Guidelines for Cytogenetic Investigations in Tumours

Rosalind J. Hastings[1], Nick Bown[2], Maria G. Tibellitti[3]; Maria Debiec-Rychter[4], Roberta Vanni[5], Blanca Espinet[6], Nadine van Roy[7], Paul Roberts[8], Eva van den Berg-de-Ruiter[9], Alain Bernheim[10], Bauke Ylstra[11], Jacqueline Schoumans[12], Steve Chatters[13], Zuzana Zemanova[14], Marian Stevens-Kroef[15], Annet Simons[15], Sverre Heim[16], Marta Salido[6], David R. Betts[17].

[1.] Women's Centre, John Radcliffe Hospital, Oxford University Hospitals NHS Trust, Oxford, OX3 9DU, UK.
[2.] Northern Genetics Service, Institute of Genomic Medicine, Central Parkway, Newcastle, NE1 3BZ, UK.
[3.] U.O Anatomia Patologica, Ospedale di Circolo-Polo Universitario, Via O.Rossi 9, 21100 Varese, Italy.
[4.] Laboratory for Genetics of Malignant Disorders, Department of Human Genetics, University Hospital Gasthuisberg, UZ Leuven, Herestraat 49, 3000 Leuven, Belgium.
[5.] Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042 Monserrato, Italy.
[6.] Laboratori de Citogenètica Molecular, Servei de Patologia, Hospital del Mar, Pg. Marítim, 25-29, 08003 Barcelona, Spain.
[7.] Centre for Medical Genetics, University Hospital Ghent, De Pintelaan 185 MRB2 9000 Gent, Belgium.
[8.] Cytogenetics Department, St James's Hospital, Beckett St, Leeds, LS9 7TF, UK.
[9.] University Groningen, University Medical Centre Groningen, Dept Genet, NL-9700 RB Groningen, The Netherlands.
[10.] Génétique des tumeurs (INSERM U985), & Laboratoire de Cytogénétique, Pathologie Moléculaire Gustave Roussy, 114 rue Edouard Vaillant 94805 Paris-Villejuif Cedex, France.
[11.] Department of Pathology, Cancer Center Amsterdam, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands.
[12.] Cancer Cytogenetic Unit, Lausanne University Hospital, CHUV, Lausanne, CH-1011, Switzerland.
[13.] Paediatric Malignancy Cytogenetics Unit, Great Ormond St Hospital, London WC1N 3JH, UK.
[14.] Center of Oncocytogenetics, Institute of Clinical Biochemistry and Laboratory Diagnostics, General University Hospital and First Faculty of Medicine, Charles University in Prague, U nemocnice 2, 128 08 Prague 2, Czech Republic.
[15.] Radboud University Nijmegen Medical Centre Department of Human Genetics - 848 P. O. Box 9101 6500 HB Nijmegen The Netherlands.
[16.] Institute for Cancer Genetics and Informatics, The Norwegian Radium Hospital, Oslo University Hospital, N-0310 Oslo, Norway.
[17.] National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12, Ireland.

Introduction

Professional guidelines for tumour genetics laboratories aim to incorporate the standards imposed by generic European guidelines[1] and regulatory bodies (ISO15189)[2] while taking into account current practice in Europe. Some elements of the tumour diagnostic service not subject to statute may be varied according to local constraints and agreements. It must be noted that these guidelines are minimum requirements and that professional judgment is of paramount importance for many circumstances.

Clinical trials may have additional requirements in order to stratify patients to the appropriate treatment regimes. These should also be considered mandatory. New techniques and clinical evidence are becoming available all the time and therefore should be kept under constant review.

The use of 'must' 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 ones should be taken to apply to all.

All diagnostic laboratories must be accredited to national or internationally accepted standards (ISO15189)[2,3]. Laboratories must participate in an External Quality Assessment Scheme[4] in all aspects of their service for which a scheme is available.

These guidelines relate to Solid Tumours and make up part of the acquired best practice guidelines. The guidelines are a result of a Tumour Best Practice Meeting with invited tumour experts held on 23rd April 2013 in Oxford, UK. These general recommendations are required

due to an increasing number of somatic cytogenetic tumour markers that have been incorporated into WHO classification[5] of solid tumours. Cytogenetic investigation of tumor tissue has been routinely introduced in the clinical practice of pathology and oncology as adjunct to diagnosis, prognosis, and treatment. These guidelines should be used in conjunction with other Professional guidelines for clinical cytogenetics, for example, Generic European guidelines[1], ISO15189[2], reporting guidelines[6], and ISCN[7].

