Association for Clinical Cytogenetics

PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS

HAEMATO-ONCOLOGY BEST PRACTICE GUIDELINES (2007) v1.01

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1 INTRODUCTION

These guidelines should be used in conjunction with the Professional Guidelines for Clinical Cytogenetics: General Best Practice Guidelines (2007) and Recommendations for FISH Scoring in Oncology (2003).

Professional guidelines for Cytogenetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA) [1] and by statute (Clinical Governance) while taking into account current practice in the U.K.

Elements of the service not subject to statute may be varied in order to comply with local constraints and agreements. It must be noted that these guidelines are minimum requirements and that professional judgement is of paramount importance for many circumstances.
National trials may have additional requirements in order to stratify patients to the appropriate treatment regimes. These should also be considered mandatory. New techniques and evidence are becoming available all the time and the balance between different types of testing should be kept under constant review.

Close and timely liaison with the referring clinician or other related pathology disciplines is strongly recommended to clarify diagnosis, ensure appropriate culture, analysis, interpretation of findings and particularly in rationalising inappropriate (e.g. reactive) samples.

Cytogenetic laboratories should provide onward referral to specialist laboratories where appropriate, or advice on where such tests are performed. When tests have been delegated to another laboratory, the originating laboratory should request and receive copies of the reports from that laboratory. The originating laboratory must retain responsibility for the interpretation of their own results in the context of the additional information obtained.

The use of 'shall' in this document indicates a requirement and the use of 'should' indicates a recommendation.

Where there appears to be contradiction between available guidelines, the most recently published should be taken to apply to all.

2 PROCESSING OF SAMPLES

Appropriate transport medium should be made available to clinicians by cytogenetic laboratories.

With the exception of high risk samples, a method for cell counting should be used to produce an optimum culture density of approximately 10^6 /ml.

When the sample contains enough cells at least two cultures should be initiated. A range of culture techniques should be available and be dependent on the referral reason (refer to disease-specific sections for details)

3 ANALYSIS AND CHECKING

Level of analysis is dependent on the reason for referral and will be covered in the disease-specific sections below. Analysis from more than one culture regimen should be considered, particularly where the lineage of the neoplastic cells is in doubt.

No minimum quality can be given for haematology samples. This means that the number of cells fully analysed has to be increased where the quality is so poor that normal chromosomes cannot be reliably identified at a glance, or where the primary analyst is not sufficiently experienced to be able to make quick judgements about whether a chromosome is normal or abnormal.

For metaphase analysis, two analysts, one being a registered clinical scientist (sub-modality cytogenetics with appropriate experience in oncology cytogenetics), must be involved in the analysis or checking of all diagnostic cases.

The morphology of leukaemic metaphase chromosomes may be inferior to that of normal cells and it is important to examine metaphases of varying quality until an abnormal clone is detected.

The possibility of co-existing apparently unrelated clones means that, particularly at diagnosis and relapse, adequate cells must be looked at in enough detail to allow detection of more than one cell line.

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.

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. FISH screening may be helpful.

Checking as for constitutional analysis cannot be applied to oncology preparations, but laboratories should have protocols in place to ensure that the result is accurate as far as is practicable. Agreement on abnormal clones should be reached by two analysts, one of whom should be a registered clinical scientist. In every case, a suitably qualified person should confirm that appropriate investigations have been carried out at an acceptable level of quality with respect to the referral reason.

4 REPORTING

Cytogenetic reports shall contain the following information:
- ISCN (for reporting of conventional cytogenetics)
- the number of cells, within the current ISCN
- brief description of clinically relevant abnormalities
- relationship of any abnormalities found to the referral reason, or other possible diagnosis
- association with prognosis if a robust association from multiple publications/international trials/trial protocols exists

The presence of significant abnormalities detected only by FISH shall be summarised in a prime position in the report if FISH ISCN is not being used.

