal zone lymphoma of mucosa associated lymphoid tissue (MALT-lymphoma)	9699/3	Clinical context	Phenotype BCL10 Genotype t(1;14) (*BCL10-IGH*) t(11;18) (*API2-MALT1*)
Nodal marginal zone B-cell lymphoma *Paediatric nodal marginal zone lymphoma*	9699/3	Clinical context Clonality	
Follicular lymphoma Grade 1 Grade 2 Grade 3A Grade 3B *Paediatric follicular lymphoma* *Primary intestinal follicular lymphoma*	9690/3 9695/3 9691/3 9698/3 9698/3 9690/3 9690/3	Grade Clinical context Clonality Genotype BCL2, BCL6 Clinical context	
Primary cutaneous follicle centre lymphoma	9597/3	Clinical context Genotype *BCL2*	
Mantle cell lymphoma	9673/3	Phenotype Proliferation (Ki67)	Phenotype SOX11
Diffuse large B-cell lymphoma (DLBCL), NOS T-cell/histiocyte rich large B-cell lymphoma Primary DLBCL of the CNS Primary cutaneous DLBCL, leg type *EBV positive DLBCL of the elderly*	9680/3 9688/3 9680/3 9680/3 9680/3	Phenotype GCB/ABC, MYC, BCL2 Genotype *MYC, BCL2,* Phenotype EBER(ish)	
DLBCL associated with chronic inflammation	9680/3		
Lymphomatoid granulomatosis	9766/1		
Primary mediastinal (thymic) large B-cell lymphoma	9679/3		
Intravascular large B-cell lymphoma	9712/3		
ALK positive large B-cell lymphoma	9737/3	Phenotype ALK	

WHO 2008 classification of lymphoid neoplasms	ICD-O3	Core data	Non-core data
Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease	9738/3	Phenotype HHV8 EBER(ish)	
Plasmablastic lymphoma	9735/3	Phenotype HHV8 EBER(ish)	
Primary effusion lymphoma	9678/3	Phenotype HHV8 EBER(ish)	
Burkitt lymphoma	9687/3	Phenotype CD10, BCL6, BCL2, Ki67	Genotype *MYC, BCL2, BCL6*
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma	9680/3	Phenotype MYC, BCL2 Genotype *MYC, BCL2, BCL6*	
B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma	9596/3		
T-cell and NK-cell neoplasms			
T-cell prolymphocytic leukaemia	9834/3		
T-cell large granular lymphocytic leukaemia	9831/3		
Chronic lymphoproliferative disorder of NK cells	9831/3	Clinical context	
Aggressive NK-cell leukaemia	9948/3	Phenotype EBER(ish)	
Systemic EBV positive T-cell lymphoproliferative disorder of childhood	9724/3	Clinical context Phenotype EBER(ish) Clonality	
Hydroa vacciniforme-like lymphoma	9725/3	Clinical context Phenotype EBER(ish) Clonality	
Adult T-cell leukaemia/lymphoma	9827/3	Clinical context HTLV1 status	
Extra-nodal NK/T-cell lymphoma, nasal type	9719/3	Phenotype EBER(ish)	
Enteropathy associated T-cell lymphoma	9717/3	Clinical context	
Hepatosplenic T-cell lymphoma	9716/3	Phenotype TCR B/G/D	
Subcutaneous panniculitis-like T-cell lymphoma	9708/3	Phenotype TCR B/G/D	
Mycosis fungoides	9700/3	Clinical context	
Sezary syndrome	9701/3	Clinical context Phenotype Flow cytometry (Sezary cells)	
Primary cutaneous CD30 positive T-cell lymphoproliferative disorder Lymphomatoid papulosis Primary cutaneous anaplastic large cell lymphoma	 9718/1 9718/3	Clinical context	

WHO 2008 classification of lymphoid neoplasms	ICD-O3	Core data	Non-core data
Primary cutaneous gamma-delta T-cell lymphoma	9726/3	Phenotype TCR B/G/D EBER (ish)	
Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma	9709/3	Clinical context	
Primary cutaneous CD4 positive small/medium T-cell lymphoma	9709/3	Clinical context	
Peripheral T-cell lymphomas, NOS	9702/3		
Angioimmunoblastic T-cell lymphoma	9705/3	Phenotype ICOS, CD10, PD1, CXCL13, CD21, EBER(ish)	
Anaplastic large cell lymphoma, ALK positive	9714/3	Phenotype ALK	
Anaplastic large cell lymphoma, ALK negative	9702/3	Phenotype ALK	
Hodgkin lymphoma			
Nodular lymphocyte predominant Hodgkin lymphoma	9659/3	Phenotype	
Classical Hodgkin lymphoma	9650/3	Clinical context Phenotype	
Nodular sclerosis classical Hodgkin lymphoma	9663/3		
Lymphocyte rich classical Hodgkin lymphoma	9651/3		
Mixed cellularity classical Hodgkin lymphoma	9652/3		
Lymphocyte-depleted classical Hodgkin lymphoma	9653/3		
Histiocytic and dendritic cell neoplasms			
Histiocytic sarcomas	9755/3		
Langerhans cell histiocytosis	9751/3		
Langerhans cell sarcoma	9756/3		
Interdigitating dendritic cell sarcoma	9757/3		
Follicular dendritic cell sarcoma	9758/3		
Fibroblastic reticular cell tumour	9759/3		
Indeterminate dendritic cell tumour	9757/3		
Disseminated juvenile xanthogranuloma	No ICD-O code	Clinical context	
Post-transplant lymphoproliferative disorders (PTLD) Early lesions Plasmacytic hyperplasia Infectious mononucleosis-like PTLD Polymorphic PTLD Monomorphic PTLD (B- and T/NK-cell types) Classical Hodgkin lymphoma type PTLD	 9971/1 9971/1 9971/3 9971/3 9971/3	Clinical context Phenotype EBER(ish) Clonality	