The provision of specific assays varies between and within countries as a range of laboratories may offer diagnostic solid tumour genetics including Cytogenetic, Pathology, Haematology, and Molecular Genetics laboratories. These guidelines are aimed principally at giving guidance on the minimum, cytogenetic and FISH analyses.
Recommendation: Laboratories should provide onward referral to specialist laboratories where appropriate, or advice on where such tests are performed.

Tumour genetic analysis is an established and routine element in the clinical investigations of a number of neoplasms[8]. Disease-specific abnormalities, particularly translocations, can provide essential information to help the Pathologist and/ or Oncologist make the correct diagnosis. In several diseases, tumour genetics correlates strongly with clinical risk, so cytogenetic information can help the Oncologist to counsel the patient and choose a specific treatment and/or to modulate treatment intensity.

Laboratory staffing
The laboratory should have either a Head of Laboratory or Senior staff member who is knowledgeable in; the cytogenetic abnormalities, most appropriate test required, and clinical significance for the tumour types that the laboratory will process. Staff members analysing these tumours should be familiar with the reason for the test and any potential findings.

Communication
It is strongly **recommended** that the laboratory performing the cytogenetic analysis has a close liaison and dialogue with the referring Pathologist and/or Oncologist to gain information regarding the quality of the specimen received, its tumour cellularity, and the suspected tumour type both pre- and post-sample receipt, since the processing and analysis of tumour samples may be time-consuming and expensive, updates on the working diagnosis allow the most effective directing of work.

Information received regarding the possible diagnoses of the tumour sample can be vital in determining the optimum culture types that can be employed or facilitating a rapid and the most appropriate FISH test.

Subsequently prompt communication regarding the nature of the sample allows for the most effective work flow and prioritisation e.g. it may prevent the analysis of samples which turn out to be reactive or non-neoplastic. Secondly, histological information on the nature of the specimen, in particular the tumour cell content, is often essential to interpret chromosome abnormalities and FISH results.

Sources of material
Tumour analysis may be done with fresh, frozen, fixed, formalin fixed paraffin embedded (FFPE) tissue or using cytological material (fine-needle aspirates, FNA) or dissociated cells or tissue. Methods for processing of tumour material will be determined by the cytogenetic laboratory based on available clinical and pathologic findings. In instances where fresh viable material is obtained, the cytogenetic laboratory should seek as much information as possible about the differential diagnosis and the tissue type at the time of sample receipt to choose the most appropriate processing techniques.

The amount of material received, typically in the form of a tumour biopsy, can vary greatly e.g. whether it derives from an open surgical procedure or a needle biopsy.

For conventional cytogenetic analysis, the tissue sample must be fresh and ideally without necrosis. It is essential to collect the tumour sample under sterile conditions and to select a representative area or areas of the tumour to improve success rate. Therefore, the laboratory should have procedures in place whereby fresh tissue can be transported and processed promptly. Many laboratories provide sterile culture medium to local surgical units or pathology departments for this purpose, and although sterile saline may also be used it is not recommended. Tumour biopsies should never be frozen prior to dispatch to the cytogenetic laboratory.

FISH analysis can be done on fixed, frozen, FFPE, FNA, or touch preparations from fresh tumour tissue. These cells will be the principal target for FISH, as they will most closely represent the cell populations in the tumour biopsy compared to cultured cells for cytogenetic analysis.

The laboratory must have clear guidelines on any subsequent retention of patient material post diagnostic testing[9].

Paraffin-embedded tissue
Formalin-fixed, paraffin-embedded tissue is acceptable for FISH analysis. Tumour sections cut 3 to 4 microns thick and mounted on a positively charged organosilane-coated (silanized) slides should be used. Before scoring a paraffin-embedded slide by FISH, it is crucial that a pathologist reviews a haematoxylin and eosin-stained (H & E) slide and delineate the region of tumour cells that should be scored because it can be difficult to differentiate normal cells from malignant cells using only DAPI counterstain. The analyst should know, before scoring the slide, where the malignant cells of interest are located on the slide.

Recommendation: *All tumour samples should have the region of tumour cells delineated on an H & E section by the Pathologist.*

Touch preparations
Touch preparations (TP) are helpful when tissue architecture is not crucial. In most instances a pathologist and/or operating oncologist will make the TP or be involved in selecting the tissue for TP. TPs should be made by lightly touching a tumour piece to a glass slide without smearing. Subsequent preparation of these slides, prior to FISH analysis, may be laboratory or tumour specific. If the laboratory receives the made slides, rather than preparing them within the laboratory, there should be communication about how the slides should be made, how many are required, and subsequently sent. The laboratory should have a system to evaluate the received slides and whether they are appropriate for the test required.

