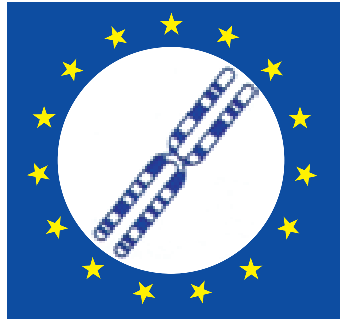
Guidelines and Quality Assurance for Acquired Cytogenetics



A common European framework for quality assessment for banded chromosome studies and molecular cytogenetic investigations of acquired abnormalities.

E.C.A. Permanent Working Group for Cytogenetics and Society

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GUIDELINES FOR CYTOGENETIC ANALYSIS OF ACQUIRED DISORDERS

These guidelines are a supplement to the General guidelines (General Guidelines and Quality Assurance for Cytogenetics, E.C.A. Newsletter, January 2012).

1. ACQUIRED ANALYSIS

1.1 General

Karyotypic investigations, including conventional chromosome analysis and fluorescence *in situ* hybridization (FISH) analyses, of haematological neoplasms have become increasingly important in the clinical management of patients with such disorders. According to WHO (2008) some cytogenetic abnormalities assist in the correct classification of leukaemia and lymphomas, and many also provide important prognostic information. Numerous examples of specific clinico-genetic associations in various neoplasms can be retrieved from, for example the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman). Furthermore, an increasing number of specific treatment approaches directly or indirectly target genetically defined subtypes of neoplasm, and so cytogenetic analysis can comprise an important element of the diagnosis.

All laboratories offering a cytogenetic service should be able to provide a robust analytical and interpretive service for all neoplasms that they deal with. The laboratory must have comprehensive standard operating procedures for all aspects of sample processing based on in-house experiments and/or published guidelines (see reference section for examples). Referral can be at diagnosis, follow up prior to or after treatment (including transplantation), relapse/transformation or as part of a clinical trial.

Rapidly developing molecular techniques using microarrays add valuable information and will support conventional karyotyping, but are as yet not able to replace it (see Simons *et al.*, 2012). In view of the frequent changes in practice in this important developing field, it is not possible to provide guidelines in this document that will be applicable for any length of time. It is important that service providers keep themselves up to date with novel applications of this technology.

Molecular genetic analyses are essential for diagnosis in some leukaemia subgroups, and several molecular mutations not detectable by cytogenetics provide important prognostic information. These are outside the scope of the current guidelines and are not covered.

The use of 'must' in this document indicates a requirement (when not in conflict with national law or regulations) and the use of 'should' or 'may' indicates a recommendation. Expertise in G-banding is assumed throughout this document. R- and Q-banding may also be used but it is essential that the resolution of banding achieved is equivalent to G-banding and that cytogenetically visible recurrent translocations can be identified. Throughout the document, the word 'score' is used with the specific meaning of checking for the presence or absence of particular structural or numerical karyotypic features in a given number of cells.

It must be noted that these guidelines are minimum requirements and that professional judgement is always of paramount importance. Cytogenetic practises and regulations differ throughout Europe so in some instances these guidelines may not be in accordance with national/federal laws and regulations. In some circumstances the competent cytogeneticist may decide that additional analysis or tests should be undertaken to increase confidence in the result. Clinical trials may have other requirements in order to stratify patients to the appropriate treatment regimes. These guidelines should therefore be used in conjunction with clinical trial data and/or information from the European Leukaemia Network (ELN). Where there appears to be contradiction between available

guidelines, the most recently published should be taken to apply to all.

At the end of this document is attached a list of national and international guidelines and policy documents as well as the other documents consulted in preparing these guidelines. This list is not exhaustive and as this is a rapidly changing area in genetics, the authors recommend that individuals working in this field keep abreast of the current literature and guidelines.

1.2 Standard Cytogenetics

Sample transport and processing

Appropriate transport medium with anti-coagulant (*e.g.* heparin) into which the sample may be taken directly may be made available to clinicians by cytogenetic laboratories as it may improve the success rate. For most haematological neoplasms, analysis of bone marrow (BM) samples is essential, and a sufficient quantity of BM should be received by the diagnostic laboratory within 24 hours after aspiration. The laboratory should be able to advise the referring oncologist as to how much material is required, A peripheral blood (PB) sample may be substituted if no BM can be obtained, provided there are sufficient neoplastic cells circulating (usually >10%) in this tissue. BM is essential in myelodysplastic syndromes (MDS) with cytopenia, and for chronic lymphocytic leukaemia (CLL) PB is frequently the better sample type. Lymph node (LN) should ideally be used for lymphoma although other lymphoma-containing tissue may be successful.

A range of culture techniques must be available dependent on the referral reason (see disease-specific sections). The culture conditions should be optimised where possible by utilising direct, short term and/or synchronised cultures to improve the mitotic index. The seeding density is usually from 1 to $3x10^6$ cells/ml. When B- or T-cell lymphoproliferative disorders are suspected, suitable mitogens should be added to additional cultures. Laboratories should be aware that culture times may affect the detection of an abnormal clone. With the exception of samples that pose a high risk of infection to laboratory staff, a method for cell counting should be used to establish an optimum culture density (which may be dependent on disease type).

Blocking agents (e.g. colcemid) are generally added to the culture medium at some point before harvest. Details of methodology are covered in individual sections relating to specific disorders

Analysis and checking

No minimum banding quality can be recommended for analysis to identify an acquired abnormality. The quality of metaphases obtained from unstimulated blood and from bone marrow samples is frequently poor, particularly in leukaemia. As normal cells with better chromosome morphology may be present, it is important to analyse cells of varying quality in order to maximise the likelihood of detecting a neoplastic clone. The number of cells analysed has to be increased when the quality is so poor that normal chromosomes cannot be reliably identified or when it is not possible to judge whether a chromosome is normal or abnormal.

The level of analysis is dependent on the reason for referral and disease (Haferlach *et al*., 2007). Analysis from more than one culture regimen should be considered if no abnormal clone is detected, particularly where the lineage of the neoplastic cells is in doubt.

Sufficient cells must be analysed and/or scored to establish the clonality of any abnormality found (see Hook's tables, Hook, 1977). The International System for Human Cytogenetic Nomenclature (ISCN) definition of clonality stipulates that an identical structural abnormality or trisomy or identical losses of one or more chromosomes with the same structural aberration(s) should be present in at least two metaphases while loss of a single chromosome should be present in at least three metaphases. The possibility of secondary or co-existing apparently unrelated clones, or clonal evolution, or sidelines means that, particularly at diagnosis and relapse, a sufficient number of cells must be looked at in enough detail to allow detection of more than one cell line, (see specific diseases below).

