Best Practice in Lymphoma Diagnosis and Reporting

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Table of Contents

Methodology ......................................................................................................................... 3
Guideline update and major changes ....................................................................................... 3
Introduction ............................................................................................................................. 3
Collection and transport of specimens ..................................................................................... 5
Specimen handling in the laboratory ......................................................................................... 8
Saving specimens ..................................................................................................................... 14
Immunodiagnosis ..................................................................................................................... 15
Cytogenetic and Molecular Genetic Analysis ............................................................................. 17
The multidisciplinary meeting .................................................................................................. 19
Common patterns of lymphoid disease .................................................................................... 20
References ............................................................................................................................... 31
Table 1: Levels of evidence ...................................................................................................... 37
Table 2: Grades of recommendation ......................................................................................... 37
Table 3: Antibodies routinely used in the investigation of lymphoma ........................................ 38
Table 4: Selection of immunohistochemistry for lymphoma diagnosis ...................................... 53
Table 5: B-cell lymphoma - antibodies available for immunophenotyping ................................. 55
Table 6: Hodgkin Lymphoma First Line Antibodies ................................................................. 57
Table 7: T-cell lymphoma first line antibodies .......................................................................... 57
Figure 1: Suggested approach for assessing lymphadenopathy ................................................ 58
Contact details of writing group .............................................................................................. 59
Methodology

These guidelines have been produced under the auspices of the Royal College of Pathologists (RCPath) and the British Committee for Standards in Haematology (BCSH). The working group consisted of 4 haematologists (AP, SD, BB, EM), 6 cellular pathologists (AR, KG, AJ, NR, BW, AW) and one cytogeneticist (FR). The group has made recommendations based on a review of key literature to December 2005 and consensus of expert opinion. This guideline sets out the best practise for handling tissue and carrying out the investigations required in the diagnosis of lymphoma. The appendices give recommendations as to the main criteria required to diagnose Lymphoma using the WHO classification (Jaffe 2001).

The recommendations were made using the Agree instrument (http://www.agreecollaboration.org) and were further reviewed by members of the BCSH and RCPPath sounding boards, representing practice in both teaching and district hospitals. The levels of evidence used were those of the US Agency for Health Care Policy and Research (see table 1 and 2).

Guideline update and major changes

This guideline is an update to the guideline published in 2008 and has been revised to incorporate the 2008 WHO guidelines as they apply to lymphoproliferative disorders.

Introduction

The National Institute for Clinical Excellence (NICE), in its report on Improving Outcomes in Haematological Cancer, identifies the need for specialist reporting of haematological malignancies (2003). This is because accurate diagnosis in lymphoreticular disease requires the synthesis of results from a combination of investigations, good quality histology, immunostaining and molecular and genetic analysis (Jaffe 2001). It also requires experience in the field of lymph node biopsy reporting, because the pit-falls are many and the consequences of misdiagnosis serious. There are many publications which
indicate that reliable reporting of lymphoreticular disease is enhanced by the multidisciplinary approach as used in those laboratories with specialist expertise in the area (Jarrett, et al 2003, Lester, et al 2003). The different forms of lymphoma now have different treatment protocols and, in future, may require the identification of therapeutic target molecules. One of the main differences in the treatment of lymphoma, as compared to most other adult tumours, is that the patient still harbours the majority of the tumour. The laboratory has to generate the maximum amount of information from the biopsy specimen to determine treatment and prognosis (Ashton-Key, et al 1995).

The organization of the laboratory may vary from a single haematological pathology service capable of performing all tests in-house, to a combination of a local laboratory and regional cytogenetics and molecular genetics services. All laboratories should conform to standards laid down by Clinical Pathology Accreditation Ltd (UK). Whatever the local arrangements and source of the different elements, the final report delivered by the laboratory to the clinical team must integrate the different modalities into a coherent diagnosis. As recommended by NICE, there must be a review of the material by a specialist in haematopathology whose work is audited. The audit can be national or regional but should be in addition to technical quality assurance. All those reporting lymphomas in a network must be on a list of named pathologists for haematopathology and attend at least 50% of the multidisciplinary team (MDT) meetings. Where haematopathology cases are included in the workload of other specialists, such as paediatric pathologists or neuropathologists, it is recommended that these specialists have access to an expert haematopathology opinion. Ideally a specialist haematopathologist should be involved in the MDT review of these cases. Where the primary diagnosis is given by a non-specialist, a definite report of benign disease should only be given if the diagnosis is clear cut, since this may curtail any further investigations. A cautious approach and early referral of doubtful cases to the specialist centre may reduce the chance of missed lymphomas. It should also be remembered that lymphomas may manifest for the first time in patients undergoing lymphadenectomy for carcinoma. These lymph nodes
will be examined by a specialist in tumours other than lymphoma who should therefore be familiar with normal reactive patterns in lymph nodes and the appearances of lymphoma (Cox, *et al* 2005, Kampalath, *et al* 2004, Sheahan, *et al* 2005).

The prognosis for the patient with lymphoma is determined by the type of lymphoma, its stage and other prognostic factors. For instance in diffuse large B cell lymphoma the patient’s prognostic score can be assessed using the International Prognostic Index, a calculation based on age, performance status, stage, extra-nodal spread and lactate dehydrogenase level (1997). Many of the contributory tests are outside the remit of this guideline. However, there are a number of prognostic indicators that must be assessed by immunohistochemistry or molecular genetic analysis. Not least of these is the expression of *BCL2* in diffuse large B-cell lymphoma, the presence of which implies a poorer prognosis than for those tumours that are *BCL2* negative (Gascoyne, *et al* 1997, Hill, *et al* 1996, Weisenburger, *et al* 2000). Diffuse large B-cell lymphoma can no longer regarded as a single disease, because the prognosis is dependent on its germinal centre or non-germinal centre phenotype, site of origin, association with low grade disease, presence of t(14;18), expression of *BCL2* and mutation of *TP53* (Barrans, *et al* 2002, Hans, *et al* 2004). Decisions about a patient’s therapy will increasingly depend on the analysis of these and other markers, consequently it is essential that provision is made for these analyses in the future, particularly given the rapid progression in micro-array technology that will undoubtedly identify novel genes contributing to diagnosis and prognosis.

**Recommendations**

- Each MDT should have at least one identified pathologist who will review material from all new diagnoses (Grade C; evidence level IV)

- The final report should integrate all investigations carried out on the sample (Grade C; evidence level IV)

- Specialist areas such as paediatrics and neuropathology should refer lymphoma cases for review by specialist haematopathologists (Grade C; evidence level IV)
Collection & Transport of Specimens

Lymphoma diagnosis may be made from a number of different specimens depending on the presenting clinical features. Specialist Haematopathology Laboratories may expect to receive lymph nodes, bone marrow aspirates, trephine biopsy cores and peripheral blood for diagnosis as well as other fluids such as cerebrospinal fluid (CSF), ascitic fluid and pleural aspirates. In other hospitals, tissue samples may be received by the Cellular Pathology department, while bone marrow aspirates and blood samples are handled in the Haematology Laboratory. There is no consensus as to whether histopathologists or haematologists should report bone marrow trephine biopsy specimens, but it is considered best practice that the final report on the sample should integrate the findings from all disciplines. Since the haematologist is the primary investigator, it would be sensible that the haematologist should be responsible for integrating the results of bone marrow investigations, while the histopathologist should be responsible for integrating reports primarily derived from tissues such as lymph node or spleen. Obviously, variation should be allowed to take into account local expertise.

The number of pathological tests needed for the precise diagnosis and classification of haematological malignancies means that whole lymph node samples are preferred, rather than needle cores or fine needle aspirate (FNA) cytology. Core biopsy is useful in the diagnosis of inaccessible tumours such as those in the retroperitoneum but the diagnosis of tumours where the architecture is important, especially low grade non-Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL), can be difficult from these samples. Fine needle aspiration, when supported by fluorescence \textit{in situ} hybridization (FISH) or flow cytometry (FC), can be helpful in evaluating suspected transformation to higher grade disease (Dunphy 2004, Jorgensen 2005), but should not generally be used to establish a primary diagnosis of lymphoma unless there is no other readily available tissue and only providing all other diagnostic criteria are met. Consent for all laboratory investigations essential for diagnosis, including DNA analysis, should be part of the initial
consultation with the patient. This is generally in the form of investigation to find what is wrong, rather than a specific list of tests that are to be performed. However, the patient may choose to limit the investigations; for example, excluding HIV serology. It is the responsibility of the clinician to ensure that the patients consent is appropriately documented. Where investigations lead to a diagnosis not initially expected, consideration should be given to obtaining additional consent from the patient (Colvin 2005). Since the field of haematopathology is changing so rapidly, it is desirable to include consent for subsequent research investigations where possible.

The easiest way to achieve optimal results is to arrange for lymph nodes to be immediately submitted fresh to the laboratory. This allows not only collection of fresh tissue samples for immediate analysis, but also optimum fixation for paraffin/plastic embedding and processing, and the preparation of imprint cytology specimens. While Giemsa-stained cytology samples may be helpful in categorizing lymphoma, the real benefit of imprints lies in their suitability for interphase FISH analysis.

Since some Cellular Pathology laboratories are not equipped to handle high risk pathogens, the possibility of tuberculosis, HIV or hepatitis B needs to be carefully considered by both the clinical team and the pathology laboratory. If the biopsy is being done for a clinical diagnosis of infectious disease, such as tuberculosis, a fresh sample can be sent directly to Microbiology.