Cytospin preparations
Cytospin preparations are useful for concentration of samples with very low cellularity, e.g. cerebrospinal fluid. These preparations should be prepared rapidly following acquisition of the sample and care must be taken to ensure that the cell morphology remains.

Fresh-frozen tumour tissues
Such tissues may be useful in sequential analysis of recurring tumours or in the evaluation of archived specimens.

Fine-needle aspirates (FNA)
Such samples are sometimes used especially in paediatric oncology, as fine-needle aspiration is minimally invasive and usually provides high amount of tumour cells, particularly in round-cell tumours.

Fixed cytogenetically prepared cells
Such preparations have multiple uses for both interphase and metaphase FISH evaluations, including confirmation and clarification of suspected chromosome abnormalities or characterization of an apparently abnormal clone. Metaphase FISH evaluation may help clarify specific chromosome rearrangements.

Sample Preparation
Each laboratory should have written details of how it processes each sample when it is received.

Fresh tissue
This must be evaluated and processed rapidly. Where possible, presumed viable tumour material should be separated from non-viable material, e.g. calcified, or non-tumour material, e.g. fat. Disaggregation of the tumour material can be performed either mechanically, e.g. with scalpels, and/or enzymatically. The choice of technique may depend on biopsy size and presumed tumour type.

Archival material e.g. Slides
The laboratory must ensure that material on slides is received in such a way that subsequent analysis is not compromised, e.g. stained or marked by felt tip or diamond marker. Depending on result urgency, the slides may be immediately pre-processed, either in the form of fixation or pepsin treatment for subsequent FISH analysis. Otherwise, slides may be stored either at -20^{o}C, or 4^{o}C in the case of FFPE slides, until required.

Techniques
For many tumour types, it is frequent that there will not be a single cytogenetic technique that generates all clinically relevant results. In

addition, there are a number of other techniques that are frequently used in conjunction with cytogenetic methods that further aid in the determination of the genetic make up of the tumour. All techniques have their strengths and weaknesses, and the choice of test may ultimately depend on local expertise, relationship to the clinical and pathology departments, published recommendations, and typical size and form of sample. Nevertheless, all laboratories should have an awareness of the strengths and weaknesses of each technique and how they may complement each other. The latter consideration is an important factor in the final interpretation of the cytogenetic features of a tumour.

Table 1 is a non-exhaustive list of the major tumour types currently investigated together with the results that should be obtained by a laboratory and a preferential method.

Conventional Cytogenetics

It is well recognised that obtaining tumour metaphases for karyotypic analysis is technically challenging. Direct harvests or short-term cultures often provide no metaphases, while long-term cultures are prone to overgrowth by non-tumour cells. Despite this, it is recommended that cultures for chromosome analysis should be initiated whenever the laboratory receives sufficient tumour material, since successful karyotyping can provide a great deal of diagnostically helpful information, including insights into translocations and chromosome partners in unbalanced events, copy number abnormalities (CNAs) and ploidy, revealing the presence of multiple ploidy levels and tumour cell heterogeneity. In addition, it is one of the few techniques that permit the reliable determination of clonal evolution and whether this is linear or divergent. It can also explain atypical or unusual FISH patterns, which would otherwise be hard to interpret. Furthermore, as a 'pan-genomic' overview, classical cytogenetics is open to unexpected results which may lead to sudden

Table 1: Current method of choice for required tests associated with specific tumour types

Tumour type	**Test Required**	**Method of choice**	**Other non-essential tests and methods**
Breast carcinoma	*ERRB2 (HER2)*	FISH (dual colour) /CISH/SISH	Microarray
Carcinoma	Disease specific rearrangement	RT-PCR, or FISH (if available probes)	G- or R-banding, microarray
Lipomatous tumours	Subtype specific changes	FISH	G- or R-banding, MLPA (dosage)
Neuroblastoma	*MYCN* status	FISH (dual colour)	1p, 11q, 17q status (FISH or MLPA), G- or R-banding, microarray, NGS
Oligodendrogliomas	1p,19q status	FISH, or qPCR, or MLPA	Microarray NGS
Other CNS tumours	*MGMT* methylation; disease or grade specific changes	FISH (e.g. *MYC* in medulloblastoma)	G- or R-banding; MGMT Methylation analysis (MS-MLPA, MS-PCR, pyrosequencing), microarray
Renal tumours	Subtype specific changes	G- or R-banding	Microarray, FISH
Soft tissue sarcoma	Sarcoma specific fusion product	RT-PCR, or FISH (if available probes)	G- or R-banding
Wilms Tumour			G- or R-banding, FISH, Microarray

changes in diagnostic direction. This may also include the detection of a constitutional abnormality.