Polyploid and hypodiploid/apparently broken metaphases should not be excluded from the analysis, although cells with loss of >6 chromosomes cannot be considered to be fully analysed unless the loss is part of the clonal change. Analysts should be aware of low hypodiploid metaphases 'doubling-up' to masquerade as a hyperdiploid/near-triploid clone. The finding of a single hyperdiploid or structurally abnormal metaphase necessitates further screening to determine whether the abnormality is clonal. This does not necessarily apply in the case of chromosome loss and will depend on the quality of the preparation, the chromosome involved and the referral reason.

Where no abnormality is found in a diagnostic sample, a minimum of twenty metaphases must be examined, to include ten fully analysed, with a further ten counted and scored for structurally abnormal chromosomes. There are exceptions to this minimum criterion, explained under specific diseases. If a normal result is based on examination of fewer than twenty cells, the report must be suitably qualified. If only ten normal cells or fewer are found, it must be stated in the report that the analysis cannot reliably exclude a significant clonal abnormality

Where cytogenetic follow-up is required, for example after treatment or in remission, the following strategies are recommended:

- If a normal result was obtained at diagnosis, further analysis is usually not appropriate.
- If an abnormal result was obtained at diagno-sis, a minimum of 20 metaphases (see excep-

tions to this under specific diseases) should be scored for the diagnostic abnormality and secondary changes in abnormal cells. In some instances, FISH or real-time quantitative (RQ-PCR) may be preferable for follow-up studies/ detection of minimal residual disease.
- For post-transplantation samples, a minimum of 20 metaphases should be scored for the presence or absence of the distinctive karyotypic feature used to differentiate between donor and recipient cells *e.g.* the Y chromosome in mixed sex transplants. FISH analysis of 100 nuclei may be an alternative here.
- For samples at possible relapse or transformation a minimum of 20 metaphases must be analysed.

For metaphase analysis, two analysts, at least one of whom is a senior practitioner with appropriate experience in oncology cytogenetics, should be involved in the analysis or checking of all diagnostic cases and must agree the result. (see European General Cytogenetic Guidelines and Quality Assurance, 2011, for definition). Checking (as it is understood for constitutional analysis) cannot be applied to oncology preparations, but laboratories should have analytical/ checking protocols in place to ensure that the result is accurate as far as is practicable. In every case, a suitably qualified person must confirm that appropriate investigations have been carried out at an acceptable level of quality with respect to the referral reason, and finally sign off the case.

1.3 Interphase FISH

Thresholds and the confidence limit should be established for all FISH probes and probe sets, where the variation of aberrant signal patterns is documented for a number of normal and abnormal samples (usually 5 of each) to give the false positive/negative ranges. It is important to confirm these thresholds/confidence limits for a variety of different preparations (e.g. fixed cells, smears, paraffin embedded sections, touch preparations etc). Any FISH system used for diagnostic purposes must be validated and should always include adequate control probes to minimise ambiguous signal patterns.

Interphase FISH can be used as a sole test for CLL and other lymphoproliferative disorders (LPD), and also for follow up samples of other neoplasms with a known clone, provided the appropriate panel of probes is used. The minimum level of analysis recommended for an interphase FISH study is 100 nuclei. However, it is recognised that an adequate positive result can often be obtained with smaller numbers while an equivocal finding (i.e. the possibility of a low-level clone close to the threshold cut-off may need more cells and/or a repeat investigation). In many diagnostic FISH studies, it is useful to examine a few metaphase cells, if present, and not depend entirely on interphase nuclei. In normal metaphases this confirms the cytogenetic location of the probes used, and abnormal metaphases can be invaluable in interpreting unusual signal patterns.

Laboratories must be aware of the different types of FISH probes and have documentation available to the analyst that explains normal signal patterns *e.g.* break-apart probes and fusion probes. The limitations of the test (probe set) must be documented in the report, if the analysis of the sample is restricted only to interphase cells (i.e. no metaphase analysis done).

The use of FISH on paraffin embedded sections or touch preparations/smears is an appropriate approach to the investigation of specific chromosomal aberrations and has the advantage that tumour tissue can be directly screened (Perry, 2006; Summersgil *et al*., 2008; Tibiletti, 2007; E.C.A. Guidelines, 2012). When an abnormality is consistently detected in a high proportion of cells, analysis can be limited to ten nuclei scored by both analyser and checker. A100 nuclei should be scored by each person before exclusion of the abnormality is suggested.

For the detection of residual disease in neoplastic disorders at least 100 interphase nuclei must be analysed. For minimal residual disease with recurrent rearrangements, real-time quantitative PCR (RQ-PCR) testing is the gold standard and may therefore be more appropriate.

1.4 Microarray

Microarray is starting to be used for acquired disorders but at the time of writing these guidelines, it is still in its infancy for diagnostic use for most laboratories (Simons *et al*., 2012). It is essential that this technique is internally validated on known abnormal samples prior to use diagnostically. Microarray techniques have been used diagnostically for CLL, however arrayCGH may have limited sensitivity for low level clones.

2. MYELOID NEOPLASIA

One day and/or two day cultures are standard for all myeloid disorders. It is recommended that a method of cell counting is used, so that final culture densities can be optimised to approximately $1x10^6$/ml. Synchronisation or elongation techniques are sometimes beneficial. Use of specific growth factors or conditioned media may improve quality but laboratories should carry out appropriate assessments of mitotic indices and abnormality rates before introducing such factors.

Single cell trisomy, most frequently chromosome 8, poses a particular problem in myeloid neoplasm. It is recommended that a further 30 metaphases be scored for the trisomy, if possible, or alternatively FISH analysis undertaken. A single monosomy chromosome 7 cell in the absence of other metaphases with chromosome loss poses a particular problem in myeloid disease. If found, it is recommended that a further 30 metaphases be scored for chromosome 7 if possible, or interphase FISH analysis considered.

2.1 CML (chronic myelogenous leukaemia)
Diagnosis of CML
A minimum of three cells must be fully analysed with a further seven cells scored for the Ph chromosome [der(22)t(9;22)(q34;q11)] and other obvious abnormalities, unless trial studies require something different (ACC guidelines, CML and other myeloproliferative neoplasms, 2011; Haferlach *et al*., 2007). If the result shows a variant translocation consideration should be given to further investigation by FISH or reverse transcription PCR (RT-PCR) studies. Before the result can be interpreted as cytogenetically normal, ten cells must be analysed and a further 10 cells counted and scored for structural abnormalities. FISH or RT-PCR is essential if no Ph chromosome can be found.