Specimens should be delivered to the laboratory immediately after collection since cytogenetic analysis requires live cells for culture and RNA degrades rapidly after tissue is removed from the body. This requires close liaison with the operating theatre staff because lymph node biopsies are frequently added to the end of surgical lists and may arrive in the laboratory at the end of the day. Where a test, such as cytogenetic analysis or FC, is to be carried out at a remote location transport or tissue culture medium should be used to preserve the tissue for diagnosis.

In all cases, adequate clinical information is essential to assess the risk of the specimen and plan the investigations. Diagnostic material may be lost if gathering of clinical information is left until the MDT meeting. Apart from
presenting features, the information should include a summary of the haematological status of the patient, including WBC and results of any preceding investigations such as peripheral blood FC. If the lymph node biopsy is being performed at the request of a haematologist/oncologist, they may be in a better position to write the request form than the surgeon performing the biopsy.

**Recommendations**

- Fine needle aspiration samples should not normally be used as the sole tissue for diagnosis (Grade B; evidence level III)
- Fresh tissue should be submitted to laboratories where a diagnosis of lymphoma is suspected. (Grade C, evidence level IV)
- Imprints of fresh lymphoid tissue should be made and stored at the time of sample receipt (Grade C; evidence level IV)
- Samples requiring transport to remote locations for analysis require appropriate transport media/ temperature control. (Grade C, evidence level IV)
- Request forms must include relevant clinical as well as laboratory information (Grade C; evidence level IV)

**Specimen Handling in the Laboratory**

The correct handling of the specimen on its arrival in the laboratory is crucial to successful sample reporting. Although many diagnoses can be reached using routinely processed tissue, ancillary tests are essential for some diagnoses such as Burkitt lymphoma. Furthermore, cytogenetic and molecular genetic analysis can provide essential information in cases where the diagnosis is unclear, as well as contributing confirmatory data to support a diagnosis in other cases. Cytogenetic analysis and flow cytometry (FC) require fresh tissue, and tissue that is thoroughly and evenly fixed is needed to ensure good quality histological sections and reliable immunohistochemical staining. In circumstances where flow cytometry, cytogenetics etc. are carried out in different departments to that of the cellular morphology it is important to
ensure that procedures are in place for results to be integrated into a final report for the sample.

Samples need to be processed such that the following investigations can be carried out if required;

- Microscopy on appropriately fixed and stained tissue samples
- Immunological investigation by immunohistochemistry and/or FC
- Cytogenetic analysis by Giemsa-banding (G-banding)
- FISH on cell suspensions, films, imprints or paraffin sections
- Molecular genetic analysis by polymerase chain reaction (PCR), real-time PCR (RT-PCR) or gene sequencing

**Lymph Node Specimens**

On receipt of the specimen the following assessments should be carried out

*Microbiological Risk*

Consideration should always be given to what category of pathogens each laboratory is equipped to handle. If tuberculosis or fungal infection is suspected, a fresh sample should be sent directly to Microbiology. The fixed sample should be placed in formalin for 48 hours. Also, if HIV is suspected most laboratories would expect the node to be fixed for 48 hours prior to processing

*Volume*

The priority is fixation in formalin to allow paraffin or plastic embedded sections for morphological and immunophenotypical analysis. In small samples such as needle core biopsies there may be insufficient material for any additional investigations. However, if the specimen received is a whole fresh lymph node, which has sufficient volume, the specimen can be divided so that part of the specimen can be fixed for histological sections and part can be used fresh for other investigations.
Samples should then be processed using the following methods.

**Tissue Fixation**

Lymph node specimens larger than 0.5 cm in diameter need to be sliced on arrival in the pathology department, to allow proper fixation. The tissue sampled for paraffin blocks needs to be 3 mm thick. If possible, one section should be placed in each cassette since multiple pieces make immunostaining more difficult. To ensure the slices of lymph node remain flat, incorporate sponges into the cassettes to help prevent folding. The blocks need to fix for 24-48 hours; less than this leads to poor preservation of cytological detail and can make the tissue uninterpretable. Standardisation of fixation makes immunochemistry more reliable, since heat and protease recovery times will be similar. Prolonged fixation makes immunochemistry more difficult and recovery of DNA from paraffin blocks unreliable.

**Cytogenetics**

A fresh sample of the specimen in tissue culture medium should be sent for preliminary cultures to allow subsequent cytogenetic analysis. Cells left for more than a few hours without being placed in culture medium will be unlikely to yield useful cytogenetic information. Haematoxylin and eosin (H&E)-stained sections need to be reviewed as soon as they are ready so that if the lymph node is obviously reactive or contains metastatic carcinoma, cytogenetic analysis can be halted at this point, saving considerable costs in unnecessary screening.

While a fresh sample of the specimen in tissue culture medium may be sent for cytogenetic analysis, the most cost-effective way to use cytogenetics on tissue biopsies is by targeted FISH for specific translocations. Tissue imprints should therefore be made from all fresh biopsies. Pathologists and geneticists should consult to ensure that these are made in a way that allows most efficient use for FISH. Most cytogenetic laboratories now offer a service for tissue section FISH. Imprints or sections can be sent for FISH when it is clear that cytogenetic input is required. If fresh samples are processed for metaphases, these should be stored and analysis only attempted after morphological assessment of the sample indicates a need for cytogenetics.
However, the cytogenetics laboratory should store cell suspensions in case subsequent analysis is required.

*Flow Cytometry*

If the sample is to be analysed promptly a dry fresh sample should be provided to the appropriate laboratory. If this is not possible due to transport times, consideration should be given to disaggregating and fixing the sample prior to transport for analysis.

*Molecular Biology*

A sample of fresh tissue should be rapidly frozen and stored for subsequent analysis if required such as DNA analysis.

**Peripheral Blood Specimens**

*Morphology*

Peripheral blood should be collected into tubes containing the appropriate amount of EDTA for blood volume.

*Cytogenetics & FISH*

For cases where blood is to be used for cytogenetic analysis preservative-free heparin is preferred. Unfixed blood films made using silane-coated slides for FISH can be used as an alternative/ addition to cytogenetically prepared material. Such films must be made fresh, but can give adequate results even after years stored at room temperature. They have the advantage that some morphological assessment can be made alongside the FISH (e.g. neutrophils can be excluded from the analysis).

*Molecular Tests*

In instances where molecular tests such as RT-PCR are required, the anticoagulant to be used will depend on the methodology of the individual laboratory (most commonly ethylene diamine tetra-acetic acid (EDTA)).

*Flow Cytometry*

Samples referred for analysis by FC should be kept at room temperature and received within 24 hours, as there can be a reduction in antigen strength or
complete loss of antigen with time. For further information on processing and storage please see the BCSH Guidelines 2002 (Bain, et al 2002)

**Bone Marrow Aspirates**

These are useful for morphology, FC, PCR, FISH and occasionally conventional cytogenetic analysis if no lymph node material is available. Most laboratories supply suitable transport medium. As for peripheral blood, bone marrow films are a highly acceptable alternative to cytogenetically prepared material if FISH testing is required. A significant disadvantage of using liquid bone marrow samples for cytogenetic or FISH testing is that the part of the sample sent for these tests may be substantially more dilute than that used for morphological assessment. As these genetic tests have no means of assessing the cells being tested, a negative result may be due to absence of the relevant cells from the sample rather than absence of the abnormality from the tumour cells.

**Bone Marrow Trephine Biopsy**

The value of bone marrow trephine biopsy in the diagnosis and staging of lymphoma is well established (Bartl, et al 1984, Burkhardt, et al 1982, Munker, et al 1995). The trephine biopsy core should preferably be taken from the posterior iliac crest and should be a minimum of 1.6 cm (ideally at least 2 cm) in length with multiple sections taken from various levels. (Bain 2001, Bishop, et al 1992, Campbell, et al 2003, Hercher, et al 2001) Bilateral trephine samples have previously been recommended (Juneja, et al 1990, Luoni, et al 1995) but, providing a single sample of sufficient length and quality has been obtained and multiple sections are examined, there is little benefit in carrying out two painful procedures (Campbell, et al 2003)

**Collection & preparation**

The sample should be collected into 4% formalin as this fixative allows the subsequent use of most staining and molecular techniques (Le Maitre, et al 2001). The sample may then be stored for 72 hours or more before preparation, sufficient for samples to be posted to the reporting laboratory where necessary (Krenacs, et al 2005). Subsequent preparation will depend on the preferred method of the laboratory – there are advocates for both
paraffin and plastic embedding techniques with advantages to both and some laboratories use a combination of the two (Gatter, et al 1987, Krenacs, et al 2005). The use of paraffin embedding after decalcification alone is cheaper and is most suitable for DNA extraction, but plastic embedding gives improved morphology and can now be used with a wide variety of antibodies as well as for DNA based tests.

**Morphology**

Bone marrow trephine sections should always be stained with haematoxylin and eosin and with a reticulin stain; a Giemsa stain can also be helpful (Bain 2001). Each slide should be examined initially for cellularity, the trephine core being the best sample for assessing this (Pasquale and Chikkappa 1986). Any abnormal infiltrate should be identified and described in terms of cellular morphology and type of infiltration. The latter can normally be described as one or a combination of the following 4 categories which may be of prognostic relevance. (Arber and George 2005)

- Interstitial
- Paratrabecular
- Nodular
- Diffuse

Light microscopy alone cannot be relied on to distinguish non-neoplastic follicles or lymphoid hyperplasia from lymphoma infiltration (Deverell, et al 1997). However, it is possible, when no other tissue is available, to diagnose lymphoma solely based on a trephine biopsy specimen, provided the appropriate immunostaining, cytogenetic and molecular studies are performed on this or the accompanying aspirate (Buhr, et al 2002, Pasquale and Chikkappa 1986, Thaler, et al 1991). The possibility of discordance between lymph node and trephine biopsy findings should always be considered, and, therefore, the appropriate investigations should be carried out on both tissues whenever possible (Arber and George 2005, Conlan, et al 1990).