This technique requires fresh material and cells in division. In many instances only long term cultures, in which the over-growth of fibroblasts is a frequent event, are available for analysis. The technique is relatively labour intensive and requires skilled and trained personnel. The tumour related karyotypes are frequently complex, which may lead to many unidentifiable chromosomal regions and/or misidentification of chromosomes (particularly with undertrained personnel). In most instances, it is not possible to achieve a result within 24-48hrs and frequently the report time would be measured in days or weeks.

Hence, while often highly informative, chromosome analysis can be unreliable as a sole approach, and all laboratories offering a solid tumour service must have access to supplementary techniques.

Recommendations:
Cultures for chromosome analysis should be initiated, where appropriate, as it can provide information on the mechanism of the abnormality, e.g. deletion, translocation and clonal evolution.

At least one culture of disaggregated cells for appropriate tumour types should be harvested within 24 hours. Alternatively, unstained tumour touch preparations or fixed primary single tumour cell suspension can provide this pre-culture baseline for FISH testing.

Fluorescence in situ hybridisation (FISH)
In many instances, FISH is used as a stand alone technique, e.g. testing for a gene fusion, but can be used in conjunction with G- or R-banding to further help with characterisation of the karyotype, with the choice of probes being directed by the latter technique. However, FISH does not require cells in division and can be used in situations where there is no viable material or where the G- or R-banding has failed.

The technique is applicable to a range of sample types, including:

- Tumour touch imprint slides prepared from fresh or frozen tumour samples
- Paraffin sections
- Intact cells released from paraffin blocks and either cytospun or dropped onto slides
- Infiltrated bone marrow
- Other infiltrated tissue e.g. ascites or cerebrospinal fluid (CSF)
- Urine
- Cytogenetic harvests of fresh tumour tissue*

*If cytogenetic preparations are used, these ideally should be either directly harvested or from very short term cultures (72 hours maximum). Longer-term cultures should only be used if it is known that there are tumour cells present and in most instances as an attempt to further characterise an abnormality that is known to be present.

An advantage of touch imprint slides or paraffin sections is that an H&E-stained slide can be prepared from the same cut surface, allowing for assessment of tumour cell content by the Pathologist.

Rapid results can be achieved, often within 24hrs, when necessary. It is frequently the method of choice to detect fusion products and genetic amplification.

Reporting negative results from analysis of infiltrated bone marrow or other tissue requires particular consideration. As a result of haemodilution, the proportion of tumour cells in the sample sent to laboratory can be much lower than that reported by the Haematologist. In addition, bone marrow aspirates may be taken from multiple sites and may show widely differing levels of tumour infiltration. A further consideration is that some hospitals will define the presence of bone marrow infiltration as determined by a trephine investigation; therefore, the diagnostic laboratory must be aware on what basis bone marrow infiltration has been defined.

Although the technique is less labour intensive and requires less training than G- or R-banding, it is too often assumed that FISH is a simple test that is just a question of spot counting. Particularly in solid tumours, where complex signal patterns and cell to cell variation is common, skilled personnel are required to ensure accurate reporting. It is not a genome wide test and will only answer the question specific to the probe(s) used. Being interphasic FISH analysis a cell to cell approach,

it is a suitable methods to investigate genetic heterogeneity in solid tumours. In situations where multiple probes are used it rapidly becomes expensive.

Chromogenic in situ hybridization (CISH) / Silver-enhanced in situ hybridization (SISH)
CISH/SISH is a valid alternative to FISH on formalin-fixed, paraffin-embedded (FFPE) slides, and can be analysed using a brightfield microscope. One advantage these techniques have is allowing evaluation of target/gene status simultaneously with tissue morphology. As the labelling is permanent, long term archiving is possible. The technique is limited to one or two colours, and turn around time is generally slower than FISH. As with FISH, skilled personnel are required to ensure accurate reporting.

Microarray (SNP / Oligo)
Microarray analysis is applicable to any tumour type. SNP arrays give both chromosomal copy number and loss of heterozygosity LOH (including copy number neutral LOH), but mostly do not perform well with FFPE derived DNA. In contrast CGH arrays cannot detect LOH, but can be reliably used with FFPE derived DNA. In addition to a 'pan-genomic' overview, microarray analysis allows detection of very small regions of loss or gain/ amplification and, in the case of SNPs, regions of loss of heterozygosity (LOH) and frequently an indication to ploidy level. Arrays are being increasingly used in a diagnostic setting, e.g. neuroblastoma and clonality assays.