Cytogenetic diagnosis of CML should be made using a BM sample (Baccarani *et al.,* 2009). If it is not possible to obtain marrow, diagnosis using PB may be successful if the level of blasts is >10%. ELN guidelines state that chromosome analysis should be undertaken for diagnostic samples, and although karyotyping remains the gold standard it may be acceptable by local agreement to use alternative molecular techniques (FISH and RT-PCR) for diagnosis and follow-up (Baccarani *et al*., 2009).

A rapid preliminary test may be undertaken with *BCR/ABL1* FISH using a direct harvest or smears. If positive by FISH, G-banded analysis should be done. The t(9;22)(q34;q11) is detected in 90-95% of CML cases at diagnosis, The remaining 5-10% have a variant t(9;22) involving another chromosome, or have a cryptic *BCR-ABL1* rearrangement undetectable on G-band metaphases. It is important to establish if additional changes are present at diagnosis since these may be considered as a potential ‘warning’ (Baccarani *et al*., 2009) and assist the interpretation of results from subsequent samples. This “warning” considers the presence of additional chromosome abnormalities (ACA) in Ph+ cells as a whole. However, the prognostic differences of individual ACA in Ph+ cells should also be taken into account (Marin *et al*., 2008; Fabarius *et al*., 2011). Those termed *major route* abnormalities (i.e. additional der(22), i(17q), trisomy 8 and trisomy 19) are associated with an inferior overall survival and increased risk of progression to accelerated phase, but *minor route* abnormalities (i.e. any other apart from those specified above) do not appear to confer a shorter overall survival or predict a poorer response to treatment with a tyrosine kinase inhibitor (TKI) in chronic phase (Fabarius *et al*., 2011; Palandri *et al*., 2009).

FISH with probes for *BCR* and *ABL1* is mandatory in cases with an insufficient number of metaphases for banded analysis, and also in cases not showing the t(9;22)(q34;q11) in order to detect a cryptic *BCR-ABL1* fusion. It is optional to perform FISH at diagnosis with probes spanning the breakpoint regions in *ABL1* and *BCR* to detect associated der(9) deletions as the adverse clinical outcome has been nullified since the introduction of imatinib (Quintas-Cardama, 2005; Castagnetti, 2010 and Baccarani *et al*., 2009). It is recommended that dual fusion probes are used as they give a more reliably informative signal pattern than the ES (extra signal) probe.

If no *BCR-ABL1* fusion can be detected at diagnosis, and CML is still clinically suspected, then RQ-PCR and/or molecular testing for mutations associated with myeloproliferative neoplasms (MPN) is required (see MPN section below).

Follow up studies in CML
Cytogenetic analysis is recommended by ELN guidelines until complete cytogenetic remission is attained when more sensitive techniques should be used to detect the Ph clone. ELN guidelines recommend cytogenetic follow up 3 and 6 months post-treatment and then every 6 months until complete cytogenetic remission (CCgR) has been achieved and confirmed in a subsequent follow up sample. A patient in whom additional abnormalities were found at diagnosis may have an adverse response to imatinib therapy and more frequent monitoring may be required. (Baccarani *et al*., 2009). Response to treatment can be determined cytogenetically by the reduction in the number of Ph cells, or if a cryptic *BCR-ABL1* rearrangement is detected at diagnosis, then follow up must be done by FISH or molecular methods. Follow up studies must be on the tissue used at diagnosis, as comparisons cannot otherwise be made.

Monitoring the levels of Ph positivity (Ph+) can be performed by karyotyping (minimum of 20 metaphases to be scored) (Baccarani *et al.,* 2006; 2009) or interphase FISH (100 nuclei) ideally on bone marrow. Some studies have shown discrepancies between *BCR-ABL1* interphase FISH scores obtained from unselected screening of whole blood and scores obtained from selective screening of neutrophil/ granulocyte nuclei (Reinhold *et al*., 2003; Takahashi *et al*., 2005). This may be due to the presence of varying proportions of normal lymphocytes in blood that are dependant on the level of myelosuppression during the initial treatment phase. It has been reported that selective scoring of neutrophils/granulocytes more accurately corresponds to the values obtained by marrow metaphase chromosome analysis. If FISH is used as a sole test, laboratories must be aware of its limitations (as karyotypic evolution may not be detected) and as data obtained previously on monitoring response was on conventional cytogenetics (Baccarani *et al.,* 2009).

Although the significance of the emergence of unrelated clones in the Ph negative (Ph-) cells is not yet understood, in order to increase the likelihood of detecting such events, it is recommended that laboratories monitoring by conventional cytogenetics score for the Ph and other obvious numerical and structural abnormalities. This approach also allows for the detection of karyotypic evolution within the Ph+

clone. Accurate interpretation of FISH follow up requires prior knowledge of the signal pattern at presentation, and cases with only a single fusion signal cannot reliably be monitored by FISH. Once CCgR has been achieved by cytogenetics/FISH, quantitative RQ-PCR on peripheral blood samples is more appropriate for subsequent study of response to treatment (Table 1). Although the major purpose of genetic analysis after therapy is now to monitor the level of Ph+ cells, it is also recognised that new clonal abnormalities are occasionally detected in Ph- cells and should be recorded and followed up in subsequent samples as a minority (2-10%) of Ph- patients develop clinically evident MDS/AML (Heim & Mitelman, 2009).

Table 1

Cytogenetic response (CgR)*	Ph+ levels
No response	96-100%
Minimal	66-95%
Minor response	36-65%
Partial response	1-35%
Complete remission/CCgR	0%

*CgR is evaluated by conventional cytogenetic analysis of at least 20 metaphases. (Baccarani *et al.,* 2006). FISH of peripheral blood (200 nuclei) should only be used if bone marrow cells cannot be obtained (Baccarani *et al.,* 2009).

If additional abnormalities specific to acute leukaemia are present in the Ph+ clone, or if it is an i(17q) and these findings are consistent with the bone marrow morphology, the interpretation is straightforward. However, unexpected additional abnormalities may be transient and of no clinical significance (Baccarani *et al*., 2009).

- ACA/Ph+ (major route) at diagnosis is a warning factor (Fabarius et al., 2011);
- ACA/Ph+ during follow-up, if previously in CCgR (i.e. 100% Ph-ve) counts as treatment failure, without it being present in two consecutive samples, because it is the renewed Ph positivity that determines the treatment failure, not the ACA;
- ACA/Ph+ for the first time in a case that has not been in CCgR at any time, requires analysis of two consecutive samples with the same clonal ACA present in both analyses before denoting it a marker of treatment failure.

Accelerated phase/blast crisis in CML

Clonal evolution may indicate selection of treatment-resistant clones within continuing chronic phase disease, or in the context of appropriate haematological changes, may indicate disease acceleration or transformation to acute leukaemia (blast phase). The requirements for the analysis of these samples are the same as those undertaken at diagnosis of acute leukaemia, looking for abnormalities secondary to the Philadelphia chromosome / translocation.