Appendix B WHO classification of lymphoid neoplasms and SNOMED morphology codes

ICD-O3	Recommended SNOMED RT code	SNOMED CT CONCEPT ID	SNOMED CT terminology
9591/3	M-95913	1929004	Non-Hodgkin lymphoma, no International Classification of Diseases for Oncology (ICD-O) subtype (morphologic abnormality)
9596/3	M-95963	128798004	Composite Hodgkin and non-Hodgkin lymphoma (morphologic abnormality)
9650/3	M-96503	14537002	Hodgkin lymphoma, no ICD-O subtype (morphologic abnormality)
9651/3	M-96513	128799007	Hodgkin lymphoma, lymphocyte-rich (morphologic abnormality)
9652/3	M-96523	41529000	Hodgkin lymphoma, mixed cellularity (morphologic abnormality)
9653/3	M-96533	112687003	Hodgkin lymphoma, lymphocyte depletion (morphologic abnormality)
9659/3	M-96593	70600005	Hodgkin lymphoma, nodular lymphocyte predominance (morphologic abnormality)
9663/3	M-96633	52248008	Hodgkin lymphoma, nodular sclerosis (morphologic abnormality)
9671/3	M-96713	19340000	Malignant lymphoma, lymphoplasmacytic (morphologic abnormality)
9673/3	M-96733	74654000	Mantle cell lymphoma (morphologic abnormality)
9678/3	M-96783	128800006	Primary effusion lymphoma (morphologic abnormality)
9679/3	M-96793	128801005	Mediastinal large B-cell lymphoma (morphologic abnormality)
9680/3	M-96803	46732000	Malignant lymphoma, large B-cell, diffuse, no ICD-O subtype (morphologic abnormality)
9687/3	M-96873	77381001	Burkitt lymphoma (morphologic abnormality)
9688/3	M-96883	450959001	T-cell/histiocyte rich large B-cell lymphoma (morphologic abnormality)
9689/3	M-96893	128802003	Splenic marginal zone B-cell lymphoma (morphologic abnormality)
9690/3	M-96903	55150002	Follicular lymphoma (morphologic abnormality)
9695/3	M-96953	46744002	Follicular lymphoma, grade 1 (morphologic abnormality)
9691/3	M-96913	55020008	Follicular lymphoma, grade 2 (morphologic abnormality)
9698/3	M-96983	40411000	Follicular lymphoma, grade 3 (morphologic abnormality)
9699/3	M-96993	128803008	Marginal zone B-cell lymphoma (morphologic abnormality)
9700/3	M-97003	90120004	Mycosis fungoides (morphologic abnormality)
9701/3	M-97013	4950009	Sezary's disease (morphologic abnormality)
9702/3	M-97023	3172003	Peripheral T-cell lymphoma, no ICD-O subtype (morphologic abnormality)

ICD-O3	Recommended SNOMED RT code	SNOMED CT CONCEPT ID	SNOMED CT terminology
9702/3	M-97023	3172003	Peripheral T-cell lymphoma, no ICD-O subtype (morphologic abnormality)
9705/3	M-97053	835009	Angioimmunoblastic T-cell lymphoma (morphologic abnormality)
9708/3	M-97083	103682005	Subcutaneous panniculitic T-cell lymphoma (morphologic abnormality)
9709/3	M-97093	28054005	Cutaneous T-cell lymphoma, no ICD-O subtype (morphologic abnormality)
9714/3	M-97143	53237008	Anaplastic large cell lymphoma, T cell and Null cell type (morphologic abnormality)
9716/3	M-97163	103685007	Hepatosplenic gamma-delta cell lymphoma (morphologic abnormality)
9717/3	M-97173	103686008	Intestinal T-cell lymphoma (morphologic abnormality)
9718/1	M-97181	397353001	Lymphomatoid papulosis (morphologic abnormality)
9718/3	M-97183	128804002	Primary cutaneous CD30 antigen positive T-cell lymphoproliferative disorder (morphologic abnormality)
9719/3	M-97193	128805001	Natural killer-/T-cell lymphoma, nasal and nasal-type (morphologic abnormality)
9724/3	M-97243	450906003	Systemic Epstein Barr virus positive T-cell lymphoproliferative disease of childhood (morphologic abnormality)
9725/3	M-97253	450907007	Hydroa vacciniforme-like lymphoma (morphologic abnormality)
9726/3	M-97263	450908002	Primary cutaneous gamma-delta T-cell lymphoma (morphologic abnormality)
9731/3	M-97313	10639003	Solitary plasmacytoma of bone (morphologic abnormality)
9732/3	M-97323	55921005	Multiple myeloma, no ICD-O subtype (morphologic abnormality)
9734/3	M-97343	128921005	Plasmacytoma, extramedullary (not occurring in bone) (morphologic abnormality)
9735/3	M-97353	450909005	Plasmablastic lymphoma (morphologic abnormality)
9737/3	M-97373	450910000	Anaplastic lymphoma kinase positive large B-cell lymphoma (morphologic abnormality)
9738/3	M-97383	450911001	Large B-cell lymphoma arising in human herpesvirus type 8 associated multicentric Castleman disease (morphologic abnormality)
9751/3	M-97511	128809007	Langerhans cell histiocytosis, no ICD-O subtype (morphologic abnormality)
9755/3	M-97553	128813000	Histiocytic sarcoma (morphologic abnormality)
9756/3	M-97563	128814006	Langerhans cell sarcoma (morphologic abnormality)
9757/3	M-97573	128815007	Interdigitating dendritic cell sarcoma (morphologic abnormality)
9758/3	M-97583	128816008	Follicular dendritic cell sarcoma (morphologic abnormality)

ICD-O3	Recommended SNOMED RT code	SNOMED CT CONCEPT ID	SNOMED CT terminology
9759/3	M-97593	450912008	Fibroblastic reticular cell tumour (morphologic abnormality)
9761/3	M-97613	35562000	Waldenstrom's macroglobulinemia (morphologic abnormality)
9762/3	M-97623	6381009	Heavy chain disease (morphologic abnormality)
9766/1	M-97661	41556003	Angiocentric immunoproliferative lesion (morphologic abnormality)
9811/3	M-98113	450949002	B lymphoblastic leukemia lymphoma, no ICD-O subtype (morphologic abnormality)
9812/3	M-98123	450950002	B lymphoblastic leukemia lymphoma with t(9;22)(q34;q11.2); BCR-ABL1 (morphologic abnormality)
9813/3	M-98133	450951003	B lymphoblastic leukemia lymphoma with t(v;11q23); MLL rearranged (morphologic abnormality)
9814/3	M-98143	450952005	B lymphoblastic leukemia lymphoma with t(12;21)(p13;q22); TEL-AML1 (ETV6-RUNX1) (morphologic abnormality)
9815/3	M-98153	450953000	B lymphoblastic leukemia lymphoma with hyperdiploidy (morphologic abnormality)
9816/3	M-98163	450954006	B lymphoblastic leukemia lymphoma with hypodiploidy (Hypodiploid ALL) (morphologic abnormality)
9817/3	M-98173	450955007	B lymphoblastic leukemia lymphoma with t(5;14)(q31;q32); IL3-IGH (morphologic abnormality)
9818/3	M-98183	450956008	B lymphoblastic leukemia lymphoma with t(1;19)(q23;p13.3); E2A-PBX1 (TCF3-PBX1) (morphologic abnormality)
9823/3	M-98233	51092000	B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (morphologic abnormality)
9827/3	M-98273	77430005	Adult T-cell leukemia/lymphoma (morphologic abnormality)
9833/3	M-98333	128820007	Prolymphocytic leukemia, B-cell type (morphologic abnormality)
9834/3	M-98343	128821006	Prolymphocytic leukemia, T-cell type (morphologic abnormality)
9837/3	M-98373	128824003	Precursor T-cell lymphoblastic leukemia (morphologic abnormality)
9940/3	M-99403	54087003	Hairy cell leukemia (morphologic abnormality)
9948/3	M-99483	128833001	Aggressive natural killer-cell leukemia (morphologic abnormality)
9971/1	M-99711	450943001	Post-transplant lymphoproliferative disorder, no ICD-O subtype (morphologic abnormality)
9971/3	M-99713	450944007	Polymorphic post-transplant lymphoproliferative disorder (morphologic abnormality)

Appendix C Lymphoma diagnostic screening protocol for referring pathologists

H&E appearances of fixed lymph node sections are assessed at referring hospital to achieve triage into the following categories:

1. Definitely not lymphoma (e.g. metastatic carcinoma, granulomatous inflammation).*
2. Definitely lymphoma.
3. Indeterminate – possibly lymphoma.

