## E: CLINICAL CYTOGENETICS

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Cell Culture, See D1.</td>
</tr>
<tr>
<td>E2</td>
<td>Records</td>
</tr>
<tr>
<td>E2.1</td>
<td><strong>Retention of Case Materials</strong></td>
</tr>
<tr>
<td>E2.1.1</td>
<td>Slides used for diagnostic tests have a limited lifespan. If stained with a &quot;permanent&quot; banding method (G-, C-, or R-banded, NOR), slides should be kept at least 3 years or in compliance with state regulations. Retention time of those with fluorochrome stained chromosomes is at the discretion of the laboratory director.</td>
</tr>
<tr>
<td>E2.1.2</td>
<td>Each laboratory should establish a policy to assure that any residual original patient specimens, cell cultures or pellets are retained until adequate metaphase preparations are available to complete the requested analysis.</td>
</tr>
<tr>
<td>E2.1.3</td>
<td>Processed patient specimens should be retained until the final report has been signed. Long-term retention time of those with abnormal results is at the discretion of the laboratory director.</td>
</tr>
<tr>
<td>E3</td>
<td>Procedural Guidelines</td>
</tr>
<tr>
<td>E3.1</td>
<td>General Analytical Standards</td>
</tr>
<tr>
<td>E3.1.1</td>
<td><strong>Terminology</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Chromosome counts</strong> are defined as the number of centric chromosomes per metaphase cell. During the establishment of the modal number for a study, all aneuploid metaphase cells should be characterized for specific gain/loss.</td>
</tr>
<tr>
<td></td>
<td><strong>Analyzed cells</strong> are defined as banded metaphase cells in which the individual chromosomes are evaluated in their entirety, either at the microscope or from intact digitized images or photographic prints of intact cells.</td>
</tr>
<tr>
<td></td>
<td><strong>Karyogrammed cells</strong> are defined as the cutout and paired chromosomes from photograph(s) or computer-generated image(s) from a single cell following the format in An International System for Human Cytogenetic Nomenclature 2009 (ISCN 2009).</td>
</tr>
<tr>
<td></td>
<td><strong>Scored cells</strong> refer to cells evaluated for the presence or absence of a specific cytogenetic feature, usually indicated by either a particular clinical history or by the finding of one or two abnormal cells during the course of a study. Numbers of cells to be scored in most situations are left to the discretion of the laboratory director, unless otherwise specified in the guidelines.</td>
</tr>
<tr>
<td></td>
<td><strong>Clone</strong> is defined as a cell population derived from a single progenitor cell. Clonal origin is inferred by the presence of at least two cells containing the same extra chromosome(s) or structural chromosome abnormality or by the presence of at least three cells that have lost the same chromosome. For the purpose of constitutional studies, the use of the terms cell line and clone are interchangeable.</td>
</tr>
<tr>
<td></td>
<td><strong>Mosaicism</strong> is the presence of two or more cytogenetically distinguishable cell lines.</td>
</tr>
</tbody>
</table>
**Pseudomosaicism** refers to the presence of an abnormal cell(s) in cultured cells that arise from an *in vitro* culture artifact and do/does not represent the true karyotype.

**Uniparental disomy** is defined as a condition in which both homologous chromosomes are derived from a single parent.

---

**E3.1.2** Slide number and microscope stage coordinates should be recorded for all metaphases analyzed or counted. If additional cells are evaluated in questions of mosaicism, slide number should be recorded for all cells that are scored and slide coordinates should be recorded for all abnormal metaphases or suspected abnormal metaphases.

**E3.1.3** All laboratories must be able to perform studies using G- and/or R-banding, in addition to special stains and/or FISH, to characterize polymorphisms, when indicated and at the discretion of the laboratory director.

**E3.1.4** ISCN 2009 must be used to describe all karyotypes.

**E3.1.5** A number of different objective methods have been described for the calculation of band stage of resolution. One or more objective and reproducible method(s) must be used to assess *banding level of resolution* and must be formally described in the laboratory standard operating procedures/protocol manual. Specific standards for resolution should be appropriate to the case and type of tissue studied. The 550-band stage should be the goal of all peripheral blood studies. A minimum of 400 bands should be reached for 90% of analyses from amniotic fluid and chorionic villi cells.

**E3.1.6** Minimum standards established for the numbers of cells to count and/or analyze and karyotype during the "routine" component of a cytogenetic study are described in specific subsections appropriate to a specific tissue type, culture method and/or reason for referral. The numbers of cells to study in individual situations is dependent on the specific abnormality observed, the tissue being examined, whether the analysis involves prenatal diagnosis, etc. General recommendations are noted in the following subsections.

**E3.1.6.1** Each laboratory should establish guidelines for procedures (e.g., numbers of cells to score) to follow for each general type of abnormality (hypodiploidy, hyperdiploidy and structural abnormality) with the recognition that uniformity among laboratories is not required.

**E3.1.6.2** The laboratory’s scoring guidelines should be based on current knowledge of the potential clinical significance of particular chromosome abnormalities and nonmodal cells.

**E3.1.6.3** Fewer cells than indicated under analytical standards may be studied in circumstances in which screening for a specific abnormality is the indication for the study (e.g., checking for a known familial abnormality) or when an abnormality is detected but no more cells are available (see following section).

**E3.1.7** Analyses should be performed and/or evaluated by at least two qualified individuals.

---

**E3.2** Abbreviated, Focused or Limited Chromosome Studies

**E3.2.1** General Considerations

**E3.2.1.1** It is acknowledged that there are specific clinical circumstances for which an abbreviated or limited cytogenetic study may be appropriate. For example, in the tissue confirmation of an abnormal prenatal chromosome result or in peripheral blood chromosome studies on extended family members to exclude an identified chromosome rearrangement, limited analyses may be suitable.