**Other Tissue Samples**
On occasions it may be appropriate to look for evidence of disease involvement in other tissues e.g. CSF, pleural fluid. These should be sent to the laboratory in sterile containers without preservative unless more than 24 hours in transit is envisaged, in which case tissue culture medium should be used. The cells in CSF are particularly labile and these samples should be sent to the laboratory for analysis within 6 hours. Cytology is often helpful in these situations, but where sufficient cells are available flow cytometry using the appropriate panels may be more sensitive. In some cases this may be the only material available on which to make a diagnosis eg. primary effusion lymphoma. However, this should only be in instances when there is no evidence of disease involvement accessible to biopsy.

**Recommendations**

- All specimens must be assessed for microbiological risk (Grade C; evidence level IV)
- Where small amounts of lymph node tissue are obtained, sample handling should be prioritised to provide sufficient sections for histological analysis before other investigations (Grade B; evidence level III)
- Lymph nodes should be fixed in sections no more than 0.5 cm thick (Grade C, evidence level IV)
- Cytogenetic analysis of biopsy specimens should be delayed until after initial assessment, and should normally be by FISH on imprints or tissue sections rather than metaphase analysis. (Grade C, evidence level IV)
- A bone marrow trephine biopsy should always be carried out if a diagnosis of lymphoma is suspected or has been made on another tissue. (Grade C, evidence level IV)
- Length of trephine specimen should be more than 1.6 cm and several sections should be made. (Grade B, evidence level III)
• Bilateral iliac crest trephines are not required provided sections from multiple levels are viewed and sample length is adequate. (Grade B, evidence level III)

• Diagnosis of lymphoma should only be based on bone marrow samples alone when no other tissue is available. (Grade B, evidence level III)

• All suspected lymphoma samples should have basic immunohistochemistry and/or FC carried out. (Grade C, evidence level IV)

Saving Specimens

No diagnostic material should be discarded until all investigations are complete. The Royal College of Pathologist recommends that paraffin blocks are stored for a minimum of 30 years. Stained slides should be stored for a minimum of 10 years, and preferably longer, especially in the case of small biopsy specimens where material permitting diagnosis may no longer be contained within the paraffin blocks. Frozen tissue should be stored at –70°C or lower for at least 10 years, preferably longer. Storage at –170°C or lower is needed where viable cells are required e.g. tissue culture. Detailed advice is available from the Royal College of Pathologists and the Institute of Biomedical Science (2009).

Recommendations

• Stained slides should be kept for a minimum of 10 years (Grade C; evidence level IV)

• Paraffin blocks and bone marrow aspirate slides should be kept for a minimum of 30 years (Grade C; evidence level IV)

Immunodiagnosis

Immunostaining should be requested as a panel of antibodies rather than individual tests so appropriate comparisons can be made. It can be performed by FC, which readily identifies single cell populations. FC is
particularly useful in cases where there are homogeneous tumour cell populations such as lymphoblastic lymphoma in which terminal deoxynucleotidyl transferase (TdT) can be readily detected or CLL and mantle cell lymphoma where the simultaneous expression of CD5 and CD20 on tumour cell surfaces can be identified. The data generated by FC are not limited to the percentage of cells positive with a marker, but extend to simultaneous expression of markers and the intensity of staining. In these aspects, FC is superior to immunohistochemistry and is probably under-used in the UK (Camacho, et al 2003). However, FC does have its limitations. Evaluation of possible Hodgkin lymphoma can be a problem because the nodal fibrosis can prevent recovery of Reed-Sternberg cells. Similarly, necrotic tumours can give negative results if the small sample used in the flow analysis for FC does not contain any viable tumour cells (Ravoet, et al 2004).

Paraffin/ plastic section immunochemistry has the advantage that the histologically abnormal cells can be seen to express or lack a particular marker. It may supplement the information generated by FC and may be the only investigation when FC is not available or there is no fresh tissue. If all tissue blocks are similar, immunostaining needs to be performed on one block only, otherwise blocks need to be selected to demonstrate all suspected diagnoses in a patient; e.g. the presence of diffuse large B-cell lymphoma on a background of follicular lymphoma. When selecting panels for immunohistochemistry and immunocytochemistry it is important to include antibodies that are expected to give negative as well as positive results. Most lymphomas are substantially defined by their immunoprofile. Where discrepancies arise between morphology and immunophenotype it may be because of technical failure, incorrect diagnosis or a genuinely aberrant result. In these circumstances further investigations are needed to clarify the results (Bain, et al 2002). The final report must highlight discrepancies and should suggest an explanation for abnormal or conflicting immunochanical findings.

All immunostaining must be supported by satisfactory laboratory performance and appropriate external quality control. With some newer antibodies it can be difficult to achieve results of adequate quality, yet these antibodies are crucial to the diagnosis of lymphoma. Furthermore, knowledge of the normal
staining pattern and cross-reactions of an antibody is crucial to the correct interpretation and diagnosis (Ott, et al 2002, Rudiger, et al 2002). There are limited data on agreement in interpretation of immunostaining between different pathologists; further work is needed in this area (Bertoni and Zucca 2005, Zu, et al 2005).

**Immunohistochemistry Diagnostic Panels**

It is difficult to envisage a situation where the patient has undergone extensive pre-operative investigation followed by lymph node biopsy, in which some form of immunostaining is not justified. Subtle diagnoses that are easily missed on the basis of standard H&E-stained sections include; interfollicular Hodgkin lymphoma, sinusoidal infiltration by anaplastic large cell lymphoma or partial nodal involvement by follicular lymphoma. These should be recognized by careful analysis at light microscopy, but a small panel of antibodies can be useful to identify these cases more readily. Table 3 details the most common antibodies used in lymphoma diagnosis, their specificity and whether they are applicable in immunohistochemistry or immunofluorescence by FC. The last column indicates whether the antibodies are recommended as a first or second line test in B-cell lymphoma (B), T-cell lymphoma (T), Hodgkin lymphoma (H) or histiocytic neoplasms (HIS). The immunohistochemical staining patterns of individual malignancies are detailed in the disease specific sections.

In some cases a full panel may not be necessary as an initial investigation. In such cases a smaller panel of antibodies can be used. Table 4 is a framework for assigning panels of antibodies based on the diagnostic setting.

**Recommendations**

- Flow cytometry should be used to aid lymphoma diagnosis (Grade B; evidence level III)
- Both FC and immunohistochemistry should be done using an appropriate predetermined panel of antibodies (Grade C; evidence level IV)
Cytogenetic and Molecular Genetic Analysis

Specific chromosome abnormalities are strongly associated with particular subtypes. In some instances (e.g. t(8;14) or variants in Burkitt lymphoma) it is essential to demonstrate the translocation before making the diagnosis; in others translocation detection can be very helpful when the diagnosis is in doubt.

Full cytogenetic analysis can also be helpful in a more general context in rare cases in which it is difficult to demonstrate the correct lineage of neoplastic lymphoid cells by other means but the general pattern of chromosome abnormalities, rather than presence or absence of a specific translocation, can clearly indicate that the neoplasm is of lymphoid origin.

The preferred tissue for cytogenetic analysis of lymphoma is nearly always involved lymph node, spleen or other primary disease tissue. Bone marrow, even when heavily involved, will often yield only normal metaphases, due to preferential growth in vitro of reactive myeloid cells, and peripheral blood samples often fail to yield mitoses, particularly in low grade lymphomas. If cytogenetic analysis is attempted using bone marrow, a substantial number of cells should be screened for obvious lymphoma-associated abnormalities, rather than a small number of cells being examined in detail, as is usually the case in leukaemias. FISH analysis of bone marrow aspirates is subject to false negative results, due to undetected haemodilution, and few laboratories can carry out FISH analysis on bone marrow trephine biopsy sections, as decalcification interferes with the processing.

Biopsy sections for FISH should be thinly sliced (2-4 microns). Protease digestion times will vary depending on the thickness of the section, and laboratories should not hesitate to repeat the analysis using sections subject to longer digestion if hybridization fails. Increasing experience usually leads to better judgement of correct protease times. Where there is only patchy involvement of a biopsy specimen by disease, this area should be clearly marked by the pathologist if the FISH analysis is being carried out in a separate cytogenetic laboratory without direct access to pathology expertise. It is difficult to scan large areas of sections by FISH and most cytogeneticists do not have any histopathology experience. Interpretation of numerical
chromosome changes can be difficult by FISH on paraffin sections but is usually simple in imprints. The latter are also much less time-consuming to process and are recommended for all cases where fresh material is received by the pathology laboratory.

In general, cytogenetic and FISH analyses are more useful at the time of diagnosis, than during disease monitoring, as they are relatively insensitive in blood and bone marrow. Molecular monitoring of a known detectable translocation is much more sensitive, although FISH will pick up a higher proportion of cases with standard translocations at diagnosis.

**Molecular techniques**

The use of PCR for specific translocations such as t(14:18) and for detection of T cell receptor clonality and B cell clonality, based on T-cell receptor and immunoglobulin heavy and light chain gene rearrangement, are all important parts of the diagnostic armamentarium. However, the results should not be made the sole basis for a diagnosis, as false positives and negatives can occur (Gebhard, et al 2001, Maes, et al 2000, Pittaluga, et al 1999).