Action

Cases in category 1 complete local diagnostic procedures. Referral for an expert opinion to the regional centre or elsewhere may be sought if wished by local pathologists; this will be outwith the remit of the formal regional MDT-linked service.

Cases in categories 2 and 3 may be investigated locally by lymphoma screening immunohistochemistry to provide a rapid provisional diagnosis (see antibody in Appendix D) or referred directly to the regional centre without immunostaining. Small specimens must be referred directly (see below). It is recommended that lymph nodes containing 'diffuse, mixed' infiltrates (composed of a polymorphous mixture of cells of different sizes, likely to be complex and/or T-NHL) should also be referred immediately without immunostaining.

If local immunohistochemistry is undertaken, all antibodies within each of the panels (Appendix D) must be available and consultants must be fully confident in their interpretation.

These panels are minimal; use of fewer antibodies is insufficiently discriminatory in many cases to justify the cost and time spent. It is recommended that complex cases, in which selection of an appropriate basic single panel is unclear, are referred directly to the regional centre. Local immunostaining must not delay referral to the regional centre by more than two working days.

Endoscopic biopsy specimens and needle biopsy cores taken specifically for clinical suspicion of lymphoma (if the H&E appearances support this suspicion) should always be referred to the regional centre <u>without</u> immunostaining locally, to conserve scarce tissue.

* Regular and frequent network-wide audit of non-referred lymph nodes is advised to ensure that subtle lymphoma masked or accompanied by other pathology is not missed. Co-existent lymphoma obscured by granulomatous inflammation must always be considered, as must the possibility of lymphoma as a second diagnosis in lymph nodes removed to assess metastatic solid malignancies.

Appendix D Specimen handling and recommended processing and reporting timeline

Specimen handling

- Placed directly into 10% neutral-buffered formalin in theatre. If fresh tissue pathway agreed, specimen placed in cytogenetic transfer medium.
- Node sliced if more than 10 mm diameter. Smaller nodes may be left intact until next day.
- Needle biopsies and other small samples (endoscopic material) embedded in separate blocks to maximise utilisation of tissue. For lymph nodes large enough to divide into two or more slices, it is good practice to prepare multiple blocks. This enables maximum diagnostic use of the tissue and facilitates contribution to the translational research studies that are an integral component of most current clinical therapeutic trials. Tissue in any single block should not be larger than approximately 15 mm x 20 mm, since processing and immunostaining artefacts are common if larger pieces are used.
- Endoscopic and needle biopsy cores processed overnight, with extended fixation cycle if required.
- Other resection specimens should be handled as per appropriate subspecialty protocols. If resections are undertaken with upfront suspicion on lymphoma, representative lymph nodes or other fragments of tissue should be managed following the lymph node protocol.
- Sectioning:
    a. Endoscopic/needle biopsy cores (and other small specimens)
        - Duplicate H&E sections prepared from a single level +/- spare unstained sections cut in anticipation of further staining, as per local protocols.
        - If the local 'non-lymphoid' protocol for small specimens includes routine levels, at least 10 unstained sections should be reserved between levels (e.g. 5 between level 1 and 2 plus 5 between level 2 and 3).
        - Small (less than 5 mm diameter and less than 3 mm thickness) **incisional** biopsy specimens should be treated as needle biopsy cores. This also refers to bone marrow trephine biopsies.
        - No up-front multiple section levelling for H&E staining should be practised for small biopsy specimens. This is detrimental for availability of tissue for various essential laboratory investigations.

    b. Lymph node and other excision specimens
        - Duplicate H&E sections prepared (single level) from each block.
        - Larger incisional biopsy specimens should be treated as excised complete nodes.

Processing and reporting timeline

Note: Days referred to below are working days. Weekends and Bank Holidays are not included. However, it should be noted that most current KPI measures do not make allowance for any delay due to intervening non-work days.

Day 1

- Lymph node excised or endoscopic/needle biopsy performed, material sent to cellular pathology department, received and booked in.
- Macroscopic description completed and tissue handled as per processing protocol (see above).
- Endoscopic and needle biopsy cores processed overnight, with extended fixation cycle if required.

Day 2

- Lymph node macroscopic description completed if not already done.
- Tissue processed routinely overnight or during daytime, as indicated by clinical urgency.

a) Endoscopic/needle biopsy cores (and other small specimens)

- H&Es examined by local pathologist.
- If definitely not lymphoma, complete diagnostic procedures as necessary locally. Pathologists should rely on their own discretion in assessing the possibility of lymphoma in each individual case. In this view, local policies should be mindful of the fact that upfront application of broad screening panels on small biopsies may jeopardise subsequent diagnosis of lymphoma. It is therefore advisable that the initial immunohistochemical interrogations are based on prior morphological differential diagnosis and are as conservative as possible.
- If possible or definite lymphoma, complete referral request form as required by reference centre.
- **No immunostaining** to be undertaken locally on endoscopic or needle biopsy specimens with suspected or definite lymphoma, to conserve tissue.
- Provisional diagnosis forwarded to caring physician or MDT to facilitate entry into clinical investigative pathway.
- One H&E retained. Duplicate sent to reference centre with completed request form, pathology report and tissue block, plus any unstained sections, by courier/tracked first-class post.

Day 3

b) Lymph node excision and other incisional biopsy specimens

- H&E examined by local pathologist.
- If definitely not lymphoma, complete diagnostic procedures as necessary locally.
- If possible or definite lymphoma, complete initial immunostaining according to lymph node screening protocol (optional).
- If no immunostains performed locally, complete lymph node regional referral request form.
- Provisional diagnosis forwarded to caring physician or MDT to facilitate entry into clinical investigative pathway.
- If no local immunostains done, one H&E retained. Duplicate sent to reference centre with tissue block(s) and completed request form by courier/tracked first-class post. A copy of any interim local report should also be included for information;
- Endoscopic and needle biopsies removed without initial clinical suspicion of lymphoma as well as materials from other resections should be processed according to local protocol and introduced into this pathway at day 3, as for a lymph node excision specimen.

Days 4–5

- If initial immunostains were carried out on lymph node excision specimens, these are examined by local pathologist.
- Provisional diagnosis forwarded to caring physician or MDT to facilitate entry into clinical investigative pathway.
- Complete lymph node regional referral request form.
- One set of H&Es and the local immunostains retained. Duplicate H&E(s) sent to reference centre with tissue block(s) and completed request form by courier/tracked first-class post. A copy of any interim local report should also be included for information.