**E3.2.2** Analytical Standards

The laboratory should have established written criteria for which focused or abbreviated studies are permissible. Criteria should specifically address the rationale for such studies, the clinical reason for referral, the tissue type, and the minimum number of cells counted, analyzed and karyotyped under such circumstances.

**E3.3** Maternal Cell Contamination (MCC)
<table>
<thead>
<tr>
<th>E3.3.1</th>
<th>Maternal cell contamination of amniotic fluid and chorionic villi cell cultures is well documented, and therefore represents a potential source of error in prenatal diagnosis. Adequate measures to minimize the inclusion of maternal cells in prenatal samples should be part of the laboratory quality assurance program.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3.3.1.1</td>
<td><strong>Amniotic Fluid</strong></td>
</tr>
<tr>
<td></td>
<td>The overall frequency of MCC is approximately 0.5% of genetic amniocenteses [4]. Factors that increase the chance of MCC include the gauge of needle used for the amniocentesis procedure [5], the length of time in culture and the presence of blood in the sample.</td>
</tr>
<tr>
<td></td>
<td>It has also been documented that cultures initiated from the first 1-2 ml of amniotic fluid drawn at amniocentesis are at an increased risk for maternal cell contamination [5]. It is recommended that the first few milliliters of fluid be labeled appropriately and kept separate from the remaining sample to minimize inclusion of maternal cells. The initial aliquot should be used for cytogenetic analysis only if absolutely necessary.</td>
</tr>
<tr>
<td>E3.3.1.2</td>
<td><strong>Chorionic Villi Sampling (CVS)</strong></td>
</tr>
<tr>
<td></td>
<td>The risk for MCC in CVS is significantly higher than for amniocentesis samples (1-2%) [6]. A CVS specimen must be viewed under a dissecting microscope to allow for the gross identification and cleaning of villi from maternal decidua, blood vessels, membrane and other materials. It is recommended that sterile instruments (e.g., probes, scissors, forceps) be used to tease apart the sample to isolate the fetal chorionic villi from maternal decidua. It may be helpful to have two laboratory technologists clean or check the dissected tissue prior to initiating cultures.</td>
</tr>
<tr>
<td>E3.3.1.3</td>
<td><strong>Products of Conception (POC)</strong></td>
</tr>
<tr>
<td></td>
<td>Due to the manner in which abortus tissue and placenta samples are obtained and handled, there is a substantial risk of MCC, particularly in early fetal loss specimens. It is recommended that appropriate measures be taken to specifically identify fetal tissues and to dissect and culture only these tissues, as described above for prenatal CVS. Consultation with the referring physician may be warranted to determine the origin of the sample and/or the appropriateness of chromosome studies, particularly in cases for which the dissection of tissue appears to yield only maternal decidua.</td>
</tr>
<tr>
<td>E3.3.2</td>
<td><strong>Analysis of Cultures with Known or Suspected MCC</strong></td>
</tr>
<tr>
<td></td>
<td>Cultures with known or suspected MCC based on the condition of the specimen at receipt, or apparent maternal cells morphologically in culture, require variation in the normal analysis procedure. If XX cells are found in an otherwise XY study, the most likely explanation is MCC. Since the true fetal cells are probably represented by the XY complement, the full analysis and cell counts should be performed on these cells whenever possible. Counting and analyzing several cells with an XX constitution is recommended for documentation purposes. For prenatal testing, further studies may be warranted to exclude chimerism. Ultrasound examination to check the gender of the fetus, second amniocentesis or confirmatory amniocentesis after CVS and/or polymorphism studies (cytogenetic or molecular) between a maternal sample and the fetal sample may be required in the investigation.</td>
</tr>
<tr>
<td></td>
<td>If cell cultures that are initiated in the cytogenetics laboratory are to be used for molecular or biochemical testing, any serious concerns about MCC in those cultures must be conveyed to the molecular or biochemical testing laboratory. In addition, if direct prenatal samples are sent out for testing, it is recommended that back-up cultures be grown and maintained until the molecular or biochemical testing is complete and reported.</td>
</tr>
<tr>
<td>E3.3.3</td>
<td><strong>MCC Reporting and Quality Assurance</strong></td>
</tr>
<tr>
<td></td>
<td>Reporting of MCC is case-dependent and is at the discretion of the laboratory director. Consultation with the referring physician is recommended, when appropriate. Any significant observation of MCC in a prenatal diagnosis sample should be interpreted in consultation with the physician who performed the procedure. For samples with a significant risk for MCC that produce a normal female karyotype, a disclaimer should be added to the report suggesting that analysis of maternal cells due to MCC cannot be</td>
</tr>
</tbody>
</table>
excluded.

Any time that MCC is suspected or confirmed, the laboratory director must ensure that an attempt to determine the cause is documented as part of the laboratory's quality assurance program. Additionally, it is recommended that the ratio of XX:XY cases be monitored as a quality control check for CVS and POC cases. Monitoring the male cases for evidence of female cells is also important for quality control of MCC. FISH with probes for X/Y or molecular methods may be used on cell suspensions prior to culturing to screen for or to estimate the amount of MCC.

E4

Prenatal Diagnosis: General Considerations

Amniotic fluid contains single cells sloughed off of the amnion, fetal skin, lung, bladder, and digestive tract. A random sample of cells from the amniotic fluid is drawn and plated. These cells form true distinct colonies. In chorionic villus sampling, villi are usually retrieved from one or two sites and are likely to not be completely disaggregated.