**Recommendations**

- Cytogenetic or FISH analysis is essential for the diagnosis of Burkitt lymphoma (Grade C; evidence level III)
- Cytogenetics or FISH should not be routinely carried out on bone marrow aspirate samples (Grade C; evidence level IV)
- Cytogenetics or FISH should be used as adjuncts to diagnosis and not requested as a routine investigation on all samples (Grade C; evidence level IV)
- PCR results should not form the sole basis for a diagnosis (Grade C; evidence level III)

**The Multidisciplinary Meeting**

Data generated from all modes of investigation need to be collated and interpreted in a clinical context. Not every case of lymphoma will have a
classical clinical presentation, immunophenotype or genetic profile. The reporting pathologist or haematologist (in the case of blood or bone marrow biopsies) remains responsible for his or her diagnosis and for ensuring appropriate additional investigations are instituted to resolve discrepancies. An individual's experience of all the different investigations, the staining patterns of immunochemistry, the interpretation of FISH and cytogenetic analysis is useful in weighing up the contribution each investigation makes to the final diagnosis. All diagnoses of haematological malignancy should be discussed by the MDT. Both the diagnosis and the clinical management decisions should be recorded at the meeting.

**Common Patterns of Lymphoid Disease**

The correct distinction between reactive lymphadenopathy and lymphoma is extremely important. The following are suggestions as to how to approach this problem (see Fig. 1)

**Reactive Lymphadenopathy**

The classical appearances of reactive lymphadenopathy include follicular hyperplasia, paracortical hyperplasia, sinus histiocytosis and medullary plasma cell hyperplasia in any combination and in variable proportions. Follicles may be large with geographic outlines and, in fragmented nodes, can be mistaken in routinely stained sections for high grade lymphoma. The paracortex may be expanded and infiltrated by histiocytes in a diffuse or granulomatous pattern. These may obscure infiltrates of B-cell lymphoma, Hodgkin lymphoma or T-cell lymphoma. The minimum investigations required to verify a benign diagnosis will vary from case to case.

A basic panel of immunohistochemical stains might help to reveal inconspicuous foci of lymphoma; BCL2 to identify follicular lymphoma (FL), CD10 for subtle infiltrates of Burkitt lymphoma (BL), CD23 for diffuse infiltrates of chronic lymphocytic leukaemia (CLL) and CD30 to identify sinusoidal involvement by anaplastic large cell lymphomas (ALCL).

*Follicular hyperplasia*
The classical appearances are of lymphoid cells usually confined to lymph node with the preservation of capsule, peripheral sinus and lymph node architecture. There are germinal centres with light and dark zones, partly or completely surrounded by a mantle zone of small lymphocytes.

The diagnosis should be substantiated by immunostaining, PCR or FISH. Useful investigations include:

**BCL2**: negative in follicular hyperplasia and positive in 90% of follicular lymphoma.

**Ki-67/Mib-1**: useful to demonstrate zonation within reactive germinal centres.

**CD10**: positive lymphoid cells should be confined to germinal centre in reactive hyperplasia (Dogan, *et al* 2000)


**FISH**: t(14;18), *(IGH-BCL2)* translocation is present in 86% of follicular lymphoma (Einerson, *et al* 2005)

**Nodular Proliferations**

Nodules of non-germinal centre cells are a frequent occurrence in lymphadenopathy and usually indicate a neoplastic process. Castleman’s disease (hyaline vascular variant) may form nodules of small lymphocytes (IgM and CD23 positive) with a central collapsed follicular dendritic cell network. Progressive transformation of germinal centres is characterized by large nodules of lymphocytes, and must be distinguished from nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). This can be a difficult differential diagnosis, and, indeed, the two processes may co-exist in the same node. Immunostaining is, therefore, essential in making a diagnosis of NLPHL (Anagnostopoulos, *et al* 2000).

**Paracortical hyperplasia**

Florid paracortical hyperplasia may be seen in reaction to drugs or viral infection, and may simulate T-cell lymphoma. It can obscure or mimic an interfollicular lymphoma; in particular, classical Hodgkin lymphoma can be
easily dismissed or over-diagnosed in this setting. Using the appropriate immunohistochemical panel for Hodgkin lymphoma (HL) should make the diagnosis obvious.

Other patterns

Granulomatous lymphadenopathy is one of the most difficult proliferations to assess, because this pattern is seen in many lymphomas, but may also occur in cases where there is a benign immune disease with symptoms and signs simulating those of lymphoma (Asakawa, et al 2001, Braylan, et al 1977, Fukuda, et al 1997, Haralambieva, et al 2004, Leach and Maclellan 1990). In toxoplasmosis there is a granulomatous reaction associated with follicular and marginal zone hyperplasia. However, one should only make the diagnosis if all the features are present and typical, since HL may be missed or over-diagnosed (Lin and Kuo 2001, Moore, et al 2003, Symmers 1968, Tuzuner, et al 1996).

Tuberculosis and sarcoidosis have typical appearances, and the diagnosis can usually be made without immunostains. Again one should be cautious and be aware of lymphomas stimulating a granulomatous reaction. A full immunohistochemical panel for DLBCL or HL may be required.

Sinus histiocytosis is a common reactive pattern seen in response to tumours but also in specific diagnoses, such as sinus histiocytosis with massive lymphadenopathy (SHML, Rosai-Dorfman Disease), and in patients with immunodeficiency (Lampert and Lennert 1976, Maric, et al 2005, Tsakraklides, et al 1975). Sinus proliferation may obscure anaplastic large cell lymphoma or marginal zone lymphoma as well as deposits of metastatic carcinoma or melanoma. Appropriate immunochemistry is advised to avoid missing these tumours.

Small B-Cell Neoplasms.

The small B-cell lymphomas form a heterogeneous collection of non-Hodgkin lymphomas, grouped together in classification because of their morphology and generally indolent clinical behaviour. They also share some overlapping features of clinical presentation. With greater knowledge of their origin and molecular pathogenesis in recent years, exceptions with more aggressive
clinical behaviour are becoming better understood (for example, mantle cell lymphoma and subsets of CLL). When examined in detail, the morphological characteristics of these lymphomas also show at least as much heterogeneity as similarity, particularly when immunophenotyping is taken into consideration. This variation in the clinical and pathological features means that the rationale for grouping such entities together is becoming weaker. Emphasis on the presence or absence of follicular or nodular architecture has also become less important in the clinicopathological assessment of these neoplasms. However, it is still helpful in their initial histological assessment, and it is important for pathologists to recognise that this group of neoplasms can be mimicked by reactive processes, and that some cases show a nodular pattern related to follicular colonisation.

An important concept within the WHO classification of these neoplasms is that some are more leukaemic in their behaviour than others, which has a major influence on clinical presentation (blood and bone marrow involvement with secondary haemopoietic effects; the occurrence of splenomegaly and hyper- or hyposplenism). Another concept relating to dissemination is that of distinction between node-based small B-cell lymphomas and those arising at extranodal sites, particularly conditions involving mucosa-associated lymphoid tissue (MALT). Different homing patterns of lymphoid cells from nodal sources and from MALT are presumed to be the basis of different patterns of spread of the lymphomas derived from them. Systemic nodal or bone marrow dissemination from a MALT-derived lymphoma, for example, is uncommon and an indication of advanced disease when found.

An additional concept underlying some of the similarities and differences within this group of lymphomas is that of somatic hypermutation. Lymphoid cells of the B lineage are defined by having rearranged immunoglobulin genes, necessary for the normal processes of generating cell surface antigen-specific receptors and effector immunoglobulin molecules for secretion. Subsequent affinity maturation by exposure to the germinal centre environment leads to additional mutations within immunoglobulin genes. This is associated physiologically with improving the effectiveness of immune responses. In lymphoid neoplasia, the footprint of somatic hypermutation can
be detected and used to help predict diagnosis and clinical behaviour. A few of the small B-cell lymphoma categories were at first thought to be derived from non-hypermutated lymphoid cells but, in general, most cases in most subtypes are now known to consist of hypermutated cell clones. The most important exception is that approximately 50% of cases of CLL lack evidence of somatic hypermutation and have a significantly worse clinical outcome than hypermutated cases. In small B-cell lymphomas, unlike DLCBL and other large B-cell lymphomas, there is usually little evidence of ongoing clonal evolution by continuation of the hypermutation process.

A diagnosis within this group, as with all lymphomas, should be made on the basis of consideration of a wide range of clinical and laboratory data, taken in conjunction with histology findings. With regard to laboratory features, histology (and/or cytology, particularly for those with blood or bone marrow involvement) always requires interpretation in the light of immunophenotyping data. In many cases, particularly if immunophenotyping is incomplete for any reason or yields non-standard results, genetic information from cytogenetic analysis or FISH is critical. Clonality analysis by PCR is also important, particularly when there is any clinical or morphological concern that an alternative diagnosis of a non-neoplastic, reactive lymphoid proliferation has not been excluded by immunophenotyping. Efficiency of PCR as an indicator of monoclonality is known to vary between disease groups. However, new developments in primer design (e.g. the EU Biomed primer sets) and PCR techniques mean that there are continuing improvements in this regard. PCR may also be valuable for identification of IGH-BCL2 gene rearrangement when expression of BCL2 protein is absent in suspected follicular lymphoma. However, the available primer sets cannot detect all potential rearrangements associated with the underlying t(14;18). FISH may prove to be superior as it becomes practised more widely with tissue sections and dispersed nuclear preparations from lymphoma specimens. It is already the gold-standard for detection of IGH-BCL1 gene rearrangement in mantle cell lymphomas, in which immunohistochemical demonstration of cyclin D1over-expression can be difficult to achieve consistently and breakpoints are too heterogeneous to be detected efficiently by PCR.
All of these topics are covered in the individual descriptions of the small B cell lymphomas in subsequent disease-specific sections in the Appendix. This is a summary of key points:

**Histology**

Determination of cell type(s) present (lymphocytes, centrocytes, centroblasts, para-immunoblasts, plasma cells, monocytoid cells etc.) and whether reactive pathology can be confirmed or excluded. The latter is the main area in which architectural features of the tissue are particularly important, although follicularity or nodularity is also present as a distinctive feature in many of the small B-cell lymphomas. Dual pathology (including coincidence of lymphoma with reactive changes, lymphoma with metastatic solid malignancy and Hodgkin with non-Hodgkin lymphoma) always requires consideration.