Appendix E Immunohistochemistry panels

	Diagnostic setting	Minimum screening panels	Expanded ('expert') panels
1	Lineage*	CD3, CD20	CD45, CD79a, PAX5
2	Proliferation	Ki67	
3	Reactive follicular hyperplasia *versus* FL (with 1+2)	BCL2, CD10	BCL6
4	NHL composed of small cells (with 1+2+3)	CD5, CD23, Cyclin D1	CD43
5	Additional characterisation of small cell B-cell NHL (with 1+2+3+4)		SOX11 **(MCL)** Zap70, CD38 **(CLL/SLL)** CD25, CD11c, DBA44, TRAP, CD103, CD123, Annexin A1 **(HCL)** CD21, CD23 **(dd MCL, FL, MZL, CLL/SLL)**
6	Precursor/lymphoblastic lymphoma (with1+2)		TdT CD34, CD10, CD1a, CD117, CD4, CD8
7	Aggressive B-cell NHL (with 1+2+3)	CD5, CD30	MYC, MUM1 **(DLBCL)** P53 **(BL)**
8	Hodgkin lymphoma (with 1)	CD15, CD30	OCT2, BOB1 **(cHL)** Fascin, PU1 **(cHL *vs* TCRBCL)** CD21, CD4, PD1, CD57, ICOS, EMA **(NLPHL)**
9	Peripheral T-cell lymphoma (with 1+2)		CD2, CD5, CD7, CD4, CD8, CD30, CD43 **(general)** TCR-B, TCR-G, TCR-D **(gamma/delta)** CD16, CD56, CD57, TIA1, Perforin, Granzyme **(cytotoxic)** ALK, EMA **(ALCL)** CD25, FOXP3 **(ATL)** CD10, CD21, PD1, CXCL13, ICOS, BCL6, CD57, EBER(ish) **(AIL)**
10	Plasmacytome, myeloma, plasmablastic lymphoma (with 1+2)		CD138, K/L, CD56, EMA, MUM1, cyclinD1 EBER(ish), HHV8 **(PBL, PEL)**
11	Histiocytic/dendritic tumours (with 1+2)		CD4, S100, langerin, CD1a, CD123, D2-40, CD21, CD23, CD35, CD11c, CD68
12	Undifferentiated malignancy (with 1+2)	CD45, CD30, S100, cytokeratin (broad spectrum)	

Note (*) No antigen is uniquely lineage specific and few are expressed constantly through the complete sequences of differentiation, maturation and activation that haemopoietic cells undergo.

Appendix F Immunohistochemical markers used in diagnosis of lymphoproliferative conditions

See explanation of abbreviations at end of table, page 46.

Immunohisto-chemical markers	Lymphoproliferative conditions
CD45 (LCA), CD45RA, CD45RO	These are a number of antibodies against tyrosine phosphatases present on the surface of almost all haematolymphoid cells. CD45 (LCA) recognises all the isoforms. Utility of this antibody is in confirming haematolymphoid origin of proliferations. Negativity for this marker is also of significant diagnostic value. The negative haematolymphoid neoplasms include a proportion of ALCLs, ALK+ LBCL, cHL and plasma cell neoplasms. There are several antibodies against CD45RA including 4KB5, MB1, KiB3 and MT2. The most commonly used MB1 and KiB3 stain most B-cell lymphomas. MT2 in reactive tissues and FL show a pattern of staining of germinal centres similar to that obtained with BCL2 immunostaining. MT2 stains a proportion of T-cell lymphomas. Antibodies against CD45RO include UCHL1, A6 and OPD4 which are used in confirming T-cell derivation.
CD79a, CD20	B-cell markers in widespread use though their spectrum of staining is slightly different. CD79a expression starts earlier in B-cell development than expression of CD20. CD20 expression is lost at the late post-follicular stage of plasma cell differentiation. Expression of CD20 may be lost in relapsed or persisting B-cell lymphomas after rituximab treatment.
PAX5	The PAX-5 gene is essential for B-cell differentiation. Four isoforms of the gene are known and PAX-5a has been most studied. It is expressed by immature and mature B cells but is down-regulated during terminal differentiation into plasma cells. Expression is nuclear. PAX-5 influences the expression of other B-cell specific genes, including CD19, CD20 and CD79a and precedes the expression of CD20. Reactive lymphoid tissue shows the same distributions of expression of PAX-5 as CD20. They are both positive in germinal centres, mantle zones, marginal zones, monocytoid B-cells (weak) and intra-epithelial lymphocytes in extranodal sites. Haematogones in the bone marrow are also PAX-5 positive. In diagnosis of precursors B-cell lymphoma/leukaemia (ALL) it is used as a surrogate of CD19 and positivity in this context outlines commitment to B-cell lineage, in the absence of any other B-cell marker. It is also helpful in differentiating HRS cells of cHL (where it typically shows weak nuclear expression) from T and 'null' cells of ALCL lymphoma. However, expression of PAX-5 is rarely seen in ALCL of T/null cell phenotype as well. It may aid distinction of B-cell lymphomas with plasmacytic components (such as CLL/SLL with lymphoplasmacytic differentiation, LPL and MZL of MALT type) from plasma cell myeloma. It is of note that PAX-5 is also commonly expressed by Merkel cell carcinoma, small cell carcinoma and alveolar rahbdomyosarcoma, small blue round cell tumours that are often in the differential diagnosis with lymphoma.
OCT2, BOB1	These two markers are used in conjunction. They aid transcription of immunoglobulin genes in B-cells and play a role in germinal centre formation and differentiation of B-cells into plasma cells. Expression of both these markers is nuclear and is uniformly distributed in reactive, non-neoplastic B-cells. Most non-Hodgkin B-cell lymphomas and NLPHL show strong nuclear expression. Most cases of cHL are negative for both or at least one of these markers.
PU1	Transcription factor that regulates the expression of immunoglobulin and other genes important for B-cell development. Absence of PU1 results in a block in the early stage of B-cell development. It is crucial for the expression of CD20, CD72 and CD79a. It is expressed in B-lymphocytes, immature and mature, including mantle cells and most cells of the germinal centre, but not in plasma cells, histiocytes or plasmacytoid dendritic cells. Lack of expression of PU1 has been shown in cHL, while NLPHL and TCRBCL retain expression. This is the likely contributing factor to the lack of immunoglobulin expression and incomplete B-cell phenotype characteristic of the HRS cells in cHL.