Prenatal cytogenetic diagnosis can be performed on various tissues, each requiring different methods of culture and analysis.

A minimum of two cultures should be analyzed on each case whenever possible. FISH analysis for the chromosome of interest can be done on uncultured amniotic fluid cells in addition to chromosome analysis of cultured cells. The Benn and Hsu (2004) guidelines of workup should be followed for potential mosaicism.

If XX and XY cells are observed: Analyze 15 male colonies if available. If a mixture of XX and XY cells is present, it may be helpful to consult with the referring physician about evidence of a twin pregnancy.

Laboratories should have a protocol stating when to reflex to additional studies, such as increased counts, and/or FISH, and/or a recommendation for high-resolution ultrasound, uniparental disomy, microarray, and study of a second tissue. While CVS may appear as colonies, they should be analyzed as any other disaggregated tissue.

Uniparental disomy testing should be considered if numeric mosaicism or structural abnormality of chromosomes 6, 7, 11, 14, or 15 is detected because these chromosomes are known to carry imprinted genes and uniparental disomy is associated with congenital abnormalities.

It is the laboratory director’s responsibility to monitor quality and to ensure that analytical practices are consistent with the guidelines presented below. (Also see C4)

E4.1

Amniotic Fluid, Chorionic Villi and Percutaneous Umbilical Blood Sampling (PUBS)

E4.1.1 At least two independent cell cultures must be initiated and grown in separate incubators with independent electric circuits or emergency power systems, backup gas sources and emergency alarms.

E4.1.2 With the exception of PUBS, there must be a plan for maintaining back-up cell culture(s) pending the need for additional studies.

E4.1.3 If studies of parental chromosomes are necessary to help interpret a fetal chromosome abnormality or heteromorphism, the same laboratory should perform these studies, if possible and reasonable.

E4.1.4 The number of test failures (defined as failure to obtain final results from an adequate submitted specimen) should not exceed 1 per 100 consecutive samples (1%).

E4.1.5 Efforts must be made to determine the cause of all test failures. These records and records of corrective actions taken must be available for external review and kept for at least 2 years.

E4.1.6 With the exception of PUBS, at least 90% of final results must be completed and reported (verbal or written) within 14 calendar days from receipt of specimen, unless
additional studies are necessary.

E4.1.7 Laboratories consistently failing to meet these standards should consider splitting or sending samples to another laboratory until the problems are resolved.

E4.1.8 Laboratories should have specific requirements for the acceptance and rejection of specimens that include the volume and quality of the specimen received.

E4.1.9 Where there is suspicion that MCC may be present (see E3.3), the laboratory director may want to consider analysis of additional cultures, increased colony counts, or molecular genetic analyses (PCR or QF-PCR) to rule out any confounding diagnosis.

E4.2 Amniotic Fluid

E4.2.1 Amniotic Fluid: Processing Standards

E4.2.1.1 If little or no cell pellet is apparent in the sample, the laboratory should consider the use of a method (e.g., assays for pH, protein, glucose, etc.) that will help to distinguish amniotic from other fluids.

E4.2.1.2 Notification of inadequate or poor cell culture growth should be made within 10 days of the amniocentesis procedure.

E4.2.1.3 A laboratory planning to establish amniotic fluid cytogenetic testing must arrange to split and successfully analyze at least 50 consecutive specimens with a laboratory performing such studies by established standards.

E4.2.1.4 The laboratory should investigate significantly increased chromosome instability in one or several concurrent patient samples.

E4.2.1.5 Sample quality and culture failures should be monitored.

E4.2.2 Amniotic Fluid: Analytical Standards (see also E3.1.6)

E4.2.2.1 Analysis of in situ cultures is the preferred method, since it is more reliable for evaluating mosaicism. 
**Count:** a minimum of 15 cells from at least 15 colonies, distributed as equally as possible between at least 2 or more independently established cultures. Single metaphase colonies should only be used when multi-metaphase colonies are unavailable. Document any numerical/structural aberrations observed.

**Analyze:** 5 cells, each from a different colony, preferably from 2 independently established cultures. Band resolution should be appropriate to the reason for testing.

**Karyotype:** 2 cells. These cells can be from the 5 analyzed cells. If more than 1 abnormal cell line (as defined in Section E3.1.1) is found, karyotype at least 1 cell representative of each cell line.

If both abnormal and normal cells are observed in a colony, the colony is generally considered normal if the same abnormality was NOT seen in other colonies.

E4.2.2.2 Analysis from a combination of mixed in situ and subcultured cells When it is impossible to complete the analysis by in situ only, subculturing may be necessary. 
**Count:** Count as many colonies as possible and then increase the count to a total of 20 cells.

**Analyze:** 5 cells, distributed between 2 independently established cultures.

E4.2.2.3 Suspension Harvest Technique

Situations in which suspension harvest technique is appropriate include: suboptimal sample or when the primary concern is growing cells for other testing methodologies.

**Count:** a minimum of 20 cells, distributed as equally as possible among independently established cultures. Document any numerical/structural aberrations observed (see E4.1.3.2 for analysis and karyogram guidelines).

E4.3 Chorionic Villus Sample (CVS)
**Villus Sample (CVS): Processing Standards**

In cases of multiple gestations particularly in those of *in vitro* fertilization, one should be aware that a deceased co-twin with remaining viable placental material may be the source of a chromosome abnormality.