Microarray analysis can also provide a more exact determination of breakpoints, something that is valuable in larger series of individual tumours to determine potential clinical and/or biological significance of specific events. However, balanced rearrangements can not be detected.

For array analysis it is important that DNA is extracted from a region with high tumour cell content (>30% tumour cells). The technique can be sensitive to DNA quality, with low quality DNA either leading to failed tests or running the risk of calling false positives. Knowledge of tumour ploidy level is important and the determination of secondary type events is difficult.

Multiplex ligation-dependent probe amplification (MLPA)
MLPA can give an indication of imbalances and copy number of the loci that are included in the reaction kit. Commercial MLPA kits are available to detect relevant CNAs in, for example, neuroblastoma, oligodendroglioma, and breast cancer. In addition, the technique can be used to investigate methylation status of significant genes such as MGMT in high grade gliomas.

MLPA requires only small quantities of DNA, but it is important that this is extracted from material with high tumour content, and is generally regarded as fast, cheap and very simple to perform. The regions tested are defined by the kit used and balanced translocations cannot be detected. MLPA analysis in cancer samples could be inaccurate due to genomic instability, due to the presence of several genetic alterations and to contamination with normal DNA.

Flow and static cytometry of total DNA content
This technique will indicate the tumour ploidy and provides an accurate determination of the DNA content of tumours and may prove valuable when used in association with microarray, and occasionally FISH. However, it would not be the first method of choice for tumour (cyto)genetic analysis and may not be advised when there is limited material available.

Supplementary Techniques

RT PCR
Reverse-transcriptase (RT)-PCR is a technique frequently used as first-line technique to identify gene fusions resulting from translocations, particularly in the diagnosis of sarcomas, when fresh or frozen material is available. It has the advantage to allow to be designed as a multiplex technique, screening all the known rearrangements in a given tumour group, for example round-cell tumours. RT-PCR can often clarify unusual or equivocal cytogenetic or FISH results by confirming or excluding key gene fusions. It is recommended that Cytogeneticists involved in solid tumour analysis should liaise closely with appropriate units offering RT-PCR analysis, in order to define the order of use of the two types of techniques.

Specific sequencing
Sanger sequencing (SS): can detect all base substitutions, small insertions and deletions, but has a modest limit of detection, which can be highly variable depending on the exact sequence, and laboratory performing the test. Using an

automated interpretation algorithm with a 10% threshold, SS yielded 11.1%. The limit of mutation detection by SS is subjective and may depend on the experience level of the person interpreting the data.

Pyrosequencing (PS) is a bioluminescence technique in which the pyrophosphate released during incorporation of a nucleotide into a growing DNA chain is converted to light through a series of enzymatic reactions. PS can identify individual basis or short stretches of nucleic acid sequence at predetermined positions.

Heterogeneity, a peculiar feature of tumours, within the tumour cells, in conjunction with the fact that all specimens will contain some percentage of non-tumour cells may result in a relatively low percentage of mutated alleles within some specimens.

Next Generation Sequencing (NGS)
NGS allows for analysis of mutations, DNA copy number variation, LOH, balanced and unbalanced translocations, and methylation.

Genome-wide high resolution for copy number analysis performs well with small amounts of tumour material (50ng DNA). The technique is suitable for DNA isolated from fresh and FFPE samples. The digital nature of the data lowers the ambiguity of interpretation since clear cut-offs can be set for diagnostic/clinical purposes. However, extensive bioinformatics skills are required. A large amount of data provides both computational, data storage and off- target information may pose ethical challenges.

Follow up samples
The choice of technique may also depend on the disease status and whether the sample is post-treatment, relapse or a potential metastasis. In most instances, the laboratory should choose a technique that will either help to demonstrate the presence of tumour cells or the emergence of a known prognostic/clinical marker. In most instances FISH would be the method of choice.

Success rates
The success rate will depend on the technique and the quality and quantity of material received.

The fresh tumour samples received in Cytogenetic laboratories can be very variable in amount and quality. Samples may be small needle cores to large surgical biopsies, and they may vary widely in terms of tumour content, viability and necrosis. Given this, and the diversity of tumour types encountered, it is unrealistic to specify target success rates for classical chromosome analysis.

In contrast, interphase FISH for the detection / exclusion of specific aberrations is consistently reliable and success rates of >95% should be aimed for.