2.2 AML (acute myeloid leukaemia)

The general principles for culture given in Section 1.2.1 apply. A 48-hour culture should be considered for cases where a t(8;21) or a t(15;17) is suspected since, in the experience of many investigators, the aberrant clone is less reliably detected after 24 hours cultivation.

Diagnosis of AML

Bone marrow is the preferred tissue for the investigation of AML, but peripheral blood can be used when there are circulating blasts seen (>10%) in the peripheral blood film. In the first instance, banding analysis must be undertaken and, if an abnormal karyotype is found, a minimum of five abnormal metaphases must be fully analysed with a further five clonal metaphases counted and scored for additional structural changes if available. In the event of a normal karyotype 20 metaphases must be examined with at least ten fully analysed and the remainder counted and scored for structural abnormalities before the issue of a normal report. If 20 metaphases cannot be examined the normal report must be qualified (see section 5 on reporting).

FISH analysis to screen for *MLL* (plus partner chromosome) and *MECOM* rearrangements is highly recommended on all diagnostic AML samples as these abnormalities have a pronounced prognostic impact, and in some cases may be cryptic by banded analysis. Additional FISH analyses should be performed to screen for monosomy 5/del(5)(q31.2) and monosomy 7/del(7)(q31.2) in the case of a failed or incomplete cytogenetic analysis where no subtype-specific abnormality was detected (i.e. where fewer than 20 normal metaphases have been analysed) and the morphological subtype is unknown. In the case of a normal karyotype, additional FISH analysis to exclude cryptic *PML-RARA*, *CBFB-MYH11* or *RUNX1-RUNX1T1* rearrangements depending on the morphological subtype. The impact of screening for molecular mutations in genes such as *NPM1*, *FLT3*, *CEPBA*, *RUNX1* is increasing especially in AML with normal karyotype as the prognostic relevance has been proven in several clinical trials. Note that AML with *NPM1* mutation and AML with *CEPBA* mutation are provisional entities in the WHO (2008) classification.
FISH may also be used where common additional abnormalities associated with specific rearrangements are found. RT-PCR may be used as an alternative to certain FISH tests. Collaboration with the referring clinician is important in establishing which additional tests should be undertaken, and information is available on the European Leukaemia Network (Dohner *et al*., 2009).

Follow up studies in AML

Banded metaphase analysis is not mandatory for establishing remission, but may be helpful for

suggesting or confirming refractory disease. If the diagnostic karyotype was abnormal, scoring for the abnormalities found at diagnosis in at least 20 banded metaphases, or interphase FISH (100 nuclei scored) may be undertaken if required (Dohner *et al*., 2009). If the diagnostic karyotype was normal, analysis of post-induction samples is rarely of value.

Relapse in AML

Full analysis of 10 metaphases containing the diagnostic clone is sufficient to allow determination of any clonal progression. If no abnormality is detected, the requirements are as for follow up studies, although FISH/RT-PCR for the diagnostic abnormalities should be considered if there is still a significant suggestion of relapse. If the original abnormality is not found and there is the possibility of a secondary malignancy, a full diagnostic analysis must be undertaken in order to exclude or confirm a different disease.

2.3 MDS (myelodysplastic syndrome)

The general principles for culture given in Section 1.2.1 apply. Collaboration with the haematologists is especially important in this referral category, where many non-neoplastic disorders may present in a similar way.

Culture

In MDS, prolonged colcemid exposure increases the success rate especially in MDS with low cell count (Haferlach *et al*., 2007)

Diagnosis

Bone marrow is the preferred tissue for the invest-tigation of MDS because the cells belonging to the MDS clone are usually not dividing in PB (Cherry *et al*., 2012). If an abnormal karyotype is found, five abnormal metaphases must be fully analysed with a further five clonal metaphases counted and scored for additional structural changes. In the event of a normal karyotype, 20 metaphases must be examined with at least ten fully analysed and the remainder scored for obvious chromosome abnormalities.

If fewer than 10 normal metaphases are found, FISH assays, including probes for the detection of monosomy 5/deletion of 5q and monosomy7/deltion of 7q must be undertaken. Probes may also be used in addition to detect other frequent aberrations, for example trisomy 8, *TP53* deletion, 20q deletion, and Y chromosome loss (Haferlach *et al.*, 2007; Greenberg *et al*., 2012; Schanz *et al*., 2012). Alternatively SNP or microarrays can be used or a fresh BM requested. Where the suspected diagnosis by morphology is MDS 5q- syndrome and no mitoses were recovered or a normal karyotype was found, then FISH for deletion of 5q must be undertaken.

2.4 Non-neoplastic disorders, MDS related (aplastic anaemia)

Aplastic anemia is not in itself a neoplasm, but may be a symptom. If analysis is required these cases should be treated the same as for MDS samples. Consideration should be given to the possibility of Fanconi anaemia in childhood aplastic anaemia and liaison with the referring clinician with regard to appropriate testing is important.

2.5 MPN (myeloproliferative neoplasms excluding CML but including MDS/MPN) and hypereosino-philic syndromes

MPN is a heterogeneous group of clonal stem cell disorders. The WHO (2008) classification represents these as different disease categories including the classic MPNs – polycythaemia vera (PV); essential thrombocythaemia (ET); and primary myelofibrosis (PMF), other MPN; MPN overlapping with MDS and the rare myeloid and lymphoid neoplasms with eosinophilia. The role of molecular analysis is particularly important for some MPN diseases (WHO, 2008). The WHO (2008) and ACC Guidelines recognise the rare hypereosinophilic syndrome/ chronic eosinophilic leukaemia as separate entities. Owing to the responsiveness of these disorders to tyrosine kinase inhibitors, their accurate diagnosis is important.

Diagnosis

Cytogenetic abnormalities are not usually specific and general abnormalities of myeloid neoplasms are found. While normal karyotype results are uninformative, the detection of an abnormality can be diagnostically useful to confirm a clonal neoplastic disorder. A *JAK2*^{v617F} mutation should be excluded (this test may be done by other pathology disciplines). The exclusion of a *BCR-ABL1* fusion is necessary for the differential diagnosis of non-classic MPN from CML. Cytogenetic studies are not essential for the diagnosis of most MPN but may be referred by local agreement with the haematologist(s). Bone marrow is the preferred tissue for the cytogenetic investigation of MPN. However if the white blood cell count in the PB is elevated and immature cells are observed, or in cases of chronic idiopathic myelofibrosis (CIMF) with BM dry tap, PB may be used. If an abnormal karyotype is found, five abnormal metaphases must be fully analysed with a further five clonal metaphases counted and scored for additional structural changes. In the event of a normal karyotype, 20 metaphases must be examined; however, it is necessary to analyse only five metaphases fully, as in the majority of cases Ph exclusion is the most important factor and other MPN-associated abnormalities can be screened for when scoring the remaining metaphases.