Where abbreviated cytogenetic results are integrated into a multidisciplinary report, the information in the abbreviated version should be consistent with the full cytogenetic report. The cytogenetic summary shall be authorised by a state-registered clinical scientist. A full version of the cytogenetic report shall be sent independently to the referring consultant.

The finding of a single abnormal metaphase, even one of potential significance, cannot define a clone (ISCN 2005). Proof of clonality may often be possible by FISH and/or molecular studies. If this is not the case, significant abnormalities may be reported with qualifications.

The term "malignancy" should not be used in the context of an abnormality of unknown significance. Terms such as "clonal proliferation" or "neoplasm" are recommended instead.

Special consideration should be given to the reporting of –Y or +15 which can be found in elderly patients with no haematological disease.

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.

4.1 Reporting times

Urgent referrals (diagnostic acute leukaemia and CML):
95% should be reported within 14 calendar days
A diagnostic FISH result is adequate in this category, with confirmatory cytogenetics treated as for routine referrals.

Rapid test by FISH/PCR: 95% reported in 3 working days

Routine referral: 95% should be reported within 21 calendar days

These guidelines are **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.

5 INDIVIDUAL DISEASE TYPES

These are intended to be very general guidelines which will be superseded by a continuing review of disease specific recommendations

5.1 Myeloid neoplasia

Standard one and/or two day cultures are appropriate for all myeloid disorders. 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 loss of chromosome 7 poses a particular problem in myeloid disease. It is recommended that a further 30 metaphases be screened for 7s if possible or FISH analysis considered.

5.1.1 CML

Although conventional cytogenetics remains the gold standard for diagnosis it is acceptable to use alternative molecular techniques for diagnosis and follow-up.

DIAGNOSIS
Bone marrow or peripheral blood may be used.
When using conventional cytogenetics, a minimum of 3 cells shall be fully analysed with a further 7 cells scored for the Ph chromosome and other obvious abnormalities. If the result shows a variant translocation or is Philadelphia chromosome (Ph) negative consideration should be given to further investigation by FISH or RT-PCR studies.

FOLLOW UP STUDIES
Monitoring the levels of Ph positivity can be performed by G-Banding (minimum of 30 metaphases to be screened) or interphase FISH (100 cells). FISH may not be reliable for monitoring unless the analysis is confined to neutrophils/granulocytes [1]; this will be clarified further in expanded disease-specific guidelines. Accurate interpretation of FISH follow up requires prior knowledge of the signal pattern at presentation and cases with only a single fusion signal should not be monitored by FISH.

Once Ph negativity has been achieved by cytogenetics/FISH, quantitative RT-PCR on peripheral blood samples may be a more appropriate method for follow up studies.

Although the major purpose of genetic analysis after therapy is now to monitor the level of Ph positivity, it is also recognised that new clonal abnormalities are occasionally detected in Ph negative cells. Although the significance is not yet understood, it is recommended that laboratories monitoring by conventional cytogenetics at least perform counts as well as screening for the Ph to increase the likelihood of detecting such changes.

ACCELERATED PHASE / BLAST CRISIS
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 between 9 and 22.

5.1.2 AML

DIAGNOSIS
Bone marrow is the preferred tissue for the investigation of AML, but peripheral blood can be used when there are circulating blasts seen in the PB film. If an abnormal karyotype is found, five abnormal metaphases should be fully analysed, with a further five metaphases scored for the same and additional obvious changes. In the event of a normal karyotype 20 metaphases shall be examined with at least ten fully analysed and the remainder scored for obvious abnormalities. FISH/RT-PCR should be considered for failed samples, where common additional abnormalities associated with specific rearrangements are found, or for other results where an expected abnormality has not been detected. Guidance for which additional tests should be undertaken may be obtained after collaboration with the haematologist or the European Leukaemia Network [2].

FOLLOW UP STUDIES
G-banded 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 diagnostic abnormalities in at least 30 G-banded metaphases or interphase FISH (100 cells) may be undertaken if required. If the diagnostic karyotype was normal, analysis of post-induction samples is rarely of value.