Immunohisto-chemical markers	Lymphoproliferative conditions
Kappa and lambda light chains IgG, IgG4, IgM, IgA, IgD	Light chain immunostains are very useful in assessing clonality but are technically difficult and must be interpreted with caution. Some laboratories prefer to use *in-situ* hybridisation for kappa and lambda mRNA, at least when there is visible plasma cell maturation. Use of *in-situ* hybridisation for the assessment of clonality of lymphocytic B-cell population is problematic. Heavy chain staining can also be useful in identifying some lymphoma sub-types and assessing suspected plasma cell myeloma. IgG4 has become important in identifying systemic IgG4-related disease.
Cyclin D1	Cyclin D1 is not normally expressed by lymphoid cells. It is expressed in MCL displaying nuclear positivity, reflecting t(11;14) translocation. However, up to 20% of MCLs may be negative and require alternative immunostaining (e.g. with SOX11, cyclin D2) or FISH for diagnostic confirmation. Cyclin D1 positive DLBCL has also been described and positivity is seen in HCL and a proportion of plasma cell myelomas. In reactive lymphoid tissue and bone marrow, normal macrophages and histiocytes show strong nuclear expression.
CD21, CD23 and CD35	These identify follicular dendritic cells (FDC); they show different patterns of staining, reflecting functional variation within the FDC population. Their use can help in the identification of follicular growth patterns and in the diagnosis of AITL. CD23 is normally expressed by many follicular dendritic cells and a minor sub-population of mantle B-cells. CD23 is also expressed by 93% of CLLs, in occasional cases of other small B-cell lymphomas (particularly in FL) and in some LBCLs (particularly mediastinal LBCL). They are robust markers for follicular dendritic cell tumours.
BCL2	Expressed in many normal T- and B-cells and many lymphomas. It is not expressed in reactive germinal centres and therefore BCL2 immunostaining is useful in distinguish follicular hyperplasia from FL. Care must be taken not to misinterpret reactive germinal centre T-cells, which are normally BCL2-positive and may be present in abundance for positive germinal centre B-cells. In systemic, nodal FL, expression of BCL2 is a consequence of the t(14;18), however in most other small cell B-cell lymphomas BCL2 is positive as a result of epigenetic control in the absence of the translocation. Therefore, BCL2 cannot be used to distinguish FL from other systemic small lymphoid cell B-cell lymphomas. Primary cutaneous follicle centre lymphoma and paediatric FL do not express BCL2 and show no evidence of t(14;18). A significant proportion of grade 3B FL are also negative for this marker and lack the translocation. In DLBCL expression of BCL2 in conjunction with MYC positivity represents a significant adverse prognostic factor.
BCL6	Nuclear antigen expressed in germinal centre cells and FL. BCL6 also reflects cellular activation and is often seen in B-cell lymphoid blasts unrelated to their germinal centre origin. This could be seen in CLL/SLL, MZLs and a proportion of DLBCLs on non-germinal centre derivation. HRS cells of cHL are usually negative while L&H cells of NLPHL express BCL6. This marker is also positive in T-cells of germinal centre derivation and hence in AITL as well.
LEF1	Nuclear expression of the lymphoid-enhancer-binding factor 1 has been shown in all cases of CLL/SLL, even those which are CD5 negative. This marker is not expressed in marginal zone lymphoma, mantle cell lymphoma or follicular lymphoma and is regarded as a robust marker of CLL/SLL.
IRTA1	The immunoglobulin superfamily receptor translocation-associated 1 (IRTA1) is selectively associated with normal (Peyer's patches) and acquired MALT. It is expressed in both extranodal (93%) and nodal MZLs (73%) but is not seen in other small B-cell NHLs and is therefore regarded as a specific marker for MZLs. It is an important aid to diagnosis of MZLs, highlighting tumour cells in colonised follicles. Expression is membranous.

Immunohisto-chemical markers	Lymphoproliferative conditions
Annexin A1	Annexin A1 is encoded by the ANXA1 gene, upregulated in HCL, in which it shows membranous or sometimes cytoplasmic expression. It is currently considered the most sensitive and most specific marker for diagnosis of HCL. By contrast, B-cell lymphomas other than HCL are negative. Importantly, splenic MZL, HCv and diffuse red pulp small B-cell lymphoma are all negative for this marker. Assessment of expression in bone marrow can be difficult due to strong background staining of the myeloid cells.
CD11c	CD11c is an integrin, member of the cellular adhesion molecule family. It is strongly expressed in HCL and, when co-expressed with CD22, was proposed a unique marker of this lymphoma. It is also variably expressed in acute myeloid leukaemia with monocytic/monoblastic differentiation and some cases of CLL. It is also one of the best markers used for identification of macrophages/histiocytes.
CD103	CD103 is an alpha-E integrin expressed by intestinal intraepithelial T-lymphocytes, mucosal B-cells, and HCL cells. In HCL the staining pattern is predominantly membranous. It is useful in differentiating HCL and HCv from other small cell lymphomas including CLL/SLL, MCL, FL, LPL and MZL. Intestinal lymphomas including EATL and FL are positive.
SOX11	SOX11 is a transcription factor up-regulated in the majority of MCLs where its nuclear expression co-localises with cyclin D1. In the context of diagnosis of MCL, lack of expression of this marker identifies a small subset of MCLs characterised by CD5 lymphocytosis, minimal lymph node involvement and low proliferation. These cases pursue an indolent clinical course and require little therapeutic intervention in comparison to conventional SOX11 positive cases. SOX11 is also expressed in lymphoblastic lymphomas, DLBCL and Burkitt lymphoma but is not seen in other small B-cell NHLs. Thus, its positivity in cases of cyclin D1 negative, CD5 positive small B-cell lymphomas indicates diagnosis of cyclin D1 negative MCL. This should be corroborated by immunostaining for cyclin D2 which is usually detectable in cyclin D1 negative cases of MCL.
ZAP70	'Zeta Associated Protein-70' is a member of the Syk family of tyrosine kinases. It is involved in T and NK cell receptor transduction and also plays a role in the transition of pro-B to pre-B cells in the bone marrow. In reactive lymph nodes nuclear ZAP70 staining is seen in paracortical T lymphocytes and rare, scattered, small lymphocytes in the mantle zones and germinal centres. Histiocytes can sometimes express ZAP70 in a granular cytoplasmic pattern. It is expressed by B-precursor lymphoblastic lymphoma and a subset of CLL/SLL. It is so far the best surrogate imunohistochemical marker of the CLL/SLL mutational status and is an independent prognostic marker in this context. Nuclear positivity correlates with the clinically more aggressive unmutated phenotype. Assessment of expression requires correlation with the immunostains for background T-cells (CD3, CD5).
Ki67	Nuclear antigen expressed in nuclei of cells in cycle but not in G0. It can help in identifying highly proliferative lymphomas such as Burkitt lymphoma, in which the Ki67-positive fraction approaches 100% and the majority of cells show uniform and very strong expression. While Burkitt lymphoma generally shows proliferation of 100%, other aggressive B-cell lymphomas do as well and this is no longer a marker that helps to distinguish between Burkitt and non-Burkitt B-cell NHLs. Proliferation assessment in MCL is of significant prognostic value. Ki67 is also useful in distinguishing reactive follicular hyperplasia from follicular and other forms of B-cell lymphomas with nodular growth patterns.