In a diagnostic setting, a laboratory should not rely on a technique, or combined techniques, that gives an informative success rate of <90%.

Reporting times
The clinical significance of Cytogenetic input, and the urgency with which it is required, is highly variable from one case to the next, even within a given disease type, and it will depend to a great extent on the Pathologist's confidence in the results from other tests such as immuno-histochemistry to provide an unequivocal diagnosis.

Currently the number of (cyto)genetic markers that will directly influence treatment decisions is limited, but this is expected to change over time. Expected report times are likely to be defined within the treatment protocol and therefore, it would be inappropriate to stipulate guidelines for expected report time for either a karyotypic or FISH test. If the test is known to influence treatment decisions then the testing laboratory must be aware of any time requirement within the treating protocol.

Where cytogenetics could have a bearing on diagnosis and/or treatment, efforts should be made to report all results within 14 days. Otherwise, unless specified, it would be realistic to expect a final result within 28 days.

Analysis and checking
For all analysis types, two analysts, one being a registered clinical scientist, or equivalent, must be involved in the analysis or checking of all diagnostic samples. 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.

Conventional Cytogenetics
The morphology of tumour 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. Agreement on abnormal clones should be reached by two analysts. It is recommended that a minimum of 10 metaphases are analysed if a clone is defined and 20 metaphases if no clone is identified. However, given the low mitotic index for some tumours, it is acceptable to analyse fewer than 10 cells if there are sufficient abnormal cells to establish the presence of an abnormal clone. If analysing post treatment material or long term cultures only, consideration must be given to the possibility of treatment induced or culture-only events.

FISH

The laboratory should define the number of individual cells that should be scored. For interphase, a minimum of 100 individual cells is recommended. This number may need to be increased, particularly in instances where tumour content is unknown and low numbers of cells with an apparently abnormal signal pattern are seen. Where possible, separate cells should be scored, but if there are overlapping cells that cannot be scored individually, the tumour cell percentage must be known.

The laboratory should define its own cut offs for individual FISH probes and tissue/sample types. For this purpose they should also take into account the probe manufactures guidelines. For FFPE material, consideration must be given to the potential for truncated cells and the percentage of tumour cells in the section as defined by the pathologist. Laboratories must also be aware of the definition of imbalance and amplification for particular tumour types and genetic markers i.e. the definition of amplification may be tumour specific[10-12]. Care should be taken in describing deletion/ duplication in cells with polysomy. The use of control probes when assessing gain/loss is strongly recommended.

Microarray

The laboratory should define their minimum DNA-quality criteria and the minimum reportable size of an event.

Reporting

The reporting of tumour cytogenetic results should be concise and unambiguous, with the result and written description to include sufficient detail to give the referring Clinician and/or Pathologist a clear understanding of the results. The individual reporting the results may be a scientist or a clinician depending on National requirements. The report should include the following:

- Type of sample, including details of e.g. fixed, frozen, etc
- Origin of sample, where appropriate
- Result and written description
- Must clearly state if result is abnormal / normal
- Conventional cytogenetics use the current version of ISCN, including cell numbers
- FISH analysis should either use current ISCN or unambiguous language to describe the result
  - Abnormal FISH result summary is essential
  - Number of cells analysed
    - Real numbers of cells should be used not percentages
  - The presence of different clones must be stated unambiguously in summary result
  - Probes used and manufacturer
- Limitations of test, especially if normal
  - Brief description of clinically relevant abnormalities
  - Clinical significance of result, if applicable, in relation to referral reason
  - Relationship of any abnormalities found to the referral reason, or other possible diagnoses
    - Association with prognosis if a robust association from multiple publications/ international trials/ trial protocols exists.
- Comment if gene rearrangement is also seen in other tumours (if differential diagnosis).
- For arrays: type of array / platform; genome reference build number; tumour cell percentage; origin of sample (should be included in all reports)
  - Minimum resolution of platform and what is the minimum size of event reported
  - Use the current ISCN where appropriate, although ISCN may be difficult for tumour arrays and other unambiguous presentation of results would be acceptable
- If FFPE FISH, identification of specific block number on report (e.g. Section B of 6)
- Cross reference other tests as appropriate

In instances where multiple (cyto)genetic tests are performed a laboratory may wish to issue a summary report in which a combined interpretation of all tests is made.

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 abbreviated cytogenetic summary shall be authorised by a state-registered clinical scientist or equivalent. A full version of the cytogenetic report must be sent independently to the referring clinician.

It is recommended that the term "malignancy" is avoided particularly in the context of an abnormality of unknown significance. Terms such as "clonal proliferation" or "neoplasm" are recommended instead.