RELAPSE
Examination of 10 metaphases is sufficient when the diagnostic abnormality is detected with full analysis to allow accurate description 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. The possibility of a second malignancy should be considered in late relapse cases where a full diagnostic analysis may be appropriate if the original abnormality is not obvious.

5.1.3 MDS

Collaboration with the haematologists is especially important in this referral category, where many non-clonal disorders may present in a similar way.

DIAGNOSIS
Bone marrow is the preferred tissue for the investigation of MDS. If an abnormal karyotype is found, 5 abnormal metaphases should be fully analysed, with a further 5 metaphases scored for the same and additional obvious changes. In the event of a normal karyotype, 20 metaphases shall be examined [3] with at least 10 fully analysed and the remainder scored for obvious abnormalities. FISH for -5/5q- and -7/7q- should be considered for failed samples.

5.1.4 Aplastic Anaemia

If analysis is required these cases should be treated the same as for MDS samples.

5.1.5 MPD (excluding CML but including MPD/MDS)

DIAGNOSIS
Bone marrow is the preferred tissue for the cytogenetic investigation of MPD. If an abnormal karyotype is found, 5 abnormal metaphases should be fully analysed with a further 5 metaphases scored for the same and other obvious changes. In the event of a normal karyotype, 20 metaphases shall be examined with only 5 metaphases fully analysed as the majority of cases are done for Ph exclusion, and other MPD-associated abnormalities are not subtle.

5.2 Lymphoid neoplasia

Lymphoid cells are generally less amenable to culture than myeloid cells.

In ALL and high grade NHL in vitro cell death can be a significant problem. Laboratories should consider harvesting one culture on the day that it is received, particularly for NHL, or the preparation of smears for interphase FISH. Synchronisation is often less successful in ALL than AML. Zero-, one- or at most two-day cultures are recommended for ALL and most NHL cases,

Blood or bone marrow cells from CLL and some low grade lymphomas eg mantle cell lymphoma (MCL) may benefit from stimulation with B-cell mitogens (usually PMA), which necessitates a longer time in culture (3 or 4 days). However, short term cultures or smears are more appropriate for interphase FISH.

Three to four day unstimulated culture (or in the presence of 1ng/ml IL6) is helpful in myeloma to reduce the number of contaminating normal mitoses.

5.2.1 ALL

DIAGNOSIS
If an abnormal karyotype is found, 5 abnormal metaphases should be fully analysed with a further 5 metaphases scored for the same or other abnormalities. In the event of a normal karyotype 20 metaphases shall be examined with at least 10 fully analysed and the remainder scored for obvious abnormalities.

FISH/RT-PCR for BCR/ABL and MLL, shall be available for all paediatric cases. FISH is required for TEL/AML1 as it will also detect the prognostically significant AML1 amplification [4] and suggest high hyperdiploidy. BCR/ABL, TEL/AML1 and MLL rearrangements have been reported to be mutually exclusive [4] and it is recognised that testing for these may be sequential and is only necessary in the absence of the visible cytogenetic abnormality or a mutually exclusive abnormality. In adult ALL, only supplementary BCR/ABL testing is mandatory and then only if no Ph chromosome or mutually exclusive cytogenetic abnormality has been found.

In paediatric ALL with an 11q23 abnormality other than the classic translocations, MLL FISH should be performed since only 50% have been shown to have a split MLL [4].

FOLLOW UP SAMPLES
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.

RELAPSE
G-banded scoring of 10 metaphases is sufficient when a previous diagnostic abnormality is detected, with adequate full analysis being undertaken if there is any suggestion of clonal progression. If no abnormality is detected 30 metaphases shall be scored. The possibility of a second malignancy should be considered in very late relapse cases with no sign of the diagnostic abnormality (see AML relapse).

5.2.2 CLL

The abnormality rate by G-banding alone is lower than for FISH analysis [5]. G-banded analysis is therefore not mandatory and, if normal, must not be considered as a stand-alone test.