Immunohisto-chemical markers	Lymphoproliferative conditions
EBV	Anti-LMP1 identifies EBV in about 20–30% of infected lymphoma cells of various types. It should be emphasised that immunostaining for LMP1 alone does not exclude EBV positivity and involvement of this virus in pathogenesis of certain lymphomas. LMP1 is expressed only in latency types 2 and 3, which include cHL and a range of other lymphomas, many associated with underlying severe immunosuppression (latency 3). LMP1 does not stain tumours that belong to latency 1 such as extranodal T/NK lymphoma of nasal type, lymphomatoid granulomatosis and Burkitt lymphoma. Therefore, the gold standard for assessment of EBV is *in-situ* hybridisation for EBER, which is present in all types of latency.
P24	HIV p24-gag viral capsid protein can be demonstrated by immunohistochemistry. The staining is localised to follicular dendritic cells. Cutaneous Langerhans cells are also immunoreactive for p24-gag in early stages of HIV infection, even prior to seroconversion. Positivity in various parenchymal cells of a variety of organs is not unusual. It is frequently used to identify the presence of the virus in patients with progressive generalised lymphadenopathy. It is highly specific and sensitive. Identified positivity indicates diagnosis of HIV infection and has equivalent value to the serological HIV test.
HHV8	An antibody reacting against the Latent Nuclear Antigen of the Kaposi Sarcoma Virus (HHV8). The immunostaining is nuclear and detected in Kaposi sarcomas of HIV positive and other immunosuppressed and elderly patients. In addition, this antibody helps diagnosis of primary effusion lymphoma and plasmablastic variant of Castleman's disease of HIV-positive patients.
CD2, CD3	Robust T-cell markers, expressed by normal T-cells and most T-cell lymphomas. T-cell lymphomas may show loss of pan-T-cell markers so application of a number of markers for general T-cell differentiation is advised. Some myeloid tumours express these antigens. In particular, systemic mastocytosis is characterised by co-expression of CD2 and CD25.
CD5, CD7	Pan T-cell markers. CD5 is also expressed by some B-cell lymphomas (see above). Expression may be lost in some T-cell lymphomas and CD7 may also be down-regulated in inflammatory conditions.
CD4, CD8	Markers of T-cell subsets are useful in the differential diagnosis of T-cell proliferations and in T-cell lymphoma typing. CD4 is abundantly expressed in histiocytes which may hamper assessment of expression in presumed T-cells. Plasmacytoid dendritic cell neoplasm is also positive for CD4.
CD16	Expressed by NK cells, some T-cells, NK neoplasms and a subset of LGL leukaemias.
CD43	One of the major glycoproteins of thymocytes and T lymphocytes, used as a pan-T-cell marker. It is also expressed by myeloid cells and macrophages. In addition, it is aberrantly expressed in MCL CLL/SLL, a proportion of MZL and some DLBCL. FL and HCL do not express this marker. In this context, CD43 may be useful in resolving differential diagnosis between FL and DLBCL or MZL.
CD56, CD57	Natural killer (NK) and NK-like T-cell markers, essential for the diagnosis of malignancies derived from these cells. CD56 is expressed in NK/T-cell lymphomas of nasal type. CD57 is expressed by germinal centre T-cells and is also a marker of LGLs; it is useful in the diagnosis of NLPHL. CD56 is also expressed in many cases of plasma cell neoplasia (but not in normal plasma cells) and in non-lymphoid tumours.

Immunohisto-chemical markers	Lymphoproliferative conditions
CD30, CD15	Useful in the diagnosis of Hodgkin lymphoma and ALCL. Interpretation of CD30 depends on the detection system used; the more sensitive the technique, the more reactive B-cell blasts will be detected. Available antibodies to CD15, a carbohydrate antigen, are all IgM clones and their detection by reagents optimised for the more usual IgG monoclonal antibodies is sometimes suboptimal. In addition, CD15 is fixation sensitive; overall, expression of CD15 is demonstrable in approximately 80% of cHL; it is expressed in 15–20% of ALCL. Co-expression of CD15 with CD30 does not mandate diagnosis of cHL. Expression of CD15 is seen in a range of EBV positive lymphomas with Hodgkin-like morphology usually associated with various types of immunosuppression. Diagnosis should rely on the assessment of a range of parameters including morphology and clinical presentation and should not rely on co-expression of CD30 and CD15 alone.
CD246 (ALK-1, 5A4)	These antibodies enable visualisation of the nucleophosmin–anaplastic lymphoma kinase (NPM-ALK) fusion protein associated with t(2:5) and variant translocations involving the *ALK* gene. Positive staining identifies a subgroup of ALCL with good prognosis and is currently the defining feature of this entity within WHO 2008. A rare variant of LBCL is also characterised by nuclear ALK expression.
CD1a	Identifies Langerhans cells in the skin and in lymph nodes. It may be significantly increased in dermatopathic lymphadenopathy. Langerhans cell histiocytosis is also positive. Most T-lymphoblastic lymphomas are positive.
TdT	Terminal deoxynucleotidyl transferase (TdT) is expressed by precursor T- and B-cells, and precursor leukaemias and lymphomas. A minority of these tumours may be negative. This is a nuclear stain; cytoplasmic staining may occur with suboptimal technique and should be ignored. TdT is also expressed in up to 10% of myeloid leukaemias. Presence of TdT-positive haematogones in bone marrow trephine sections should not be mistaken for neoplasia. A rare but diagnostically important setting of aberrant expression of this marker is in a small proportion of small cell lung carcinomas.
CD38, CD138	Both are markers of plasma cells. CD138 is not expressed on mature circulating B-cells but may be expressed on lymphomas with plasma cell and plasmablastic differentiation. CD138 is also a robust epithelial marker. In addition CD38 is positive in a proportion of chronic lymphocytic leukaemia/small lymphocytic lymphomas where its expression to an extent correlates with the unmutated genotype.
EMA	This is one of several glycoproteins found in human milk fat globule membranes (HMFGP). Because HMFGP are packaged in the Golgi apparatus, dot-type reactivity in the Golgi zone may be seen. The glycoprotein identified with EMA is now known to be one of a series of glycoproteins or mucins designated as MUC1. This marker is found on a wide range of epithelial and soft tissue tumours but also in a range of normal haematopoietic cells, lymphocytes and plasma cells. In the context of diagnosis of haematolymphoid malignancies, EMA is seen expressed in myelomas and plasmacytomas, all subtypes of ALCL, ALK1+ LBCL and nodular lymphocyte predominant Hodgkin lymphoma. Importantly, it is not seen in RS cells of cHL or TCRBCL.
IRF4/MUM1	Nuclear positivity for MUM1 is a consequence of activity of the NFkB pathway. This is seen in late post follicular B-cells including plasma cells. However, its expression should not be interpreted as a definite indicator of plasmacytic differentiation. MUM1 is positive in non-germinal centre type of DLBCL and is part of the Hans and Choi algorithms for typing of DLBCL. HRS cells of cHL are usually positive but not L&H cells of NLPHL. This marker is also expressed in a range of T-cell lymphomas and has recently been suggested as a useful discriminator between cutaneous ALCL and lymphomatoid papulosis.