**Confined placental mosaicism** (CPM) is defined as at least two cell lines from a single fertilized egg seen in chorionic villi analysis that are only present in the placenta, not in the fetus itself. It is observed in about 1-2% of CVS. Laboratories should have protocols to distinguish CPM from true mosaicism in the fetus. Additional studies may be recommended depending on the chromosome involved and the type of abnormality. These may include analysis of additional cultures and in some cases analysis of amniotic fluid cultures or fetal cord blood. *In situ* culture strategy is appropriate for CVS or any other tissues that grow in a monolayer, but the analysis protocols may differ from amniotic fluid.

The significance of mosaicism in CVS may differ based on the distribution of the abnormal cells in the direct and cultured preparations, as well as the chromosomes involved. When mosaicism is documented, in general, amniocentesis is recommended, since the amniotic fluid cells are more likely to represent the fetus.

<table>
<thead>
<tr>
<th>E4.3.1.1</th>
<th>When direct (uncultured) preparations are used clinically, a cell culture technique (defined as longer than 48 hours) must also be used.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4.3.1.2</td>
<td>Final written reports should include a summary of the analysis results of the cultured cells and direct preparation, if performed.</td>
</tr>
<tr>
<td>E4.3.1.3</td>
<td>A laboratory planning to establish CVS cytogenetics should already be testing amniotic fluid cells by established standards and methods. Prior to independent CVS analysis, the laboratory must split and confirm at least 25 samples (with an adequate volume) with a laboratory already performing CVS cytogenetics by established standards and methods. Note: During this period, samples that are too small to split should be sent to a qualified reference laboratory for culturing and analysis.</td>
</tr>
</tbody>
</table>

**Chorionic Villi: Analytical Standards (see also E3.1.6)**

| E4.3.2.1 | Direct (Uncultured) Preparations: should not be exclusively used in obtaining final results. (See Section E4.3.2.3 below.) Interphase FISH or molecular screening for sex chromosomes and common aneuploidies should be used to generate alternative preliminary results. |
| E4.3.2.2 | Cultured Preparations |
| Count:  | a minimum of 20 cells distributed as equally as possible between at least 2 independently established cultures. Document any numerical/structural aberrations observed (see E 4.2.2.2 for analysis and karyogram guidelines). |
| E4.3.2.3 | Combination of Direct Preparation and Culture Technique |
| Count:  | a minimum of 20 cells, at least 10 of which come from cultured preparations. Document any numerical/structural aberrations observed. |
| Analyze: | 5 cells, preferably at least 4 cells from cultured preparations. Resolution should be appropriate to the reason for testing (see E 4.2.2.2 for karyogram guidelines). |
| E4.3.2.4 | If mosaicism is documented in a CVS sample, cytogenetic studies of amniotic fluid are recommended. |

**Fetal Blood: Percutaneous Blood Sampling (PUBS)**

| E4.4.1.1 | Final results of PUBS should not be released until the sample has been confirmed to be fetal in origin, |
| E4.4.1.2 | A minimum of 2 cultures should be established, if adequate specimen is submitted. |
| E4.4.1.3 | Processing after 48 and 72 hours in culture is recommended. |
| E4.4.1.4 | Final reports (verbal or written) should be available within 7 calendar days. |
| E4.4.2 | **Fetal Blood: Analytical Standards (see also E3.1.6)**  
  **Count:** a minimum of 20 cells (see E 4.2.2.2 for analysis and karyogram guidelines). |
| E5 | **Peripheral Blood and Solid Tissue Constitutional Chromosome Study** |
| E5.1 | **Peripheral Blood (Stimulated Lymphocytes): Routine Studies** |
| E5.1.1 | **Processing Standards** |
| E5.1.1.1 | At least 2 cultures should be established for each specimen. |
| E5.1.1.2 | At least 90% of all routine peripheral blood analyses must have final written reports completed within 28 calendar days (21 calendar days is recommended) from receipt of the specimen. Clinical indications may dictate more rapid turn-around time. Specialized stains and studies may take longer. |
| E5.1.1.3 | Test failures should not exceed 2% per year. |
| E5.1.1.4 | The 550-band stage should be the goal of all constitutional studies to rule out a structural abnormality, particularly in cases of mental retardation, birth defects, dysmorphology, or couples with recurrent pregnancy loss. |
| E5.1.2 | **Analytical Standards (see also E3.1.6)** |
| E5.1.2.1 | **a. Count:** a minimum of 20 cells, documenting any numerical/structural abnormalities observed.  
  **b. Analyze:** 5 cells. Resolution should be appropriate to the reason for testing.  
  **c. Karyotype:** 2 cells. If more than 1 clone (as defined in Section E3.1.1) is found, karyotype 1 cell representative of each clone. |
| E5.1.2.2 | Cases being studied for possible sex chromosome abnormalities, in which mosaicism is common, should include the standard 20-cell assessment. If mosaicism is confirmed, the analysis is complete. A minimum of 10 additional metaphase cells should be evaluated when one cell with a sex chromosome loss, gain or rearrangement is observed within the first 20 cells analyzed. |
| E5.2 | **Peripheral Blood (Stimulated Lymphocytes): Focused High Resolution Analysis** |
| E5.2.1 | **Analytical Standards** |
| E5.2.1.1 | Focused high resolution analysis should be reserved for cases in which a specific microabnormality syndrome is being considered, the diagnosis of which generally requires chromosomes above the 650-band stage (resolution at the 850 level is recommended). In addition, it can be applied to cases requiring the improved characterization of an identified chromosome abnormality. With respect to microdeletion/microduplication syndromes for which FISH probes are widely available, FISH analysis should be an integral component of diagnostic testing. Examples include Prader-Willi/Angelman syndrome, DiGeorge/velocardiofacial syndrome and Williams syndrome. |
| E5.2.1.2 | General processing and analytical standards for routine peripheral blood studies apply. In addition, in a focused analysis, the primary analytical standard must be that the specific chromosomal region in question is visible and clearly separated from adjoining bands in both homologues, or in abnormal cases, in the normal homolog. |
| E5.3 | **Peripheral Blood (Stimulated Lymphocytes): Complete High Resolution Analysis** |
| E5.3.1 | **Analytical Standards** |
| E5.3.1.1 | General processing and analytical standards for routine peripheral blood studies apply. In addition, complete high resolution chromosome analysis should include detailed evaluation of all regions on all chromosome pairs at a level of resolution above the 650-band stage (resolution at the 850 level is recommended). |
E5.4 Peripheral Blood (Stimulated Lymphocytes): Heritable Fragile Sites (Including Fragile X)
This section initially provided guidelines for the evaluation of patients for fragile X syndrome using the cytogenetic expression of the Xq27.3 (FRAXA) fragile site. Such chromosome testing has been replaced by molecular genetic DNA evaluation of the FMR1 locus, and specific College recommendations have been published to cover such testing (see Section FX, "Technical Standards and Guidelines for Fragile X"). However, because some laboratories may still include fragile site screening in the chromosome study portion of fragile X testing, and cytogenetic testing for other heritable fragile sites is requested under some clinical circumstances, the following guidelines are recommended.