FISH is the most appropriate test and is particularly useful for the differential diagnosis with MCL. FISH for t(11;14) should be offered in all cases with atypical morphology or with an immunophenotyping score for CLL of 3/5 or less [6]. In CLL 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 affect treatment.

Blood or bone marrow is likely to be suitable for the investigation of CLL. Smears may be used: the quality of FISH is comparable and additional information on cell morphology is available.

5.2.3 Non-Hodgkins Lymphoma

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

For all lymphomas the preferred tissue is lymph node or other relevant biopsy material. If fresh material is available, G-banded karyotyping is recommended.

Where G-banding on lymph node is attempted, 20 metaphases shall be screened for the key abnormalities. Full analysis is not necessary unless an abnormality is found. If analysis is on infiltrated bone marrow, the number of cells screened should be at least 50 if normal. Where an abnormal clone is detected, 5 abnormal metaphases should be fully analysed and a further 5 scored for the same and additional abnormalities.

FISH testing using all necessary commercially available probes should 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.

5.2.4 Myeloma

The detection of -13/13q- by conventional cytogenetic analysis is a significant prognostic marker in myeloma [7]. If cytogenetic studies are undertaken full analysis is not necessary unless an abnormality is found. Where possible at least 50 metaphases should be screened for key abnormalities if no abnormality is found. This should be increased to 100 if only one or two day cultures are used. If an abnormal clone is found, 5 abnormal metaphases should be fully analysed if available. Key abnormalities are 14q32 translocations, deletion/monosomy 13 (although not in isolation) and high hyperdiploidy [8]

Single cells with classic myeloma karyotypes may be reported with appropriate qualification but should not be confused with random abnormalities which also occur frequently.

In the absence of a reliable means of identifying plasma cells, totally normal FISH results shall be qualified, explaining that the possibility of a false negative result is much higher than might be anticipated from assessment of the morphology smears. [9]

5.3 Bone marrows from solid tumour cases

Bone marrows infiltrated with 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's 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) prompt or overnight harvest and (ii) flasks to allow growth of adherent cells. Where no abnormalities are obvious, examination should tend towards screening large numbers of cells for likely abnormalities rather than detailed analysis of small numbers of cells.

6 STORAGE OF SAMPLES

Refer to General Best Practice guidelines (2007).

7 DEFINITION OF TERMS

Analyse: to count and compare every chromosome, band for band, with its homologue in a metaphase and to verify the banding pattern of the X and Y-chromosomes in male karyotypes.
Score/Screen: to check for the presence or absence of abnormalities in a cell or metaphase without full analysis.
Count: to enumerate the total number of chromosomes in any given metaphase, or in FISH analysis, to enumerate the number of signals in an interphase nucleus.
Examine: to look for the presence or absence of any abnormality in a case.

8 GLOSSARY

ALL	acute lymphoblastic leukaemia (may be B or T cell ALL)
AML	acute myeloid leukaemia
CLL	chronic lymphocytic leukaemia
CML	chronic myelogenous leukaemia
FISH	fluorescence in situ hybridisation
LPD	Lymphoproliferative disorder
MCL	Mantle cell lymphoma
MDS	myelodysplastic syndrome
MPD	myeloproliferative disease
PHA	Phytohaemagglutinin
PMA	Phorbol-12-myristate-13-acetate (also abbreviated as TPA)
RT-PCR	reverse transcriptase PCR

9 USEFUL REFERENCES / WEBSITES

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http://atlasgeneticsoncology.org/

The British Committee for Standards in Haematology (BCSH) Guidelines.
www.bcshguidelines.com

Mitelman database of chromosome aberrations in cancer
http://cgap.nci.nih.gov/Chromosomes/Mitelman

Improving outcomes in Haemato-oncology cancer
http://www.nice.org.uk/page.aspx?o=89908

10 Version Control

issue date	Current document	summary of changes	Version replaced
03/09/2007	**Haemato-Oncology Best Practice Guidelines 2007 v1.01**	updated referencing between ACC Best Practice Documents	Haemato-Oncology Best Practice Guidelines 2007
	Previous versions		
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