Immunohisto-chemical markers	Lymphoproliferative conditions
GCET1 and FOXP1	GCET1 is highly expressed in normal germinal centre B-cells and B-cell lymphomas of germinal centre derivation including FL and germinal centre type DLBCL. Expression is cytoplasmic and membranous. FOXP1 is a transcription factor essential for transcriptional regulation of B cell development. By gene expression profiling it has been shown to be highly expressed by activated B-cell type DLBCL. Expression is nuclear. These two markers are part of the Choi algorithm for typing of DLBCL with CD10, MUM1 and BCL6.
MYC	Participates in the regulation of gene transcription and cell cycle. The antibody displays nuclear expression of MYC which could be seen in normal cells in cycle. It must be emphasised that evidence of expression of MYC does not equate with the translocational status of the MYC gene as the protein could be overexpressed in the absence of the translocation as a result of epigenetic regulation. DLBCL, which co-express MYC and BCL2 regardless of the presence or absence of the MYC gene translocation, pursue an aggressive course. In this context, the cut-off point for positivity is 40% nuclei positive for MYC.
Cytotoxic molecules: TIA1, Granzyme B, Perforin	T-cell intracellular antigen 1 (TIA1), granzyme B and perforin are cytotoxic molecules stored in cytoplasmic granules. TIA1 is present in all cytotoxic cells while granzyme B and perforin expression depends on the activation status. The expression of cytotoxic molecules is helpful in typing of T-cell and NK-cell neoplasms. Aggressive NK-cell leukaemia, extranodal NK/T-cell lymphoma of nasal type, subcutaneous panniculitis-like T-cell lymphoma and EATL express TIA1, granzyme B and perforin. Hepatosplenic T-cell lymphoma, T-cell large granular lymphocytic leukaemia and T-cell prolymphocytic leukaemia usually express TIA1 but not granzyme/perforin. HTLV-associated ATLL does not express cytotoxic molecules. These are also expressed by CD8+ PTCL.
TCR	The T-cell receptor (TCR) performs an antigen-recognition function on the surface of T-cells, analogous to that of immunoglobulins on the surface of B-cells. The TCR associated with the CD3 complex and comes in two forms: the alpha/beta and gamma/delta heterodimers. The alpha/beta heterodimer is present on ~90% of thymocytes and mature peripheral T-cells, ~60% of precursor T-cell lymphoblastic lymphomas and ~70% of PTCLs. The gamma/delta heterodimer is present on a small numbers of thymocytes, a small number of cells in the skin and mucosa-associated lymphoid tissue and in rare types of T-cell lymphomas: hepatosplenic T-cell lymphoma and primary cutaneous γδ T-cell lymphoma.
ICOS	The inducible T-cell co-stimulator (ICOS) protein is a member of the CD28 co-stimulatory receptor family and identifies TFH cells, a specialised subpopulation of T helper cells residing primarily in germinal centre. ICOS expression appears to be restricted to certain subsets of T-cells, with the highest expression on CD4-positive T-cells and moderate expression on T regulatory cells (Tregs). The expression of ICOS has been described in AITL, follicular variant of PTCL, primary cutaneous CD4+ lymphoma, and cases of PTCL, NOS with borderline AITL features. Expression of ICOS is variably associated with other markers of follicular T-helper cell differentiation including CD10, BCL6, CXCL13 and PD-1. In the context of AIL, ICOS is at present considered most sensitive.
PD1	Similar to ICOS, this is a member of the CD28 family of receptors and is expressed on all CD4+ T-cells and half of CD8+ T-cells. It is expressed by activated T-cells, B-cells and myeloid cells. There are at least 2 ligands for PD-1, PD-L1, and PD-L2, which are expressed on a range of cells. The expression of PD1 has been widely reported in AITL; it is also expressed by T cells associated with neoplastic B cells in NLPHL. It is considered to be a more specific marker for AITL as, unlike CD10 and BCL-6, PD-1 is expressed by few B cells and it appears to stain a higher number of CD3+ neoplastic cells compared to CD10 and BCL-6. It is also found to be useful in diagnosis of cutaneous CD4+ small/medium size T-cell lymphoma.

Immunohisto-chemical markers	Lymphoproliferative conditions
FOXP3	The protein encoded by FOXP3 is a member of the forkhead/winged-helix family of transcriptional regulators. It is a marker of T regulatory cells and is expressed in adult ATLL. In FL and cHL a high numbers of FOXP3+Tregs correlate with better overall survival. However, both in FL or cHL, this is not part of routine investigations or basis for specific therapeutic decisions.
CD25	CD25 is the interleukin-2 receptor, widely expressed on activated T-cells, B-cells and macrophages and in both non-Hodgkin and Hodgkin lymphomas. CD25 is expressed in ATLL associated with HTLV, some peripheral T cell lymphoma NOS, mycosis fungoides, especially in the large cell transformation, and in some ALCLs. It is also a widely used marker of HCL and is expressed in some cases of splenic MZLs. Neoplastic mast cells show CD25 immunoreactivity together with CD2. This pattern of co-expression is highly specific for diagnosis of systemic mastocytosis (reactive mast cells in the marrow are CD25/CD2 negative). CD25 can be expressed in HRS cells as well as by reactive T-cells in the tumour microenvironment.
TCL1	The TCL1 gene (14q32.1) is involved in the leukaemogenesis of mature T cells and its overexpression is observed in more than 90% of T-PLLs. Chromosomal rearrangements bring the TCL1 gene in close proximity to the T-cell antigen receptor (TCR)-alpha or TCR-beta regulatory elements. In normal T cells TCL1 is expressed in CD4-/CD8- cells, but not in cells at later stages of differentiation.
CD123	CD123 is the interleukin-3 receptor alpha chain regarded as a reliable marker of plasmacytoid dendritic cells (PDCs). These represent one of the three subsets of normal dendritic cells, originate from CD34+ bone marrow progenitor cells and have been identified in the thymus and lymphoid tissues, including tonsil, bone marrow, peripheral blood and spleen. It is a useful marker in the diagnosis of blastic plasmacytoid dendritic cell neoplasms (formerly known as ‘blastic NK-cell lymphoma’ or ‘CD4-positive/CD56-positive neoplasm’) and reactive proliferations of plasmacytoid dendritic cells including Kikuchi lymphadenitis.
D2-40 (podoplanin)	D2-40 is directed against the M2A antigen, a sialoglycoprotein found on the cell surface of testicular gonocytes, germ cell tumours, lymphatic endothelium and mesothelial cells. Using a human podoplanin-Fc fusion protein, it has been shown that the commercially available mouse monoclonal antibody D2-40 specifically recognised human podoplanin. This antibody was initially described as specific and sensitive for diagnosis of follicular dendritic cell tumours, however a large spectrum of tumours have been shown to express this marker including mesothelioma, a range of vascular tumours, carcinomas and benign epithelial tumours.
S100	This marker identifies interdigitating dendritic cells and their tumours. It is also positive in a small proportion of T-cell lymphomas.
Langerin	Langerin is a type II transmembrane C-type lectin specific to normal Langerhans, Langerhans cell histoocytosis and langerhans cell sarcoma.
Fascin	Fascin is an actin-binding protein which is specifically expressed by some dendritic cells and high percentage of HRS cells of cHL.
CDw75 (LN1)	CDw75 is a neuraminidase-sensitive sialoprotein, present on cell membrane and cytoplasm of germinal centre B-cells and derived lymphomas. LN1 reacts with erythroid precursors, ductal and ciliated epithelial cells of kidney, breast, prostate, pancreas, lung, and with glioblastomas, astrocytomas, and L&H cells of NLPHL. LN1 is shown to be a reliable antibody for ascribing a B-cell phenotype in known lymphoid tissues.

Abbreviations

ALL	acute lymphoblastic leukaemia
AITL	angioimmunoblastic T-cell lymphoma
ALCL	anaplastic large cell lymphoma
ATLL	adult T cell leukaemia/lymphoma
CLL/SLL	chronic lymphocytic leukaemia/small lymphocytic lymphoma
cHL	classical Hodgkin lymphoma
DLBCL	diffuse large B-cell lymphoma
EATL	enteropathy associated T-cell lymphoma
FL	follicular lymphoma
HCL	hairy cell leukaemia
HCv	hairy cell variant
HRS	Hodgkin/Reed-Sternberg
LBCL	large B-cell lymphoma
LCA	leukocyte common antigen
LGL	large granular lymphocyte
LPL	lymphoplasmacytic lymphoma
MALT	mucosa associated lymphoid tissue
MCL	mantle cell lymphoma
MZL	marginal zone lymphoma
NLPHL	nodular lymphocyte predominant Hodgkin lymphoma
PLL	prolymphocytic leukaemia
PTCL	peripheral T-cell lymphoma
TCRBCL	T-cell rich B-cell lymphoma
TFH	T-follicular helper

Appendix G Reporting proforma for lymphoma specimens

Surname: ..... Forenames: .....Date of birth: .....