E5.4.1 Processing Standards

E5.4.1.1 Fragile site expression should be assessed using at least two different culture induction systems; i.e., low folate and an anti-metabolite for folate-sensitive fragile sites, BrdU and distamycin A for BrdU-inducible sites.

E5.4.2 Analytical Standards

E5.4.2.1 Scoring for a fragile site is analogous to the exclusion of chromosomal mosaicism, and at least 50 cells total from the two cultures should be scored.

E5.4.2.2 In reporting of results, fragile site nomenclature must follow ISCN 2005, and it is recommended that the report contain both the methods of induction and frequency of fragile site expression.

E5.4.2.3 For FRAXA and FRAXE, all positive cytogenetic results should be confirmed with molecular genetic testing. Fragile site expression must not be used as a basis for premutation carrier evaluation.

E5.5 Solid Tissues (Skin, Organs, Products of Conception, etc.)

E5.5.1 Processing Standards

E5.5.1.1 Tissue biopsy specimens and small specimens should be transported in sterile cell culture medium with or without serum. Sterile saline solution may be used if medium is not available. Larger specimens should be transported according to written guidelines in each laboratory.

E5.5.1.2 At least two independent cultures should be established (three are recommended for resolving questions of mosaicism). These can be from explants of tissue grown in flasks or from enzyme-dissociated cells that can be processed in flasks or in situ.

E5.5.2 Analytical Standards
See amniotic fluid guidelines (E4.1.3) for analytical standards.

E5.5.2.1 When sex chromosome anomalies are suspected, at least 30 cells should be counted and scored for sex chromosome complement.

E5.5.3 Except for products of conception (POC), test failure rates should not exceed 5% per year, in total. It is suggested that periodic monitoring of POCs be done to assure that the ratio of 46,XX: 46,XY results approximates 1:1.

E5.6 Bone marrow studies for constitutional disorders. In most laboratories, these studies have been replaced by analysis of short term (overnight) blood cultures and/or FISH analysis performed on interphase nuclei.

E5.7 Chromosome Instability Syndromes: Peripheral Blood Breakage Analyses

E5.7.1 General Standards
The rarity of chromosome instability syndromes requires that inexperienced laboratories should refer cases to reference laboratories with experience in diagnosing such disorders. Additionally, as research leads to the identification and cloning of the putative disease genes, molecular testing is recommended to supplement cytogenetic analysis.

E5.7.1.1 G-banded or unbanded preparations may be applied, depending upon the particular goal
of the study. Unbanded preparations are acceptable only if there is no need to identify abnormalities such as translocations or inversions that will not be visible in unbanded preparations. All abnormalities should be recorded using appropriate ISCN 2005 designations.

**E5.7.2 Fanconi Anemia**

Cytogenetic evaluation for Fanconi anemia (FA) should include analysis of crosslinking agent (e.g., mitomycin C [MMC], diepoxybutane [DEB]) induction of breakage in addition to baseline chromosome breakage.

**E5.7.2.1 Culture Conditions**

Each laboratory should have well-established negative control (non-Fanconi) and positive control (Fanconi) ranges for each culture (with and without mutagen) condition. Each new lot number of crosslinking agent should be appropriately quality controlled for its efficacy and potency for inducing chromosomal breakage. Given variability between drug lots, and the need to routinely prepare fresh stock and working solutions for most of the crosslinking agents, parallel testing of control specimens is recommended, as necessary. When a sufficient amount of blood specimen (and cell count) is available, two drug-treated cultures (e.g., either two different concentrations of either DEB or MMC, or one culture each of MMC and DEB) are recommended.

**E5.7.2.2 Chromosome Breakage Analysis**

Optimally, 50 metaphase cells (banded or unbanded) should be scored from each culture condition. The average rate of chromosomal aberrations per cell or the distribution of aberrations among cells should be compared to negative and positive control reference ranges. The percentage of cells demonstrating aberrations should be reported to enable identification of those patients who are mosaic for mutant and wild type cells.

**E5.7.3 Bloom Syndrome**

Cytogenetic evaluation for Bloom syndrome must include assessment of baseline sister chromatid exchange (SCE) rates.

As the Bloom syndrome gene BLM has been cloned, molecular evaluation to identify the mutation may be possible to supplement a positive cytogenetic result.