**Immunophenotyping**

Most examples of small B cell lymphomas can readily be classified once their cellular immunophenotype is known, in the context of appropriate cytological/histological features and clinical background. The table (Table 3) and diagnostic algorithm (Fig 1) provide details of the characteristics of each of the main lymphomas that can be distinguished in this way. Notably, lymphoplasmacytic lymphoma cannot be separated immunophenotypically from the various subtypes of marginal zone lymphoma, nor can the individual marginal zone lymphoma subtypes (extranodal of MALT type, nodal and splenic) be distinguished on this basis. Clinico-pathological considerations are essential in each case.

**Genetic Analysis**

PCR-based clonality studies have become well established to aid the distinction of neoplastic from reactive lymphoid proliferations. In all cases, TCR as well as IGH gene rearrangements should be analysed, since there may be lineage infidelity in such genetic processes in lymphomas. The latter is a more significant problem with more-aggressive neoplasms and with those of presumed T/NK cell origin but should not be discounted in small B-cell lymphomas. Hypermutational analysis, of particular importance in sub-categorisation of CLL, should be undertaken where available; current
techniques require DNA from unfixed cells and the methods cannot yet be accurately reproduced for fixed tissue. Immunofluorescent detection of Zap70 expression by FACS appears to be a reasonable surrogate for hypermutation detection but the results of these two approaches do not correlate exactly; immunohistochemical staining for Zap70 in tissue sections has received only limited evaluation currently in diagnostic practice and poses further technical challenges.

Cytogenetic analysis by karyotyping also has an established role in assessment of those small B-cell proliferations that have a leukaemic phase or bone marrow involvement. This approach is frequently uninformative, however, due to limited proliferative activity of the neoplastic cells in many cases. Specific translocations of interest and gains or losses of particular chromosomes/chromosome segments can increasingly be studied successfully by FISH.

Investigation of Infective Agents

Some of the small B-cell lymphomas are associated with immune disturbances caused by exposure to particular infective agents, most notably *Helicobacter pylori* and extranodal marginal zone lymphomas of MALT type in the stomach. Cutaneous marginal zone lymphomas are associated with the organism responsible for Lyme disease, *Borrelia burgdorferi*. Association of Hepatitis C virus with lymphoplasmacytic and other small B-cell lymphomas has been suggested and detection of this virus in such lymphomas may become important as its role becomes better understood. In all of these examples, and others that seem likely to emerge in future years, detection by serology and by molecular analysis of lymphoma tissue is of increasing interest.

Hodgkin Lymphoma

Hodgkin lymphomas are currently considered to consist of two major disease types. The major entity is classical Hodgkin lymphoma (CHL) and the minor is nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The relationship between them is historic rather than biological as they are no more closely related to each other than to several other lymphoma types e.g.,
follicular lymphoma. These diseases characteristically involve lymph nodes rather than extranodal sites. They typically affect young adults and CHL shows a second smaller peak of incidence in late middle age.

**Classical Hodgkin lymphoma (CHL)**

This may be defined as a lymphoma containing a number of scattered large abnormal cells called Hodgkin cells, if mononuclear, and Reed-Sternberg cells, if bi- or multinucleated. These cells have characteristic cytology and reside in an abundant heterogeneous admixture of non-neoplastic inflammatory, stromal and accessory cells. These abnormal cells are considered to be derived from germinal centre B cells as they possess monoclonal but non-functional immunoglobulin gene rearrangements.

Based on the nature of the accompanying tissue reaction and the numbers of abnormal cells four distinct subtypes of CHL can be recognised. These are*

1) **Lymphocyte rich** (LRCHL)
2) **Nodular sclerosis** (NSCHL)
3) **Mixed cellularity** (MCCHL)
4) **Lymphocyte depleted** (LDCHL)

*abbreviations from the WHO Classification 2008 (Swerdlow et al., 2008)

**Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)**

This is a monoclonal B-cell neoplasm characterised by an at least partly nodular proliferation within a lymph node. Within and around these nodules are a varying number of characteristic abnormal cells with hyperlobated nuclei usually known as L&H (lymphocytic and histiocytic) or “popcorn” cells. Informal debate continues whether or not this condition should be regarded as a Hodgkin lymphoma (rather than a non-Hodgkin lymphoma) but for the moment the consensus, especially clinically, is that it should remain where it is in the WHO classification.

The diagnosis of CHL and NLPHL is primarily based on morphology and immunophenotype. This still mainly involves a lymph node biopsy which, for preference, should be the whole node rather a needle core or fine needle
biopsy. Distinction between anaplastic large cell lymphoma and CHL, and between follicular lymphoma/reactive hyperplasia and NLPHL, can at times be challenging. Many cases of CHL are associated with Epstein-Barr virus (EBV) latent infection of neoplastic cells, which is absent in NLPHL except occasionally in accompanying reactive stromal or lymphocytic cells.

**Histology**

This is a good predictor of clinical behaviour in these neoplasms. This has been consistently confirmed in clinical studies over many decades. Cytological examination may be useful in suggesting a diagnosis but this should be confirmed histologically by biopsy. There is no current role for flow cytometry in the diagnosis or management of CHL or NLPHL.

**T-cell and NK-cell neoplasms**

T-cell and natural killer (NK) cell neoplasms comprise a variety of disease entities derived respectively from the clonal proliferation of T or NK cells at different stages of differentiation and/or activation. Clinically these neoplasms form a diverse group, and patients may present with the features of either leukaemia or lymphoma. The lymphomas may arise at nodal or extranodal sites. Pathologically, the tumours show a broad range of cytological appearances, with some neoplasms composed of small uniform cells and others containing large, pleomorphic, anaplastic cells.

The diagnosis of these disease entities should be based on a constellation of clinical and laboratory features. Assessment of these tumours involves blood cell morphology, histology, immunophenotyping and, where appropriate, molecular genetic analysis. Immunohistochemistry is a key investigation and is used to detect a variety of T-cell and NK-cell associated antigens. The biology of these conditions is poorly understood, however, and few immunophenotypic or genetic markers are fully diagnostic. Distinction from B-cell neoplasms may be problematic, as T-cell lymphomas can show aberrant expression of B-cell antigens, and some are associated with secondary proliferations of B cells. A number of these lymphomas show a specific geographical distribution and some are associated with EBV or human T-cell lymphotropic virus I (HTLV-I) infection.
In general, histology is a poor predictor of clinical behaviour for these neoplasms and few of the clinical studies in the literature have been based on well-characterised histopathological features.

The following account describes the laboratory tests used for investigation and diagnosis of the various T- and NK-cell derived neoplasms in this group.

**Histology**

Histological assessment is necessary for the diagnosis of the majority the lymphomas in this group but is rarely diagnostic on its own. Whatever the morphological appearance, immunophenotyping is required to confirm the T-cell or NK phenotype, and molecular studies to confirm a clonal T-cell proliferation are recommended. This is particularly true in those conditions in which the disease consists predominantly of small cells.

**Immunophenotyping**

Neoplastic T-cells can express any combination of T-cell antigens and cytotoxic markers, and NK cells may express some of the less specific T-cell antigens. T-cell lymphomas can also show aberrant expression of B-cell antigens. Immunophenotyping of the cells is mandatory for the diagnosis of all tumours in this group, and both flow cytometry and immunohistochemistry may be used. The complex nature of these neoplasms means that use of a broad panel of antibodies is recommended. Table 7 lists antibodies that are useful in the diagnosis of T-cell and NK-cell neoplasms.

**Cytogenetic analysis**

Cytogenetic studies have a limited role in this group of tumours. Cytogenetic information may provide diagnostic and prognostic information in cases of anaplastic large cell lymphoma. It also has a role in confirming the diagnosis in cases of smouldering T-prolymphocytic leukaemia and hepatosplenic T-cell lymphoma and establishing clonality in NK cell derived tumours. In other T-cell and NK-cell tumours, cytogenetic studies are rarely of diagnostic significance.

**Molecular tests**

Clonality studies are important in confirming the neoplastic nature and lineage of a T-cell proliferation. Such studies may not be required in florid aggressive disease but in indolent cases with low-grade disease clonality investigations
are recommended. Clonality investigations must include assessment of both T- and B-cell lineages; T-cell lymphomas may express some B-cell characteristics, and secondary B-cell lymphomas may arise in the setting of a T-cell neoplasm.

Viral studies

Viral investigations are important in many of the conditions in this group. In particular, in situ hybridisation for EBERs is necessary in many cases. HTLV-I testing, by serological, immunohistochemical or molecular techniques, is also an important investigation.