Patient identifier (CHI/NHS no):..... Referring organisation: .....Hospital no: .....

Biopsy taker: ..... Caring physician: .....

Specimen number (referring organisation): .....…… Dispatch date from referring organisation .....

Reporting organisation:..... Date of receipt:..... Date of reporting: .....

Specimen number (reporting organisation): .....…… Pathologist: .....

Clinical context, relevant clinical history, including immunosuppression status

Indication for investigation

Primary diagnosis ☐ Staging ☐ Relapse/progression ☐
Re-staging ☐ Review ☐ Clinical trial ☐ Post mortem ☐

Specimen type

Excision biopsy ☐ Needle core biopsy ☐ Punch biopsy ☐
Endoscopic biopsy ☐ Extranodal resection (including splenectomy) ☐
Bone marrow trephine ☐ Other biopsy (specify).....

Fresh tissue sampling

Imprint: Yes ☐ No ☐ Frozen tissue: Yes ☐ No ☐
Flow cytometry / genetic / molecular testing: Yes ☐ No ☐

Specimen description

Site ……. Size ….. x ……. x …… mm
Weight …… g Macroscopic description:

Provisional (referring) diagnosis

Tumour type

WHO entity diagnosis, including grade, where applicable:
ICD-O morphology code:
(If diagnosis is incomplete/uncertain, provide reasons):

Clinical context:

Corroborated by clinical context ☐ Not corroborated by clinical context ☐ Not applicable ☐

Microscopic description

Components of integrated report

Immunophenotype (immunohistochemistry and/or flow cytometry):

Genotype and clonality (karyotype, FISH, PCR for clonality, mutational analysis):

Other investigations (specify):

Integrated report interpretation and summary

SNOMED codes T……..… M……..

Pathologist .....…….. Date…….../……...../…….

Appendix H Proforma in list format

Element name	Values	Implementation comments
Full name; date of birth; sex; NHS number; hospital number; referring organisation; reporting organisation; date of biopsy/surgery; biopsy taker (surgeon, interventional radiologist or other physician); caring physician (haematologist, oncologist or other, if known); date of dispatch from referring organisation; date received at reporting organisation.	Standard data formats	
Clinical context, relevant clinical history, including immunosuppression status	Free text	
Indication for investigation	Multiple selection value list: • primary diagnosis • staging • relapse/progression • re-staging • review • clinical trial • post mortem	
Specimen type	Single selection value list: • excision biopsy • needle core biopsy • punch biopsy • endoscopic biopsy • extranodal resection (including splenectomy) • bone marrow trephine • other biopsy (specify)	
Fresh tissue sampling	Multiple selection value list: • imprint • frozen tissue • flow cytometry/genetic/molecular testing	
Site	Free text	
Size	Size in mm x 3	
Weight	Weight in g	
Macroscopic description	Free text	
Provisional referring diagnosis	Free text	

Element name	Values	Implementation comments
Tumour type	Free text	
ICD-O morphology code	Look up from ICD-O tables.	
Grade (nodal follicular lymphoma (FL) and lymphomatoid granulomatosis (LyG) only)	For FL: • 3A, 3B For LyG: • 1, 2, 3 • not applicable	For LyG, grades 1, 2 and 3 are only applicable to ICD-O morphology 9766/1. For FL grades 3A and 3B are only applicable to ICD-O morphology 9698/3. Not applicable is the selected value for all other ICD-O morphologies.
If diagnosis is incomplete/uncertain, provide reasons	Free text	
Clinical context	Single selection value list: • corroborated by clinical context • not corroborated by clinical context • not applicable	
Microscopic description	Free text	
Immunophenotype	Free text	
Genotype and clonality	Free text	
Other investigations	Free text	
Pure heterologous sarcoma, specify	Free text	
Integrated report interpretation and summary	Free text	
SNOMED Topography code	May have multiple codes. Look up from SNOMED tables.	
SNOMED Morphology code	May have multiple codes. Look up from SNOMED tables.	

Appendix I Summary table – Explanation of level of evidence

(Adopted from Palmer K *et al. BMJ* 2008; 337:1832.)

Level of evidence	Nature of evidence
Level A	At least one high-quality meta-analysis, systematic review of randomised controlled trials or a randomised controlled trial with a very low risk of bias and directly attributable to the target cancer type or A body of evidence demonstrating consistency of results and comprising mainly well-conducted meta-analyses, systematic reviews of randomised controlled trials or randomised controlled trials with a low risk of bias, directly applicable to the target cancer type.
Level B	A body of evidence demonstrating consistency of results and comprising mainly high-quality systematic reviews of case-control or cohort studies and high-quality case-control or cohort studies with a very low risk of confounding or bias and a high probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in A.
Level C	A body of evidence demonstrating consistency of results and including well-conducted case-control or cohort studies and high quality case control or cohort studies with a low risk of confounding or bias and a moderate probability that the relation is causal and which are directly applicable to the target cancer type or Extrapolation evidence from studies described in B.
Level D	Non-analytic studies such as case reports, case series or expert opinion or Extrapolation evidence from studies described in C.
Good practice point (GPP)	Recommended best practice based on the clinical experience of the authors of the writing group

Appendix J AGREE standards

The cancer datasets of The Royal College of Pathologists comply with the AGREE standards for good quality clinical guidelines. The sections of this dataset that indicate compliance with each of the AGREE standards are indicated in the table.

AGREE standard	Section of dataset
Scope and purpose	
1. The overall objective(s) of the guideline is (are) specifically described	1
2. The clinical question(s) covered by the guidelines is (are) specifically described	1, 7, 12, 14
3. The patients to whom the guideline is meant to apply are specifically described	Foreword
Stakeholder involvement	
4. The guideline development group includes individuals from all the relevant professional groups	Foreword
5. The patients' views and preferences have been sought	Not applicable
6. The target users of the guideline are clearly defined	1
7. The guideline has been piloted among target users	Foreword
Rigour of development	
8. Systematic methods were used to search for evidence	Foreword
9. The criteria for selecting the evidence are clearly described	Foreword
10. The methods used for formulating the recommendations are clearly described	Foreword
11. The health benefits, side effects and risks have been considered in formulating the recommendations	Foreword
12. There is an explicit link between the recommendations and the supporting evidence	All sections
13. The guideline has been externally reviewed by experts prior to its publication	Foreword
14. A procedure for updating the guideline is provided	Foreword
Clarity of presentation	
15. The recommendations are specific and unambiguous	All sections
16. The different options for management of the condition are clearly presented	All sections
17. Key recommendations are easily identifiable	All sections
18. The guideline is supported with tools for application	Appendices
Applicability	
19. The potential organisational barriers in applying the recommendations have been discussed	Foreword, 1
20. The potential cost implications of applying the recommendations have been considered	Foreword, 1
21. The guideline presents key review criteria for monitoring and/or audit purposes	15
Editorial independence	
22. The guideline is editorially independent from the funding body	Foreword
23. Conflicts of interest of guideline development members have been recorded	Foreword