Normal karyotypic results in particular must always be regarded with suspicion, and these reports must include caveats about the likelihood of non-tumour cells having been analysed, if analysed from long term cultures, or reactive cells if analysed from short term cultures.

Similarly, if the results of interphase FISH are normal (negative), then consideration should be given to the potential tumour cell content of the sample analysed, and the potential clinical significance of the negative results should be discussed promptly with the referring Clinician and/or Pathologist. When reporting negative FISH results, knowledge of tumour cell content in the material analysed should be regarded as essential. If the tumour content of the tested material is unknown or in doubt, reports of negative results must be strongly qualified.

Normal results from DNA-based methods should consider whether the DNA has been extracted from tumour-rich material.

The laboratory should have a policy of the issuing of preliminary or verbal reports in instances of the detection or exclusion of an important clinical marker, but other testing is still ongoing, thereby delaying the final report.

If a potential constitutional abnormality is detected, analysis of a PHA-stimulated blood sample may be appropriate. There should be consultation with the patient's clinician and reference the potential need for genetic counselling for either the patient or their family.

Laboratories should follow their guidelines for sign-off of the report; however, it is recommended that at least one signature is a staff member of senior grade who is defined as competent to analyse and interpret tumour cytogenetic results.

Acknowledgements

The authors wish to acknowledge feedback from Barbara Dewaele, Belinda Carleer, Jérome Couturier, Vanessa Vanspauwen, and other colleagues in their departments on these guidelines. These guidelines resulted from a Tumour Best Practice meeting held on 23rd April 2013 in Oxford that that was funded by Eurogentest (FP7- HEALTH-F4-2010-261469). We would like to thank Cheryl Guiver for her help in the organisation of the Best Practice meeting.

References

1. European Cytogenetic Guidelines, ECA Newsletter, Jan 2012 http://e-c-a.eu/files/downloads/E.C.A._General_Guidelines_Version%202.0.pdf
2. ISO 15189:2012 Medical laboratories - Requirements for quality and competence
3. European co-operation for accreditation http://www.european-accreditation.org/
4. UK NEQAS Executive Office: Sheffield S10 2PB. http://www.ukneqas.org.uk/
5. Fletcher CDM, Bridge JA, Hogendoorn PCW, Mertens F eds. World Health Organization classification of tumours of soft tissue and bone, 4th edn. Lyon: IARC Press, 2013.
6. Claustres M, Kozˇich V, Dequeker E, Fowler B, Hehir-Kwa JY, Miller K, Oosterwijk C, Peterlin B, van Ravenswaaij-Arts C, Zimmermann U, Zuffardi O, Hastings RJ, Barton DE, on behalf of the ESHG Quality committee. Recommendations for reporting results of diagnostic genetic testing (biochemical, cytogenetic and molecular genetic) (2013). Eur J Hum Genet 2013; 22:160-170.
7. Shaffer LG, McGowan-Jordan J, Schmid M. (2013), ISCN 2013: An International System for Human Cytogenetic Nomenclature. S. Karger, Basel.
8. Heim S, Mitelman F (eds). Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells. 3rd Edition. Ed. Wiley-Blackwell.
9. The Retention and Storage of Pathological Records and Archives. 3rd Edition. 2006 The Royal College of Pathologists, London.
10. Ambros PF, Ambros IM, Brodeur GM, Haber M, Khan J, Nakagawara A, Schleiermacher G,

Speleman F, Spitz R, London WB, Cohn SL, Pearson AD, Maris JM. International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee. Br J Cancer. 2009; 100(9):1471-82.

11. Walker RA, Bartlett JM, Dowsett M, Ellis IO, Hanby AM, Jasani B, Miller K, Pinder SE. HER2 testing in the UK: further update to re-commendations. J Clin Pathol. 2008; 61:818-24.
12. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JMS, Bilous M, Fitzgibbons P, Hanna W, Jemkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF. Recommendations for Human Epidermal Growth Factor Receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. J Clin Oncol 2013; 31(31):3997-4014.