FISH on interphase nuclei and/or metaphases with probes for *BCR* and *ABL1* is recommended in cases with an insufficient number of metaphases, or in cases not showing a specific abnormality, since a proportion of CML may be misclassified as MPN by cytomorphology only.

For diagnosis of hypereosinophilic syndrome/chronic eosinophilic leukaemia FISH (or RT-PCR) investi-

gations for *FIP1L1- PDGFRA* fusions and *PDGFRB* rearrangements are recommended,

There are no specific cytogenetic abnormalities that will confirm transformation to acute leukaemia or CIMF. However, karyotype evolution to complex karyotypes with abnormalities of 5q, 7q and 17p is suggestive of transformation.

3. LYMPHOID NEOPLASIA

Lymphoid cells are generally less amenable to culture than myeloid cells. In acute lymphoblastic leukaemia (ALL) and high grade lymphoma *in vitro* cell death can be a significant problem. Laboratories should consider harvesting one culture on the day that it is received (particularly for high grade lymphoma) and the preparation of smears or cell suspensions for interphase FISH. Use of synchronising agents in culture is often less successful in ALL than AML. Same day, overnight and two-day cultures are recommended for ALL and most B cell lymphoma cases, and 72 hour cultures for T-cell lymphomas. Where sufficient material is available, a selection of appropriate cultures is recommended.

Blood or bone marrow cells from chronic lymphocytic leukaemia (CLL) and some lymphomas e.g. mantle cell lymphoma (MCL) may benefit from stimulation with B-cell mitogens that necessitates a longer time in culture (3 or 4 days). However, short term cultures or smears are more appropriate for interphase FISH.

3.1 Acute lymphoblastic leukaemia - B or T cell ALL

B-lineage ALL is more frequent, accounting for 85% of childhood ALL and 75% of adult ALL (WHO, 2008). Bone marrow from lymphoid leukaemias is generally cultured overnight with short exposure to colcemid before harvesting. If bone marrow is not available then trephine biopsies or PB (if circulating blasts) may be cultured. The recommended seeding cell density is $1x10^6$/ml. If sufficient material is available a second 24hour or an overnight culture is a useful back up. In B-cell ALL, growth factor supplements may improve the quality of the chromosome preparations for analysis. For T-cell lineages, PHA stimulated cultures may be appropriate. There is evidence that cell cycle synchronization techniques as well as high colcemid concentrations and prolonged colcemid exposure may have a negative impact on metaphase yield in ALL due to cell poisoning. Optimisation of the culture techniques is therefore important and multiple cultures with different culture times are recommended where practical.

WHO (2008) and other publications (for example ACC guidelines on acute lymphoblastic leukaemia; Heim & Mitelman 2009, Moorman 2012, Moorman *et al.*, 2010) give an overview of the non-random abnormalities found in precursor B-ALL detailing the chromosome abnormality, gene(s) involved, FISH probes and prognosis. The most significant genetic prognostic factors for paediatric B-ALL are: t(9;22)(q34;q11), MLL rearrangements, *ETV6-RUNX1* fusion, amplification of the RUNX1 locus (iAMP21), t(17;19)(q22;p13), hyperdiploidy and, rarely, near-haploidy.
It is recommended that FISH probes for *BCR/ABL1*, *ETV6/RUNX1*, and *MLL* are used in all cases of B-ALL depending on the age of the patient. Rapid diagnosis is important and multiple FISH tests may be run simultaneously. Alternatively sequential testing may be considered as the abnormalities are almost mutually exclusive. If this approach is taken then a suggested order for infants (<1 year old) is MLL, *ETV6-RUNX1, BCR-ABL1*. For paediatric/adolescent ALL it would be *ETV6-RUNX1, BCR-ABL1*, then *MLL*. For adult ALL *BCR-ABL1* then *MLL; ETV6-RUNX1* in adult ALL is optional. Further FISH testing may be considered for potential high-hyperdiploidy if the karyotype is normal or fails e.g. 4, 10, 17 and 18 (Moorman *et al*., 2003). FISH for T-ALL is optional but could include *CDKN2A, TCRb, TLX3, TLX1, MLL, SIL-TAL1* and *NUP214-ABL1*. If Burkitt leukaemia/lymphoma is suspected a break apart MYC probe and IGH@/MYC should be used (see Heim & Mitelman 2009, p330) and if rearrangement positive, FISH for an IG-MYC rearrangement (to exclude non IG-MYC rearrangements in adults), *BCL2* and/or *BCL6 (*see Section 3.2.2) should be undertaken.

For T-cell lineage ALL, an abnormal karyotype is reported in 50-70% of cases (WHO, 2008). Numerical abnormalities are less frequently observed than in B-cell ALL with the exception of paratetraploidy, which is present in approximately 5% of cases. Around 35% of T-cell ALL have rearrangements involving the TCR loci at 7q34 (*TCRB*) or 14q11 (*TCRA/D*) (Graux *et al*., 2006, Heim & Mitelman 2009). *TLX3* and *TLX1* abnormalities are observed in 25% and 5% of childhood T-ALL patients respectively.

Diagnosis

If an abnormal karyotype is found, ten abnormal metaphases should be fully analysed. If necessary, the abnormal result can be reported on fewer cells provided the abnormality is clonal. In the event of a normal karyotype, 20 metaphases must be examined with at least ten fully analysed and the remainder counted and scored for structural abnormalities before issue of a normal result. If 20 metaphases cannot be examined the normal report must be qualified (see section 5 on reporting).

In cases where extra RUNX1 signals are found, intrachromosomal amplification of chromosome 21 (iAMP21) typically presents as 5 or more copies and shows clustering of signals (although individual cells may display apparently distinct signals). On the other hand, extra RUNX1 signals that are not obviously clustered are more likely to be indicative of a hyperdiploid karyotype. The hyperdiploid karyotype will rarely display more than five RUNX1 signals.

In paediatric ALL with an 11q23 abnormality other than the classic translocations, *MLL* FISH should be performed, since only 50% have a rearranged abnormal signal pattern.

When a TCF3 breakpoint probe detects a rearrangement, it is important to distinguish between a t(17;19) and a t(1;19) as the prognosis is different.

Analysts should also be aware of the different mechanisms that may give rise to a hyperdiploid clone. While most hyperdiploids originate from simple gain of chromosomes in a diploid cell line, a near-haploid or low hypodiploid clone can ‘double up’ and appear as hyperdiploid/near-triploid metaphases. The prognosis for these two entities is different.