Recommendations

- A basic antibody panel (see Table 4) should be applied to all lymph nodes even if thought to be reactive. (Grade C; evidence level IV)
- Table 5 lists a suggested basic panel for diagnosis of B-cell lymphoma. (Grade C; evidence level IV)
- Table 6 lists a suggested basic panel for diagnosis of Hodgkin lymphoma. (Grade C; evidence level IV)
- Table 7 lists a suggested basic panel for diagnosis of NK/ T cell lymphoma. (Grade C; evidence level IV)
- FISH/ PCR/ cytogenetics are useful, but negative results do not exclude lymphoma. (Grade C; evidence level IV)
- Flow cytometry is not helpful in the diagnosis of Hodgkin lymphoma. (Grade C, evidence level IV)
- FNA should not be used as the sole basis for diagnosis in Hodgkin lymphoma. (Grade C, evidence level IV)
- Both T and B cell clonality should be assessed in parallel when confirmation of clonality by PCR is required, irrespective of suspected lineage. (Grade B, evidence level III)
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### Table 1: Levels of Evidence

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>Evidence obtained from meta-analysis of randomised controlled trials</td>
</tr>
<tr>
<td>1b</td>
<td>Evidence obtained from at least one randomised controlled trial</td>
</tr>
<tr>
<td>IIa</td>
<td>Evidence obtained from at least one well-designed, non-randomised study, including phase II trials and case-control studies</td>
</tr>
<tr>
<td>IIb</td>
<td>Evidence obtained from at least one other type of well-designed, quasi-experimental study, i.e. studies without planned intervention, including observational studies</td>
</tr>
<tr>
<td>III</td>
<td>Evidence obtained from well-designed, non-experimental descriptive studies. Evidence obtained from meta-analysis or randomised controlled trials or phase II studies which is published only in abstract form</td>
</tr>
<tr>
<td>IV</td>
<td>Evidence obtained from expert committee reports or opinions and/or clinical experience of respected Authorities</td>
</tr>
</tbody>
</table>

### Table 2: Grades of Recommendation

<table>
<thead>
<tr>
<th>Grade</th>
<th>Evidence level</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ia, Ib</td>
<td>Recommendation based on at least one randomised controlled trial of good quality and consistency addressing specific recommendation</td>
</tr>
<tr>
<td>B</td>
<td>IIa, IIb</td>
<td>Recommendation based on well conducted studies but no randomised controlled trials on the topic of recommendation</td>
</tr>
<tr>
<td>C</td>
<td>IV</td>
<td>Evidence from expert committee reports and/or clinical experience of respected authorities</td>
</tr>
</tbody>
</table>
Table 3: Antibodies routinely used in the Investigation of Lymphoma

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Main specificity</th>
<th>Flow cytometry</th>
<th>Immunohistochemistry (IHC)</th>
<th>IHC staining pattern</th>
<th>Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD1a</td>
<td>Langerhans’ cells and cortical thymic T-cells (positive in some cases of precursor T-ALL)</td>
<td>N/A</td>
<td>Yes</td>
<td>Membrane</td>
<td>T2, HIS</td>
</tr>
<tr>
<td>CD2</td>
<td>Pan T-cell marker, cortical and late thymocytes, and NK cells. Expressed in T-lineage neoplasms, some AML and some B-lineage neoplasms.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B1, T1, H1</td>
</tr>
<tr>
<td>CD3</td>
<td>Pan T-cell marker, late thymocytes and mature T-cells. Expressed on many neoplasms of mature T cells. There is surface membrane expression in a few cases of precursor T-cell leukaemia/lymphoma and cytoplasmic expression in most cases. Most antibodies used with paraffin-embedded tissue recognise the epsilon chain of the CD3 molecule, and show cytoplasmic positivity in NK cells.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane Cytoplasmic</td>
<td>B1, T1, H1</td>
</tr>
<tr>
<td>CD4</td>
<td>T-cell helper subset; expressed at a low level on monocytes and macrophages. Expressed</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T1</td>
</tr>
</tbody>
</table>
in some cases of T-cell precursor leukaemia/lymphoma and some mature T-cell neoplasm. Positive in some cases of AML, particularly if there is monocytic differentiation.

| CD5 | Pan T-cell marker; expressed by B-cells in B-CLL, mantle cell lymphoma, splenic marginal zone lymphoma and up to 20% of DLBCL. | Yes | Yes | Membrane | B1, T1 |
| CD7 | Pan T-cell marker also expressed on myeloid cells and NK cells. Positive on blast cells in a minority of cases of AML. | Yes | Yes | Membrane | T1 |
| CD8 | T-cell cytotoxic subset. Expressed in some T cell precursor leukaemia/lymphoma, T-cell large granular lymphocytic leukaemia and some cases of T-PLL and some mature T-cell lymphomas. | Yes | Yes | Membrane | T1 |
| CD10 | Common ALL antigen. Expressed by germinal centre B cells and some normal B-cell precursors. Stains cells of many cases of precursor B lymphoblastic lymphoma/leukaemia, follicular lymphoma, Burkitt lymphoma and | Yes | Yes | Membrane | B1, T2 |
~40% of diffuse large B-cell lymphoma. Expressed by T-cells in precursor T lymphoblastic lymphoma/leukaemia, often more weakly than in B-lineage cases. Stains neoplastic T cells in 50-60% of angioimmunoblastic T-cell lymphoma cases and in some cases peripheral T-cell lymphoma, unspecified cases. Stains bone marrow stromal cells and shows spurious binding to ischaemic and infarcted lymphoid cells.

<table>
<thead>
<tr>
<th>CD11b</th>
<th>NK cells, granulocytes and monocytes; expressed in some cases of AML-M5 (monocytic/monoblastic)</th>
<th>Yes</th>
<th>N/A</th>
<th>N/A</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11c</td>
<td>Monocytes, NK cells and neutrophils (also expressed in hairy cell leukaemia, some cases of variant hairy cell leukaemia, splenic marginal zone lymphoma, some cases of CLL and other B-NHLs)</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
<td>B2</td>
</tr>
<tr>
<td>CD13</td>
<td>Myeloid cell marker. Expressed in some cases of ALCL.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T2</td>
</tr>
<tr>
<td>CD15</td>
<td>Stains mature myeloid cells, and can stain macrophages when using</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T1, H1</td>
</tr>
</tbody>
</table>
sensitive methods and the IgM monoclonal LeuM1. Expressed on Reed-Sternberg cells, a proportion of large T-cell lymphomas and rare cases of DLBCL. Also positive in some cases of AML and B-cell precursor leukaemia/lymphoma.

<table>
<thead>
<tr>
<th>CD16</th>
<th>Marks NK cells and macrophages; stains NK proliferative disorders, T-cell large granular lymphocytic leukaemia, and hepatosplenic T-cell lymphoma</th>
<th>Yes</th>
<th>Yes</th>
<th>Membrane &amp; cytoplasmic</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>B-cell antigen</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B1</td>
</tr>
<tr>
<td>CD20</td>
<td>B-cell antigen expressed on neoplasms of mature B cells (including nodular lymphocyte predominant Hodgkin lymphoma) and some precursor B-cell neoplasms. Positive on cells of some cases of plasma cell myeloma.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic Membrane</td>
<td>B1, T1, H1</td>
</tr>
<tr>
<td>CD21</td>
<td>Follicular dendritic cell marker. Marks mantle lymphocytes and shows low density expression by a wide range of B-cell lymphomas.</td>
<td>N/A</td>
<td>Yes</td>
<td>Membrane</td>
<td>B1, T1, H2, HIS</td>
</tr>
<tr>
<td>CD22</td>
<td>B-cell marker. Expressed by mature B-cell neoplasms except CLL. Expressed in the</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
<td>B1</td>
</tr>
<tr>
<td><strong>CD23</strong></td>
<td>Follicular dendritic cell marker, mantle lymphocytes and activated B-cells. Expressed in typical chronic lymphocytic leukaemia, a proportion of follicular lymphomas and some DLCL, particularly mediastinal DLBCL.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B1, T2</td>
</tr>
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<tr>
<td><strong>CD25</strong></td>
<td>Detects an activation antigen (IL-2 receptor alpha chain) on T cells and B cells. Positive in hairy cell leukaemia, anaplastic large cell lymphoma, adult T-cell leukaemia and less often in other T- and B-cell neoplasms.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B2, T2</td>
</tr>
<tr>
<td><strong>CD30</strong></td>
<td>Activated T and B lymphoid cell marker; also stains embryonal carcinoma and malignant melanoma cells. Expressed in Reed-Sternberg cells and Hodgkin cells in classical Hodgkin lymphoma, anaplastic large cell lymphoma, some diffuse large B-cell lymphomas (in particular mediastinal B-cell lymphoma),</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic Membrane</td>
<td>B2, T1, H1</td>
</tr>
<tr>
<td>CD33</td>
<td>Myeloid cell marker</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T2</td>
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<tr>
<td>CD34</td>
<td>Haemopoietic stem cells. Also stains endothelial cells (can highlight intravascular lymphoma).</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic Membrane</td>
<td></td>
</tr>
<tr>
<td>CD35</td>
<td>Follicular dendritic cell marker. May be expressed in AML, particularly where there is monocytic differentiation.</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
<td>B2</td>
</tr>
<tr>
<td>CD38</td>
<td>Plasma cells and plasma cell neoplasms including “plasmablastic” lymphomas and lymphoplasmacytic lymphoma. Stains follicle centre cells, follicular lymphoma, mantle cell lymphoma, a proportion of cases of CLL, primary effusion lymphoma and malignant melanoma. In CLL it stains a subset of cases with a worse prognosis.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
<td>B2</td>
</tr>
<tr>
<td>CD43</td>
<td>Stains normal T-cells, myeloid cells, 40-50% of DLBCL, T-cell lymphomas, ALCL, CLL, mantle cell lymphoma and proportion of marginal zone lymphomas. Strongly</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B2</td>
</tr>
<tr>
<td>CD45</td>
<td>Leucocyte common antigen (LCA)</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>B2, T2, H2</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>CD45R0</td>
<td>LCA subset expressed predominantly on T-cells</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T2</td>
</tr>
<tr>
<td>CD56</td>
<td>NK cell marker; also stains a subset of T-cells, myeloid cells, neuroendocrine cells (N-CAM) and most cases of plasma cell myeloma. Positive in AML, mainly AML-M5. Expressed in plasmacytoid dendritic cell leukaemia, NK LGL leukaemias and some T-cell LGL leukaemias.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T1</td>
</tr>
<tr>
<td>CD57</td>
<td>NK cell and T-cell marker, in particular intrafollicular T-cells.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
<td>T2, H2</td>
</tr>
<tr>
<td>CD68</td>
<td>Monocytes and macrophages; some epitopes are expressed on cells of granulocytic lineage.</td>
<td>Yes</td>
<td></td>
<td>Cytoplasmic</td>
<td>T2, HIS</td>
</tr>
<tr>
<td>CD75</td>
<td>Cell adhesion molecule. Pan-B cell marker strongly expressed by follicle centre cells. Stains L&amp;H cells in nodular lymphocyte predominant Hodgkin lymphoma</td>
<td>N/A</td>
<td>Yes</td>
<td>Membrane &amp; Cytoplasmic</td>
<td>H2</td>
</tr>
<tr>
<td>CD79a</td>
<td>B-cell antigen receptor alpha chain. Marks a</td>
<td>Yes</td>
<td></td>
<td>Membrane &amp; Cytoplasmic</td>
<td>B1, T1,</td>
</tr>
</tbody>
</table>