**E5.7.3.1 Culture Conditions**

Each laboratory should have well-established negative (non-Bloom syndrome) and positive (Bloom syndrome) control SCE ranges. Duplicate cultures should be established both from the patient and a concurrently processed negative control. Cultures should be supplemented with bromodeoxyuridine and maintained in darkness prior to harvesting. Cells should be cultured for the period of time predetermined by the laboratory to yield a high percentage of cells in second division. At the discretion of the laboratory director, cultures for evaluation of baseline chromosome breakage may also be established.

**E5.7.3.2 Analysis**

For confirmation of a negative result, fifty metaphase cells (25 from each of the two duplicate cultures) should be evaluated. For a positive result, fewer cells may be evaluated. However, it is recommended that both cultures from the patient and at least one from the concurrent control be sampled in the evaluation.

**E5.7.4 Ataxia Telangiectasia and Nijmegen Breakage Syndrome**

Cytogenetic evaluation for ataxia telangiectasia (A-T) and Nijmegen breakage syndrome (NBS) should include specific screening for rearrangements involving chromosomes 7 and/or 14.

Evaluation for A-T and NBS should include evaluation of sensitivity to radiation. Although such sensitivity can be assessed by cytogenetic methods, it generally is evaluated by survival assays on lymphoblastoid or fibroblast cells.

As the (A-T) gene (ATM) and the NBS gene (NBN) have been cloned, molecular evaluation should be performed for confirming the diagnosis in patients who have positive cytogenetic and/or radiosensitivity assays.

**E5.7.4.1 Culture Conditions**

Each laboratory should have well-established negative (non-A-T, non-NBS) and positive (A-T or NBS) control chromosome 7/14 rearrangement and chromosome
breakage rates. Duplicate stimulated cultures should be established for each patient case, and cultured for 48-96 hours identical to the conditions utilized for the establishment of the laboratory's control ranges.

**E5.7.4.2 Analysis**
Analysis should include 50 G-banded metaphase cells screened for rearrangements involving chromosomes 7 and/or 14. Overall breakage and rearrangement rates may also be helpful. It should be noted that, among confirmed A-T patients, the frequency of 7/14 rearrangements increases with age. Thus, negative results should be interpreted with caution. Among healthy controls, rearrangements of chromosomes 7 and 14 are well documented in PHA-stimulated cultures. Thus, comparison to laboratory norms for age-matched controls is essential.

**E5.7.5 Miscellaneous**
Cytogenetic evaluation of chromosome breakage may also be undertaken for other reasons, e.g., prior exposure to clastogens. The specific culture methods utilized (e.g., timing of cultures) and the methods of analysis (G-banded vs. unbanded chromosomes) should be appropriate to the referral. The laboratory should have well established positive and negative control ranges for the specific analyses being conducted.

**E6 Chromosome Studies for Acquired Abnormalities**

- **a.** A patient with an acquired clonal chromosomal abnormality or one who is at high risk for developing such an abnormality may have multiple cytogenetic studies during the course of his or her disease.
- **b.** Tissue processing, analytical variables and turn-around times should be determined by the laboratory based on the indication for the cytogenetic referral (e.g., initial diagnosis versus follow-up studies; pre- versus post-transplant studies) and the clinical application of the cytogenetic results (e.g., selection of therapy).
- **c.** All results should, to the extent possible, be interpreted in the context of other clinical and laboratory findings.
- **d.** The laboratory director and staff should be familiar with the various recurrent chromosome abnormalities associated with specific subtypes of malignancies that may be crucial for differential diagnosis.
- **e.** Monitoring the percentage of cases for which an abnormal clone has been identified is recommended for quality assurance assessment for the laboratory. Such monitoring is particularly valuable for cases of documented acute leukemia, in which the expected percentage with abnormalities is greater than 50%; a lower than expected rate can alert the laboratory to issues such as tissue processing or analysis guidelines.
- **f.** For studies performed, the cytogenetics laboratory should obtain, at the time of receipt of the specimen, as much information as possible about the suspected pathologic diagnosis, preliminary morphologic, immunophenotypic and clinical findings, results of any previous cytogenetic testing and indication for the requested study.

**E6.1 Bone Marrow/Blood: Specimen Procurement and Processing**
Only those cells involved in the disease process will harbor the abnormalities being sought. The culture techniques and methodologies utilized should optimize the probability of detecting an abnormal clone.

**E6.1.1 In most cases, bone marrow is the tissue of choice for analysis of suspected premalignant or malignant hematologic disorders.**
In circumstances in which adequate bone marrow aspirate can not be obtained, alternative specimens may include:

- **a.** Peripheral blood: (Blood specimens may yield informative results when the circulating blast count is higher than 10% to 20%.) In general, the abnormal clone can be identified in such specimens, albeit not as often as in bone marrow.
- **b.** Bone marrow core biopsy (discussed under specimen collection, Section E6.1.2):

**E6.1.2 Specimen Collection**

- **a.** Bone marrow and blood specimens should be collected under sterile conditions in sodium heparin. The concentration of sodium heparin should be approximately 20 units per ml of specimen (per either bone marrow volume alone, or per total volume of bone marrow and transport medium combined). If collected in a green
top tube, the smaller sized sodium heparin tube should be used.
b. The volume of bone marrow available will differ for adults and children. An approximate specimen of 1 to 3 ml should be requested when possible. During the procurement of the specimen, several draws are likely to be taken from the patient. As the first draw is more concentrated with blasts, it is recommended that cytogenetics receive first or second draw whenever possible.
c. Specimens should be received by the laboratory as soon as possible (ideally within 24 hours).
d. It is generally recommended that specimens be maintained at ambient temperature during transit. Extreme temperatures should be avoided.
e. If a bone marrow core biopsy is obtained, it should be minced to generate cell suspensions. This can be done mechanically or enzymatically (e.g., with collagenase), if it is resistant to mechanical dissociation. Culture conditions are the same as those for bone marrow aspirates.