Follow up studies

Follow up needs to employ the same method that was used to make the diagnosis. G-banded analysis of post-induction samples is not mandatory and is unlikely to be clinically useful. Follow-up studies by FISH analysis may be helpful in some circumstances. Minimal residual disease (MRD) is best monitored by molecular means or by flow cytometry.

Relapse

G-banded scoring of ten metaphases is sufficient when a previous diagnostic abnormality is detected. However, if there is any suggestion of clonal progression, additional analysis must be undertaken. If no abnormality is detected, 20 metaphases must be scored. The possibility of a secondary malignancy should be considered in very late relapse cases with no sign of the diagnostic abnormality (see AML relapse). In the case of relapse involving *ETV6-RUNX1* rearrangement, FISH must be undertaken.

3.2 Mature B-cell neoplasms

3.2.1 CLL (chronic lymphocytic leukaemia)

CLL is diagnosed on B cell count, morphology and immunophenotype, not on cytogenetics.
Interphase FISH has been established as the routine diagnostic procedure for cytogenetic classification of CLL, as a lower abnormality rate is detected by banding alone. Banded chromosomal analysis is therefore not mandatory and, if normal, must not be considered as a stand-alone test. However, chromosome analysis is useful in this group as a prognostic indicator and to differentiate CLL from mantle cell lymphoma (MCL) or other B-cell lymphomas.

For culturing in CLL, PB has been found to be more successful than BM. A low proliferation rate of CLL cells *in vitro* is expected, and in order to maximise the yield of metaphases at least two cultures should be performed, one for 24 hours, and a three day culture supplemented with the oligonucleotide DSP30 and the cytokine IL2. Smears may be used for FISH, where the quality is comparable to that in cell suspensions and has the added advantage of giving additional information on cell morphology.
FISH probes detect prognostically significant abnormalities that may affect clinical management. A variety of commercial probes are available and a CLL service should offer tests that are clinically relevant. FISH is particularly useful for the differential diagnosis with MCL. FISH for either t(11;14)(q13;q32) or IGH@ breakapart should be offered in all cases with atypical morphology or with an immunophenotyping score for CLL of 3/5 or less. If an IGH@ rearrangement is found, additional FISH probes should be used to identify the partner chromosome (it may be a t(11;14) or t(14;18) or t(14;19)).

To assign the patients into clinically relevant prognostic subgroups, FISH is recommended for the following loci and chromosome regions: minimal regions of loss at 11q22 (*ATM*); *D13S319* at 13q14 (plus control probe); *TP53* at 17p13; probes to detect full and partial trisomy 12. In addition, FISH for the detection of a 6q21and/or 6q23 deletion can be performed.

3.2.2 B- and T- cell lymphomas

For all lymphomas the preferred tissue is lymph node or other relevant biopsy material. If fresh material is available, banded karyotyping is recommended. For analysis see Section 1.2.2.

Karyotyping and/or FISH may be used as a diagnostic test or to add information to the immunophenotyping and morphology data.

BM or PB analysis is not appropriate unless there is morphological/immunophenotypic evidence of infiltration, and clinical liaison is essential to exclude inappropriate samples. For FISH analysis, BM smears may be preferable to BM cultures in allowing better selection of cells with relevant morphology and being less prone to cell degradation in poorly viable cases.

Where banding on lymphatic tissue is attempted, 20 metaphases must be scored for the key abnormalities. Full analysis is not necessary unless an abnormality is found. If analysis is on infiltrated BM, the number of cells scored must be at least 50 before issue of a normal report. Where an abnormal clone is detected, five abnormal metaphases must be fully analysed and a further 5 counted and scored for the same and additional abnormalities.

FISH testing, using all the necessary validated probes, must be available if a lymphoma service is offered. Collaboration between cytogenetic laboratories is encouraged when using probes for rare abnormalities to maximise efficiency. The use of tissue sections allows the selection of clinically relevant samples, unlike fresh tissue where up to 50% of samples may turn out to be non-lymphomatous.

Karyotyping and FISH may be used either as a diagnostic test or as an adjunct to the immunophenotyping and morphology data. Guidelines on the diagnosis and reporting of LPD and Lymphoma have been published that also give valuable recommendations for all laboratories/Multi-disciplinary teams (MDTs) (Parker *et al*., 2008). Laboratories should refer to the WHO classification of tumours (WHO, 2008).

Some examples of most common recurrent chromosome abnormalities in NHL are given below (this is not an exhaustive list, see WHO, 2008 or Heim & Mitelman, 2009 for a comprehensive list).

(i) Recurrent translocations associated with MALT lymphomas include t(11;18)(q21;q21); t(1;14)(p22;q23); t(14;18)(q32;q21) and t(3;14)(p14.1;q32) (WHO, 2008). Trisomy 3 and/or trisomy 18 are non-specific but not infrequent findings in MALT lymphomas. The frequency at which these abnormalities occur varies markedly with the primary site of the disease. Transformation to diffuse large B-cell lymphoma (DLBCL) may occur.

(ii) The recurrent translocation associated with follicular lymphoma (FL) is characterised by t(14;18)(q32;q21) and BCL2 gene rearrangements. FISH for a BCL2 rearrangement is the most widely used method of detection (WHO, 2008). Alternative BCL2 translocations involving the immunoglobulin light chain have been reported. BCL2 rearrangements are much less frequent in grade 3B FL and if BCL2 negative should be checked for BCL6 gain and rearrangements (WHO, 2008). Additional abnormalities may involve loss of 1p, 6q,10q and 17p and gains of chromosome 1, 6p, 7, 8, 12q, 18q and X. Rare cases involving a t(14;18)(q32;q21) in conjunction with a t(8;14)(q24;q32) or variants constitute a separate disease entity (WHO, 2008). Transformation to DLBCL may occur.

(iii) The recurrent translocation associated with mantle cell lymphoma (MCL) is t(11;14)(q13;q32) involving the CCND1 and IGH@ loci. Variant translocations involving the immunoglobulin light chain have rarely been reported and may not present with typical MCL (WHO, 2008). Trisomy 12 and several other non-random secondary chromosomal abnormalities have been reported. The presence of a t(8;14)(q24;q32) with MYC translocation occurs rarely and is associated with an aggressive clinical course.

(iv) In diffuse large B-cell lymphoma (DLBCL), 50% have rearrangements involving the IGH@ locus (Heim & Mitelman, 2009) while 30% have abnormalities of BCL6 (WHO, 2008). Translocations involving the BCL2 gene e.g. t(14;18)(q32;q21), which is a hallmark of FL, also occurs in 20-30% cases of DLBCL (WHO, 2008). MYC rearrangements are observed in 10% of cases; the MYC partner is an IG gene in 60% and a non-IG gene in 40% of cases (WHO, 2008). Gain of 3q and 18q is common in the ABC subtype, while gain of 1q, 7 and 12 is common in the GCB subtype.