Appendix

Commercially available FISH probes that may assist in cytogenetic investigation of specific tumours

Disease	**Chromosomal abnormality**	**Commercially available FISH probes**
Alveolar Rhabdomyosarcoma	t(1;13)(p36;q14)	FOXO1, BA; PAX7/FOXO1 DF
Alveolar Rhabdomyosarcoma	t(2;13)(q37;q14)	FOXO1, BA; PAX3/FOXO1 DF
Alveolar soft parts sarcoma	t(X;17)(p11;q25)	TFE3, BA
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	FUS, BA
Angiomatoid fibrous histiocytoma	t(12;22)(q13;q12)	EWSR1, BA
Breast carcinoma	*ERBB2 (HER2)* amplification	ERRB2 (HER2) and D17Z1
Clear cell sarcoma	t(12;22)(q13;q12)	EWSR1, BA
Dermatofibrosarcoma protuberansand giant cell fibroblastoma	t(17;22)(q22;q13)	PDGFB, BA
Desmoplastic small-round-cell tumour	t(11;22)(p13;q12)	EWSR1, BA; WT1, BA
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12)	EWSR1, BA; NR4A3, BA
Extraskeletal myxoid chondrosarcoma	t(9;17)(q22;q11)	NR4A3, BA

Disease	Chromosomal abnormality	Commercially available FISH probes
Ewing tumour	t(2;22)(q33;q12)	EWSR1, BA
Ewing tumour	t(7;22)(p22;q12)	EWSR1, BA.
Ewing tumour	t(11;22)(q24;q12)	EWSR1, BA; EWSR1/FLI1, DF
Ewing tumour	t(17;22)(q21;q12)	EWSR1, BA
Ewing tumour	t(21;22)(q22;q12)	EWSR1, BA; EWSR1/ERG, DF
Ewing tumour	inv(22q)	EWSR1, BA
Ewing tumour	t(16;21)(p11;q22)	FUS, BA
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1, BA
Extraskeletal myoepithelial tumours	t(22q12)	EWSR1, BA
Gastric carcinoma	*ERRB2* (*HER2*) amplification	ERBB2 (HER2) and D17Z1
Hyalinizing clear cell carcinoma (salivary gland)	t(12;22)(q13;q12)	EWSR1, BA
Infantile fibrosarcoma, congenital mesoblastic nephroma	t(12;15)(p13;q26)	ETV6, BA
Inflammatory myofibroblastic tumour	t(1;2)(q25;p23)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;17)(p23;q23)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;19)(p23;p13)	ALK, BA
Inflammatory myofibroblastic tumour	t(2;11)(p23;p15)	ALK, BA; CARS, BA
Liposarcoma	*MDM2* amplification	MDM2, D12Z1
Low grade myxoid fibrosarcoma	t(7;16)(q34;p11)	FUS, BA
Lung adenocarcinoma	inv(2)(p23p21) or other 2p23 rearrangements	ALK
Lung adenocarcinoma	6q22.1	ROS1
Lung adenocarcinoma	10q11	RET
Lung adenocarcinoma		EGFR, MET, ERBB2

Disease	Chromosomal abnormality	Commercially available FISH probes
Medulloblastoma	i(17)(q10)	17p13.3 and RARA
Mucoepidermoid carcinoma and Hidradenoma	t(11;17)(q21;p13)	MAML2, BA
Myxoid liposarcoma	t(12;16)(q13;p11)	DDIT3, BA; FUS, BA
Myxoid liposarcoma	t(12;22)(q13;q12)	DDIT3, BA; EWSR1, BA
Neuroblastoma	*MYCN* amplification / del(1p) / del(11q)	Various, combinations available to determine *MYCN* copy number, 1p and 11q status
Non-small cell lung carcinoma	inv(2)(p21p23)	ALK, BA
Non-small cell lung carcinoma	t(6q22)	ROS1, BA
Oligodendroglioma	del(1p) / del(19q)	1p36/1q25, 19q13/19p13
Other Carcinomas		EGFR, MET, ALK, ROS1, RET
Papillary Renal Cell Carcinoma	Trisomy 7 and 17, disomy 1	Chromosome enumerator probes for chr. 1, 7 and 17
Pilocytic astrocytoma	putative inv(7)(q34)	BRAF BA*
Renal cell carcinoma with Xp11 translocation	t(Xp11.2), usually t(X;1)(p11.2;q21)	TFE3, BA
Schwannoma	22q deletion	22q11
Secretory carcinoma (breast, salivary gland)	t(12;15)(p13;q26)	ETV6, BA
Synovial sarcoma	t(X;18)(p11;q11)	SS18, BA

**BRAF* activation through the *KIAA1549-BRAF* fusion has also been described in other paediatric low-grade gliomas (e.g. pilomyxoid astrocytoma). *BRAF* point mutations (V600E) are observed in non-pilocytic paediatric low-grade gliomas as well, including approximately two-thirds of pleomorphic xanthoastrocytoma cases and in ganglioglioma and desmoplastic infantile ganglioglioma.

Abbreviations:
BA: break-apart; DF: dual fusion