E6.1.2.2 Culture conditions should be optimized for the specific hematologic disorder suspected:
a. Acute leukemias: Unstimulated short-term cultures are recommended. If sufficient specimen is received, at least two cultures should be initiated, one of which should be designated as a 24-hour culture.
b. Aplastic anemia and chronic myeloproliferative disorders: Same as acute leukemias above.
c. Chronic lymphoproliferative disorders: Depending on the immunophenotype, additional cultures with B- or T-cell mitogens may be helpful.
d. Well-differentiated T-cell disorders (e.g., T-cell leukemia, T-cell lymphoma, Sezary syndrome, mycosis fungoides): T-cell mitogens may be helpful.
e. Mature B-cell disorders (e.g., plasma cell leukemia, multiple myeloma): Although there is no consensus on this point, some laboratories have had success in identifying abnormal clones with the addition of B-cell mitogens.

E6.2 Analytical Standards

E6.2.1 Cell Selection
Cells selected for analysis should not be selected solely on the basis of good chromosome morphology. In general, the technologist should select an area of a slide to begin the analysis, and then examine cells as they appear consecutively in the microscope field, only skipping cells for which extremely poor morphology precludes chromosome identification.

When cells are skipped because of poor morphology, it is important to attempt to count the number of chromosomes (particularly for possible hyper- or hypodiploidy relevant in pediatric ALL). In addition, attempts should be made to identify possible structurally abnormal chromosomes, particularly if the disease under consideration is associated with a specific recurring chromosome abnormality such as t(9;22) in CML.

E6.2.2 Number of Cells Evaluated
The number of metaphase cells analyzed versus the number of metaphase cells counted or scored should be appropriate to the type of study (e.g., initial diagnosis versus follow-up); the purpose of the study (e.g., detection of residual disease or response to therapy, monitoring for cytogenetic evolution or monitoring of engraftment of allogeneic transplant); and/or the characteristics of the specific chromosomal abnormalities present or suspected.

E6.2.2.1 Initial Diagnostic Studies
a. G-band analysis

Analysis
Analyze a minimum of 20 cells from unstimulated cultures. A combination of unstimulated and mitogen-stimulated cultures may be appropriate for the mature B- and T-cell disorders as described above (Section E6.1.2.2). For all other diagnoses (e.g., acute leukemias), only when the abnormal clone is identified in the mitogen-stimulated cultures can the G-band analysis include less than 20 metaphases from the unstimulated cultures.
Documentation
For the abnormal cells:
*If only one abnormal clone is present:* two karyotypes.
*If more than one related abnormal clone is present:* Two karyotypes of the stemline and one of each sideline.

*If unrelated clones are present:* Two karyotypes for each stemline and one for each associated pertinent sideline.

For the normal cells:
*If only normal cells are present:* two karyotypes.
*If normal and abnormal cells are present:* one karyotype of a normal cell.

b. Supplemental FISH Analysis
Studies using FISH may be indicated to (1) provide a rapid result that will aid in the differential diagnosis or for planning induction therapy, or (2) to rule out a cryptic abnormality. Characterization of the initial diagnostic FISH pattern will also allow for future monitoring of the patient’s disease. Examples of such cases include, but are not limited to:

i. Acute promyelocytic leukemia (AML-M3): FISH with PML/RARA gene probes to detect t(15;17).

ii. Chronic myelogenous leukemia: FISH with BCR/ABL gene probes for t(9;22) or variants thereof.

iii. Acute myelomonocytic leukemia with abnormal eosinophils (AML-M4eo): FISH with CBFB gene probe or other informative probe for inv(16) or t(16;16).

iv. Infant or childhood acute leukemia with high risk features: FISH with BCR/ABL and/or MLL gene probes.

v. Precursor B-cell acute lymphoblastic leukemia most frequently seen in children ages 1 - 10 years: FISH with TEL/AML1 gene probes to detect a cryptic 12;21 translocation.

vi. Suspected Burkitt leukemia/lymphoma (acute lymphoblastic leukemia L3), FISH with CMYC/IgH gene probes or others to detect t(8;14) or variant translocations.

vii. B-cell CLL when a normal cytogenetic result is obtained by analysis of unstimulated and B-cell mitogen stimulated cultures to detect +12, del(13)(q14), deletions of the ATM (11q22.3) and p53(17p13.1) genes.

Other applications of FISH include (but are not limited to) interphase FISH when there is insufficient material for G-banded analysis and there is a suspected pathologic diagnosis associated with a recurring chromosomal abnormality; metaphase or interphase FISH to investigate the presence of a variant translocation suspected in the G-banding analysis by an abnormality involving a recurring chromosomal breakpoint.

Documentation
Documentation of FISH results should be in accord with Sections E9 and E10 of these Standards and Guidelines for Clinical Genetics Laboratories.

E6.2.2.2 Follow-up studies of patients who have had a previous cytogenetic study
For the following analytic guidelines, it is assumed that the laboratory has documentation of the patient’s previous cytogenetic results. If the study has been performed elsewhere, and there is minimal information available, it is recommended that, except for patients seen for the first time post-transplant, the analysis be considered the same as an initial diagnostic workup (see above).