(v) ALK-positive DLBCL, All cases contain rearrangements involving the ALK gene, typically the t(2;17)(p23;q23) but rarely other translocations including the t(2;5)(p23;q35) translocation. FISH with the ALK break apart probe is recommended as some cryptic insertions have been reported with the ALK gene (WHO, 2008).

(vi) Burkitt lymphoma (BL) is characterised typically by t(8;14)(q24;q32) involving MYC and IGH@ with no additional involvement of BCL2 or BCL6. The less common partners of MYC (8q24) involve the immunoglobulin light chains at 2p12 and 22q11 (WHO, 2008). There is a Burkitt leukaemic variant in patients with bulky disease and rare cases present as a pure leukaemia with PB and BM involvement. Translocations involving MYC are NOT specific to BL and BCL2 and BCL6 rearrangements must be excluded as well as non-IG-MYC translocations. WHO (2008, p265) compares the features that may be used in distinguishing BL from DLBCL.

(vii) B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL is a new disease category in WHO (2008). When both MYC plus BCL2 and/or a BCL6 are rearranged these lymphomas are classified as double or triple hit lymphoma. Frequently, the MYC partner gene is a non-IG locus. Double hit lymphomas often show a complex karyotype with multiple abnormalities (WHO, 2008).

3.2.3 Plasma cell myeloma

Plasma cell myeloma or multiple myeloma (MM) is an orphan disorder of end stage plasma cells with acquired genetic abnormalities of clinical importance not captured by conventional cytogenetic analysis (Ross *et al*., 2012).

Three to four day unstimulated culture is helpful in myeloma as fewer contaminating normal mitoses are found. Some laboratories use phobol stimulated cultures as an alternative method for chromosomal analysis.

FISH is recommended as the first line of analysis for plasma cell myeloma. However, if there are more than 30% plasma cells, banding analysis may be undertaken. If there are <30% plasma cells it is recommended to separate them using magnetic beads (the CD138+ fraction is isolated) followed by FISH for *TP53*, *IGH@*, 1p gain and 1q loss. If the sample is positive for an *IGH@* rearrangement then the sample must be scored (either simultaneously or sequentially)

for t(4;14), and t(14;16) (Dimopoulous *et al*., 2011; Munshi *et al*., 2011; Ross *et al*., 2012). An extended panel may include testing for t(11;14) (q13;q32), t(14;20)(q32;q12), ploidy status, and chromosome 12 and 13 abnormalities. (Ross *et al*., 2012) and testing to establish aneuploidy for any two chromosomes out of 5, 9, 11 and 15. Positive cut-off levels should be relatively conservative: 10% for fusion or break-apart probes, 20% for numerical abnormalities (Ross *et al.,* 2012).

In the absence of a reliable method of identifying and selecting plasma cells, totally normal FISH results must be qualified, explaining that the possibility of a false negative result is much higher than might be anticipated from assessment of the morphology smears.

If karyotyping is undertaken the emphasis should be on scoring for the key abnormalities e.g. 14q32 translocations, loss of 17p, deletion/monosomy 13 (although not as a sole abnormality) and high hyperdiploidy (Heim & Mitelman, 2009). Analysis should be verified by FISH or other methods before reporting (Ross *et al.,* 2012). Note that random (non-clonal) abnormalities also occur frequently and must not be reported.

4. BONE MARROWS FROM SOLID TUMOUR CASES

These guidelines only briefly cover some of the soft tissue sarcomas and oligodendroglial neoplasms. Further information on these and other solid tumours can be found in Heim & Mitelman (2009) as well as the E.C.A. Guidelines: FISH on histological sections of solid tumours (2012).

Bone marrows with confirmed infiltration of metastatic solid tumours can provide a very valuable source of tumour cells for clinically important genetic studies, particularly when biopsies of the primary tumour may be of very limited size and/or of poor quality. This can be particularly important in paediatric cancers including Ewing tumour, rhabdomyosarcoma and neuroblastoma. It is strongly recommended that the morphological result is obtained as soon as possible to establish the degree of infiltration. If this is significant, multiple cultures should be established including (i) direct or overnight harvest and (ii) flasks to allow growth of adherent cells. Where no abnormalities are obvious, examination should be directed to screening of large numbers of cells for likely abnormalities rather than detailed analysis of small numbers of cells.

(i) There are limits of reliance on the *EWSR1* break apart FISH probe in the differential diagnosis of sarcomas, as *EWSR1* is rearranged in a number of tumours (Ewing sarcoma family tumours, extra-skeletal myxoid chondrosarcoma, desmoplastic small round cell tumour, angiomatoid fibrous histiocytoma and clear-cell sarcoma). The *EWSR1* gene has been shown to be involved in translocations with a number of different partner genes (most not identifiable using FISH). The specific partner gene involved in *EWSR1* rearrangements has been shown to correlate strongly with specific clinical pathology entities such as Ewing's tumour/ primitive neuroectodermal tumour; extraskeletal myxoid chondrosarcoma; clear cell sarcoma; desmoplastic small round cell tumour; myxoid liposarcoma and angiomatoid fibrous histiocytoma.

(ii) Rhabdomyosarcoma
Tumour genetics in alveolar and embryonal rhabdomyosarcoma is not currently used for prognostic stratification. *FOXO1* rearrangement and *3'FOXO1* amplification occur in alveolar rhabdomyosarcoma (aRMS) tumour sample, while additional copies of *FOXO1* but no rearrangement can occur in an embryonal rhabdomyosarcoma (eRMS) tumour sample. There are also reports in the literature of aRMS with no evidence of a *FOXO1* rearrangement (Watchel *et al*., 2004). When reporting these FISH results in the ISCN it is important to remember that *3'FOXO1* is proximal to *5'FOXO1*. It is not the cytogeneticist's role to discuss clinical prognostic factors, for example, that eRMS carries a better prognosis than aRMS. FISH for *MYCN* is optional as it is not currently used in establishing treatment intensity. Published data regarding the poor prognosis of *MYCN* are conflicting.

(iii) Neuroblastoma
The most frequent abnormalities detected in neuroblastoma are *MYCN* amplification, deletion of 1p, unbalanced gain of 17q and loss of 11q. FISH analysis is the recommended for this tumour type and is often referred to specialist centre. The European Neuroblastoma Quality Assurance Group guidelines provide nomenclature to distinguish between 'deletion' and 'imbalance' (Ambros *et al*., 2003).