CD79a

B-cell antigen receptor alpha chain. Marks a
<p>| CD79b | B-cell antigen receptor beta chain. Expressed in most mature B-cell malignancies except CLL; not seen in precursor B-ALL. | Yes | N/A | N/A | B2 |
| CD103 | Intra-epithelial/mucosal T-cells, monocytes and a small subset of peripheral blood T cells. Useful in enteropathy type T-cell intestinal lymphoma and adult T-cell leukaemia/lymphoma. Also expressed in hairy cell leukaemia. | Yes | Yes (frozen section) | Membrane | T2 |
| CD123 | Detects the alpha chain of the IL3 receptor. Expressed in plasmacytoid dendritic cells, NK cells eosinophils, basophils and monocytes. Positive in hairy cell leukaemia, 50% of AML cases and precursor B-lymphoblastic lymphoma/leukaemia | Yes | Yes | Membrane | B2 |
| CD138 | Stains mature plasma cells, and pre-B cells. | Yes | Yes | Cytoplasmic | B2 |</p>
<table>
<thead>
<tr>
<th>Protein</th>
<th>Expression/Function</th>
<th>Immunohistochemical Staining</th>
<th>Localization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD163</strong></td>
<td>Haemoglobin scavenger receptor glycoprotein. Expressed on monocytes and macrophages. Positive in myelomonocytic leukaemia and histiocytic malignancies.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td><strong>CD207</strong></td>
<td>Langerhans’ cells</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td><strong>BCL2</strong></td>
<td>Apoptosis regulator. Overexpressed in most cases of follicular lymphoma, but not expressed in reactive follicle centres. Also stains a wide range of normal and neoplastic T and B cells</td>
<td>N/A</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td><strong>BCL6</strong></td>
<td>Transcription factor expressed in the nuclei of germinal centre cells. Positive in follicular lymphoma and in a subset of diffuse large B-cell lymphoma</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td><strong>BOB1</strong></td>
<td>Lymphocyte transcription co-factor. Marks L&amp;H cells in nodular</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>Antibody</td>
<td>Target</td>
<td>Staining Pattern</td>
<td>Location</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-----------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td><strong>Lymphocyte predominant Hodgkin lymphoma; generally negative in the Reed-Sternberg cells of classical Hodgkin lymphoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cyclin D1</strong></td>
<td>Mantle cell lymphoma and a percentage of hairy cell leukaemia cases; marks neoplastic cells in some cases of plasma cell myeloma.</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td><strong>Desmoplakin</strong></td>
<td>Cytoskeletal component of desmosomes; seen in epithelia and follicular dendritic cells</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td><strong>EBV-LMP1/EBER ISH</strong></td>
<td>Epstein-Barr virus latent membrane protein (LMP1) or EB-encoded viral RNA (EBER)</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic/LMP1, Nuclear/EBER</td>
</tr>
<tr>
<td><strong>Epithelial membrane antigen (EMA)</strong></td>
<td>Cells of epithelial origin; also expressed by plasma cells, some cases of myeloma, some histiocytic lymphomas, a proportion of T-cell lymphomas (particularly ALCL), L&amp;H cells in NLPHL, some cases of primary effusion lymphoma and the T-cell rich variant of DLCBL.</td>
<td>N/A</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td><strong>Factor XIIIa</strong></td>
<td>Histiocytes and dermal dendrocytes.</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td><strong>Fascin</strong></td>
<td>Actin-binding protein found in dendritic cells (and Reed-Sternberg cells)</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td><strong>FMC7</strong></td>
<td>A subset of B cells but not B-cell</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Precursor:</td>
<td>Expression Pattern</td>
<td>Detection Method</td>
<td>Markers</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------</td>
<td>-----------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Precursors. Expressed in hairy cell leukaemia and most B-cell lymphomas with the exception of CLL.</td>
<td>Yes Yes</td>
<td>Cytoplasmic</td>
<td>T2</td>
<td></td>
</tr>
<tr>
<td>Granzyme B</td>
<td>Cytotoxic T-cell marker, positive in a proportion of T cell lymphomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Antigen presenting cells including B cells; activation marker on T cells. Expressed in most B-cell precursor leukaemia/lymphoma and AML with the exception of megakaryoblastic and erythroid leukaemias.</td>
<td>Yes Yes</td>
<td>Membrane Cytoplasmic</td>
<td>HIS</td>
</tr>
<tr>
<td>IRF4 (MUM1)</td>
<td>Detects MUM1/IRF4 protein, a B-cell proliferation/differentiation marker. Widely expressed in haematolymphoid neoplasms (and malignant melanoma). MUM1/IRF4 expression is of prognostic value in diffuse large B-cell lymphoma, and is useful in distinguishing classical Hodgkin lymphoma (positive) from NLPHL (negative).</td>
<td>N/A Yes</td>
<td>Nuclear</td>
<td>B1,</td>
</tr>
<tr>
<td>ISH for cytoplasmic kappa &amp; lambda light chains</td>
<td>Detection of light chain restriction</td>
<td>N/A Yes</td>
<td>Cytoplasmic</td>
<td>B2</td>
</tr>
<tr>
<td>J chain</td>
<td>Positive in the large cells</td>
<td>N/A Yes</td>
<td>Membrane</td>
<td>H2</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>-------</td>
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<td>-------</td>
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<td>-------</td>
</tr>
<tr>
<td>Ki67/MIB1</td>
<td>Proliferation marker</td>
<td>Yes</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>KIRs (CD158a, CD158b and CD158e)</td>
<td>Killer immunoglobulin-like receptors. Detects clonality in T and NK LGL leukaemia</td>
<td>Yes</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Monocytes and macrophages; cells of granulocytic lineage. Expressed on many cases of AML.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>Myeloid cell marker (mature myeloid cells); weaker staining of cells in the monocytic lineage than those of granulocytic lineage. Expressed on most cases of AML.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>OCT2</td>
<td>B-cell immunoglobulin regulation transcription factor, strongly expressed in nodular lymphocyte predominant Hodgkin lymphoma but downregulated in classical Hodgkin lymphoma.</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>p21(WAF1)</td>
<td>Cyclin dependent kinase inhibitor; prognostic marker in multiple myeloma.</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
</tbody>
</table>