(iv) Synovial sarcoma
Synovial sarcoma is characterised by an *SS18* rearrangement (18q21).

(v) Oligodendroglial neoplasms
Oligodendroglial neoplasms are characterised by loss of heterozygosity at 1p and 19q (Perry, 2006, Scheie *et al*., 2006).

5. REPORTING

The most recent version of the International System for Human Cytogenetic Nomenclature (ISCN) must be used to report the results of the chromosome banding. The presence of significant abnormalities detected only by FISH may be given in ISCN but in any case must be summarised in a prime position in the report. Cell numbers must be given in the ISCN for all

cytogenetic investigations in neoplastic disorders, both normal and abnormal results. It is preferable to describe the FISH results as normal or abnormal. The term 'positive' or 'negative' must not be used to describe the FISH result as the referring clinician may not understand the difference between a fusion or break apart probe. Abnormal FISH results should be described as for example '*MLL* rearrangement is present' or '3 copies of *RUNX1* present' so that it is clear the result is not normal.

Where abbreviated cytogenetic results are integrated into a multidisciplinary report, the information in the abbreviated version must be consistent with the full cytogenetic report. The cytogenetic summary must be authorised by a suitably qualified senior/supervising practitioner. A full version of the cytogenetic report must be sent independently to the referring consultant.

It is advisable to provide information regarding the clinical consequences of the observed genetic aberrations in the report (see Mitelman *et al.,* 2006; Swerdlow *et al*., 2008), or if a purely technical report is issued it should be made clear to the referring clinician that the interpretation of the results must be clearly documented in the patient notes.

The generic term "malignancy" should not be used in the context of a clone of unknown significance. The term 'chromosomal aberrant clone' is recommended instead. In relation to the disease category, WHO (2008) nomenclature should be used.

If a potential non-mosaic constitutional abnormality is detected, analysis of a PHA stimulated sample or remission sample may be appropriate. Consideration should be given to the wider implications for the patient and their family members. Although mosaic trisomy 8 can be constitutional, it is not considered necessary to attempt to exclude this in the majority of circumstances where an extra chromosome 8 is found as the sole abnormality in a myeloid disorder. When reporting –Y or +15 it should be made clear that these changes can be found in elderly patients with no haematological neoplasm (Guttenbach *et al.,* 1995; Wiktor *et al*., 2000).

The finding of a single abnormal metaphase, even one of potential significance, cannot define a clone (ISCN, 2013). Proof of clonality may often be possible by FISH and/or molecular studies. If this is not the case, potentially significant abnormalities may be reported with qualifications. When there is fusion or rearrangement the genes can be written as *IGH@-CCND1* (i.e. use a – sign rather than a /) to distinguish the fusion product from a mixed probe kit.

Qualified reports: It is helpful to draw attention to limitations of the analysis and uncertainties of the result especially when the extent of analysis has not reached the standard given in guidance documents.

Reports of acquired cytogenetic abnormalities must contain the following information:

- information for laboratory identification;
- patient identification (using two different identifiers; date of birth, full name - not initials);
- referring physician/scientist identification;
- sample information (type of sample, date of sample referral, date of report and unique sample identification);
- referral information (reason for referral and clinical indication for test);
- limitations of the test used when appropriate for FISH and chromosome analysis;
- summary statement, whether or not a clinically significant abnormality was detected;
- reference to other investigations (e.g. FISH) to clarify significance;
- ISCN (including number of cells examined – refer ISCN, 2013 for reporting FISH results);
- modal number of chromosomes in all cell lines
- description of numerical abnormalities includeing the number of copies of any chromosome missing or extra;
- description of clinically relevant structural abnormalities including the chromosomes, arms and band locations involved in the rearrangement;
- names of significant genes at loci involved in any established recurrent rearrangement;
- description of any karyotype imbalance resulting from unbalanced rearrangements and aneuploidy;
- disease given according to WHO classifycation;
- relationship of any abnormalities found to the referral reason, or other possible disease association;
- association with prognosis if a robust association from multiple publications/ international trials/trial protocols exists (e.g. Ambros et al., 2003; Baccarani et al., 2009; Greenberg et al., 2012; Grimwade et al., 2010; Moorman et al., 2010; Munshi et al., 2011; Schanz et *al*., 2012; Varella-Garcia 2003; Zenz *et al.,* 2011).

5.1 Reporting times

The guidance in Table 2 (below) is for maximum reporting times and it is expected that the majority of referrals will be reported well within these times. The laboratory should have contingencies for providing more rapid reporting of some results.

Table 2

Urgent referrals (*e.g.* acute leukaemia):	95% should be reported within 10 calendar days. A diagnostic FISH result is adequate in this category, with confirmatory cytogenetics treated as for routine referrals.
Rapid test by FISH/PCR [*e.g.* t(15;17)]:	95% reported in 3 working days.
Routine referral (*e.g.* follow up):	95% should be reported within 21 calendar days.

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APPENDIX

A. INDICATIONS FOR CYTOGENETIC ANALYSIS

Whenever a clinician suspects a patients' disease is associated with a recurrent chromosomal abnormality or would benefit from cytogenetic analysis. Although these conditions are well known to most clinicians referring patients to a cytogenetics laboratory, this list of indications may be helpful to delineate the type of patients eligible, especially if these indications are used in conjunction with the ICD-10 nomenclature of diagnoses. These indications are given as a guideline to enable stakeholders to monitor the referral pattern and the expected workload of a cytogenetics laboratory. The local referral pattern may also be dependant on local clinical need and/or national clinical trials (see also section 1.1).

CLINICAL INDICATIONS FOR CANCER CYTOGENETICS

(in the absence of alternative local referral policies) (bone marrow, lymph node, solid tumour, aspirates, fluids)

- Acute leukaemia: at diagnosis. If an abnormality is present, follow up prior to or after treatment or at relapse may be indicated. If an abnormal clone is not detected, re-investigation at relapse may be indicated;
- Myelodysplastic syndrome (MDS): at diagnosis, for disease progression and after treatment;
- Chronic myelogenous leukaemia (CML): at diagnosis. Follow up may be indicated for staging purposes or to monitor therapy efficiency;
- Other chronic myeloproliferative neoplasms (MPN): at diagnosis in selected cases, to rule out CML and to assess for possible acute leukaemic transformation;
- Malignant lymphoma and lymphoproliferative disorders (LPD): at diagnosis in selected cases, follow up or relapse;
- Solid tumours: may be indicated at diagnosis for small round cell tumours of childhood, selected sarcomas, lipomatous tumours, and other tumours in consultation with the pathologist/clinician;
- CLL for differential diagnosis (CLL vs MCL) or prognostic indications or follow up or relapse.

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