of nodular lymphocyte predominant Hodgkin lymphoma (negative in classical Hodgkin lymphoma Reed-Sternberg cells)
<table>
<thead>
<tr>
<th>Antigen</th>
<th>Protein Function</th>
<th>Staining Location</th>
<th>Expression</th>
<th>Staining Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>p27&lt;sub&gt;Kip1&lt;/sub&gt;</td>
<td>Nuclear cell cycle regulatory protein present in normal B-cells. Expression is regulated through the cyclin-D1 pathway, so it is reciprocally expressed with cyclin-D1. Can be positive in atypical CLL when CD23 is absent. Is helpful in the identification of rare cyclin-D1 negative mantle cell lymphoma cases, where p27 is still suppressed.</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>p53</td>
<td>p53 protein is part of a tumour suppressor pathway; abnormal expression of p53 protein is prognostically significant in B-cell CLL and other lymphoid neoplasms.</td>
<td>Yes</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>p63 (VS38c)</td>
<td>p63 protein is associated with rough endoplasmic reticulum. Labels plasma cells and B-cells with plasmacytic differentiation. Also stains melanoma cells and some epithelial cells.</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>CD246 (p80; ALK1)</td>
<td>Anaplastic large cell lymphoma; ALK+ve; also ALKc antibody directed against</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear and/or cytoplasm</td>
</tr>
<tr>
<td>Gene/Marker</td>
<td>Description</td>
<td>Localization</td>
<td>Cytoplasmic</td>
<td>Nuclear</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
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<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>PAX5</td>
<td>Transcription factor regulating gene expression and differentiation in B lymphocytes. (Positive in Reed-Sternberg cells.)</td>
<td>N/A</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>Perforin</td>
<td>Cytotoxic T-cell marker, positive in a proportion of T cell lymphomas.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>S100</td>
<td>Interdigitating reticulum cells and Langerhans' cells, most histiocytic sarcomas, 30% of Reed-Sternberg cells, gamma-delta T-cells and (also melanoma cells and Schwann cells.</td>
<td>N/A</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>Smlg heavy chain D, M, G</td>
<td>Surface membrane immunoglobulin heavy chains.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td>Smlg light chain K, L</td>
<td>Surface membrane immunoglobulin light chains.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td>TCRβ</td>
<td>Pan T-cell marker.</td>
<td>Yes</td>
<td>Yes</td>
<td>Membrane</td>
</tr>
<tr>
<td>TdT</td>
<td>Terminal deoxynucleotidyl transferase. Expressed by precursor T-cells and B-cells. Positive in most precursor lymphoblastic leukaemia/lymphoma and some AML.</td>
<td>Yes</td>
<td>Yes</td>
<td>Nuclear</td>
</tr>
<tr>
<td>TIA-1</td>
<td>Cytotoxic T-cell marker, positive in a proportion of T cell lymphomas, including hepatosplenic T cell lymphoma and T-cell LGL leukaemia.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-------------</td>
</tr>
<tr>
<td>ZAP70</td>
<td>One of the Syk/ZAP protein tyrosine kinase family. Expressed in T cells and NK cells but not in normal mature B cells. Cases of CLL with non-mutated immunoglobulin V genes frequently show ZAP-70 expression (weaker than that seen in T cells), and its expression is of prognostic significance. Some B-PLL cases are also positive.</td>
<td>Yes</td>
<td>Yes</td>
<td>Cytoplasmic</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>(Basic Panel)</td>
<td>Basic Panel plus</td>
<td>(Large B cell panel)</td>
<td>(Undifferentiated malignancy panel)</td>
<td>(Hodgkin panel)</td>
</tr>
<tr>
<td>CD20</td>
<td>BCL6</td>
<td>CD45</td>
<td>CD2/CD3</td>
<td>CD2</td>
</tr>
<tr>
<td>CD79a</td>
<td>CD10</td>
<td>Cytokeratins</td>
<td>CD20</td>
<td>CD4</td>
</tr>
<tr>
<td>CD2/CD3</td>
<td>(CD138)</td>
<td>S100</td>
<td>CD30</td>
<td>CD5</td>
</tr>
<tr>
<td>(CD45RO)</td>
<td>p21</td>
<td>MelanA</td>
<td>CD15</td>
<td>CD7</td>
</tr>
<tr>
<td>Ki67</td>
<td>p53</td>
<td>( +/- basic panel)</td>
<td>CD15</td>
<td>CD8</td>
</tr>
<tr>
<td>BCL2</td>
<td>IRF4/MUM1</td>
<td>EBV-LMP1/ EBER (ISH)</td>
<td>MUM1</td>
<td>(CD3epsilon)</td>
</tr>
<tr>
<td>(add EBV-EBER ISH if acute EBV suspected)</td>
<td></td>
<td></td>
<td></td>
<td>(add TdT and CD43 if T-LBL suspected)</td>
</tr>
</tbody>
</table>

(add CD57, CD75, BOB1 and OCT2 if NLPHL suspected)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic Panel plus</td>
<td>Basic &amp; Small B cell Panels plus</td>
<td>Basic Panel plus</td>
<td>Basic, Hodgkin &amp; T cell Panels plus</td>
</tr>
<tr>
<td><em>(Small B cell panel)</em></td>
<td><em>(Blastoid panel)</em></td>
<td><em>(Myeloid panel)</em></td>
<td></td>
</tr>
<tr>
<td>CD15</td>
<td>TdT</td>
<td>Myeloperoxidase</td>
<td>CD246 (ALK-1;p80)</td>
</tr>
<tr>
<td>CD10</td>
<td>CD5</td>
<td>CD68 (KP1)</td>
<td></td>
</tr>
<tr>
<td>CD23</td>
<td>CD43</td>
<td>CD68R (PGM1)</td>
<td></td>
</tr>
<tr>
<td>Cyclin D1</td>
<td></td>
<td>CD34</td>
<td></td>
</tr>
<tr>
<td>BCL6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(add cytokeratins if lympho-epithelial lesions suspected)</td>
<td>(add EBV-EBER ISH if immunodeficiency or immunosuppression suspected)</td>
<td>(add Mac387, CD14, CD15, CD34, CD42b, CD61, CD117, glycophorin C later, as needed)</td>
<td>(add CD56 and CD57 later, if null)</td>
</tr>
</tbody>
</table>
Table 5. B Cell Lymphomas – Antibodies available for immunophenotyping

<table>
<thead>
<tr>
<th></th>
<th>Immunofluorescence</th>
<th>Immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>SmIg light chain K, L</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>SmIg heavy chain D, M, G</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>CD19</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>CD20</td>
<td>Available</td>
<td>1</td>
</tr>
<tr>
<td>CD79a</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>CD79b</td>
<td>Available</td>
<td>N/A</td>
</tr>
<tr>
<td>CD22</td>
<td>Available</td>
<td>N/A</td>
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<tr>
<td>CD23</td>
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<tr>
<td>FMC7</td>
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<td>N/A</td>
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<td>CD10</td>
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<tr>
<td>CD2/CD3</td>
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<td>1</td>
</tr>
<tr>
<td>CD5</td>
<td>Available</td>
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<tr>
<td>CD43</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>BCL2</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>BCL6</td>
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<td>1</td>
</tr>
<tr>
<td>Ki67</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>IRF4/MUM1</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>CD21</td>
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<tr>
<td>p53</td>
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<td>2</td>
</tr>
<tr>
<td>p21(WAF1)</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>TdT</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>PAX5</td>
<td>N/A</td>
<td>1</td>
</tr>
<tr>
<td>p63 (antibody VS38c)</td>
<td>N/A</td>
<td>3</td>
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<tr>
<td>CD38</td>
<td>Available</td>
<td>2</td>
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<tr>
<td>ZAP70</td>
<td>Available</td>
<td>3</td>
</tr>
<tr>
<td>CD138</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>CD56</td>
<td>Available</td>
<td>2</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----------</td>
<td>------------</td>
</tr>
<tr>
<td>ISH for cytoplasmic K &amp; L</td>
<td>Available</td>
<td>1 (in plasma cell proliferations)</td>
</tr>
</tbody>
</table>

1. **First line antibody**
2. **Second line antibody**
3. **Third line antibody**

N/A. Technique not applicable with this antibody
<table>
<thead>
<tr>
<th>CD2/CD3</th>
<th>CD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20</td>
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</tr>
<tr>
<td>CD30</td>
<td>CD4</td>
</tr>
<tr>
<td>CD15</td>
<td>CD5</td>
</tr>
<tr>
<td>EBV-LMP1/EBER (ISH)</td>
<td>CD7</td>
</tr>
<tr>
<td>IRF4/MUM1</td>
<td>CD8</td>
</tr>
<tr>
<td>PAX5</td>
<td>CD56</td>
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<td>Ki67</td>
</tr>
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<td>CD20</td>
</tr>
<tr>
<td></td>
<td>CD79a</td>
</tr>
<tr>
<td></td>
<td>CD15</td>
</tr>
</tbody>
</table>

Table 6: Hodgkin Lymphoma First Line Antibodies

Table 7: T-Cell Lymphoma First Line Antibodies
Figure 1: Suggested approach for assessing lymphadenopathy

Differential Diagnosis
Reactive Lymphadenopathy

Follicular Proliferation
- Germinal centre zoned (use Ki67)
- Bcl-2 negative
- CD10 confined to germinal centres
- No spread beyond capsule
- Otherwise Follicular lymphoma

Nodular Proliferation
- Small lymphocytes
  - NILPH
  - Castleman disease (hyaline-vascular subtype)
  - CLL
- Mixed cells
  - Classical Hodgkin lymphoma
  - CLL with proliferation centres
- Plasma cells
  - Castleman disease
  - Rheumatoid disease
  - Plasmablastic lymphoma
- Metastases
  - Classical Hodgkin lymphoma
  - Anaplastic large cell lymphoma
  - Rosai-Dorfman disease

Paracortical expansion
- Mixed cells
  - Drugs, viruses
  - Interfollicular CHL
  - Extramedullary haemopoiesis
- Diffuse involvement
  - Sarcoidosis
  - Tuberculosis
  - With follicular hyperplasia
  - Toxoplasmosis

Granulomatous lymphadenopathy
- Metastases
- Anaplastic large cell lymphoma
- Rosai-Dorfman disease

Sinus expansion
- Mixed cells
  - Drugs, viruses
  - Interfollicular CHL
  - Extramedullary haemopoiesis
- Diffuse involvement
  - Sarcoidosis
  - Tuberculosis
  - With nodules of small lymphocytes
  - NILPH – nodules of small B lymphocytes with rosettes of T cells around L&H cells

Large cells
- Metastases
  - NS classical Hodgkin lymphoma
  - Large cell lymphomas
- With follicles
  - Marginal zone lymphomas

With nodules of small lymphocytes
- NILPH – nodules of small B lymphocytes with rosettes of T cells around L&H cells
Declaration of Interest:
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