E6.2.2.2 (a) Patients who have NOT received allogeneic hematopoietic cell transplant (e.g., history of previous radiation and/or chemotherapy, autologous transplant, no prior therapy, etc.):

Analysis
Analyze 20 cells. If all cells are normal, additional cells may be scored for a specific
abnormality by G-banding or FISH.

*Note: For some patients, follow-up cytogenetic study is ordered to rule out a therapy-associated malignancy (e.g., MDS) rather than disease recurrence.

**Documentation**
For cases with both normal and abnormal cells or only abnormal cells:
One karyotype of a normal cell, if such a karyotype was not documented in a previous study by the laboratory; otherwise, one normal metaphase spread.
One or two karyotypes from each abnormal clone for a minimum total of two karyotypes.

For cases with all normal cells: Two karyotypes.

<table>
<thead>
<tr>
<th>E6.2.2.2 (b)</th>
<th>Patients who are post-allogeneic hematopoietic cell transplant for whom donor versus recipient origin of cells can be determined (by sex chromosome complement or cytogenetic heteromorphisms):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>For studies aimed solely at determining engraftment status, molecular methods and/or interphase FISH (in the case of opposite sex transplant) are more sensitive than G-banded analysis and are the preferred methodologies. Therefore, in consultation with the referring physician, cancellation of test requests for G-band analysis for engraftment status should be considered.</td>
</tr>
<tr>
<td></td>
<td>During the course of the cytogenetic analysis it will become evident whether there is chimerism for donor and recipient cells. It is expected that there will be different approaches used by different laboratories to address these studies.</td>
</tr>
</tbody>
</table>

If only donor cells are present:

**Analysis**
Analyze 20 cells.

**Documentation**
Document two karyotypes for each cell line. (In such cases, one is documenting either the constitutional karyotype (normal or abnormal of the donor) or the rare event of a malignant process arising in a donor cell.)

If donor and recipient cells are present:

**Analysis**
Analyze recipient cells completely for previously identified clonal chromosome abnormalities and any newly acquired abnormalities. In some cases there may be structural chromosomal abnormalities secondary to chromosome breakage or rearrangement induced by the pretransplant conditioning regimen. The laboratory should distinguish clonal from nonclonal changes and determine the significance of new abnormalities as possible.

Analyze all recipient cells present out of 20 cells. Evaluate each recipient cell for the presence of the abnormality present prior to transplant (i.e., the diagnostic abnormality). Depending on the number of recipient cells present among the initial 20 metaphase cells scored, additional recipient cells may be analyzed completely and/or scored for the presence of the diagnostic abnormality.

Donor cells: Analyze two donor cells if donor cells have not been analyzed in previous studies. Otherwise simply score these cells as being of donor origin, and count.

**Documentation**
For the *recipient* cells: Two karyotypes of the stem line and one of each sideline.
For the *donor* cells: If donor cells have been documented previously, then provide a single metaphase spread. If donor cells have not been documented previously, provide two karyotypes.

If only recipient cells are present:

**Analysis**
Analyze 20 cells, following the guidelines set forth above with respect to the characterization of secondary abnormalities.

**Documentation**
Same as above for abnormal recipient cells.

E6.2.2.2 (c) Patients post-hematopoietic transplant for whom donor and recipient cells cannot be differentiated by G-banding:

Analysis
Analyse 20 cells. As in case scenarios outlined above, follow guidelines for recipient cells as set forth above.

E6.3 Turnaround time (TAT) should be appropriate for clinical utility.
It should generally be recognized that under certain clinical circumstances, specific chromosome abnormalities, or lack thereof, may be crucial in establishing a diagnosis and have direct relevance to specific treatment offered to the patient. Every effort should be made to expedite conveying such chromosome information. It is strongly recommended that the cytogenetics laboratory director have a written policy describing how cases are prioritized in the laboratory, with associated TATs, and addressing the particular specimen processing and analytical needs.

E6.3.1 For initial diagnostic work-up, to rule out, confirm, or further characterize a diagnosis of acute lymphoblastic leukemia, acute myeloid leukemia, or chronic myelogenous leukemia, it is strongly recommended that these studies have preliminary results reported within 7 calendar days. Final results should be reported out within 21 calendar days.

E6.3.2 In the initial diagnostic work-up of other hematologic disorders, and for longitudinal studies on a patient, it is strongly recommended that final results be reported within 21 calendar days.

E6.3.3 Specific exceptions to these guidelines are: Diagnostic studies for acute promyelocytic leukemia should be approached on a STAT basis, as selection of induction chemotherapy (including or excluding the use of ATRA as part of the regimen) depends largely upon cytogenetic or molecular findings. Therefore, if APL is suspected in a patient, it is highly desirable for preliminary results of FISH and/or G-banding to be reported within 2 working days of receipt of the specimen.

E6.3.4 Specific exceptions to these guidelines are: Diagnostic studies for acute promyelocytic leukemia should be approached on a STAT basis, as selection of induction chemotherapy (including or excluding the use of ATRA as part of the regimen) depends largely upon cytogenetic or molecular findings. Therefore, if APL is suspected in a patient, it is highly desirable for preliminary results of FISH and/or G-banding to be reported within 2 working days of receipt of the specimen.

E6.4 Lymph Nodes

E6.4.1 Specimen Procurement and Processing
Common diagnoses include Hodgkin and non-Hodgkin lymphomas, including follicular, diffuse large B-cell, marginal zone, mantle cell, T-cell, and anaplastic large cell lymphoma.

E6.4.1 (a) Information regarding the pathologic examination of the tissue (e.g., whether or not a malignant process was identified, any subtyping that is available, including immunophenotype) can provide valuable information regarding the processing and analytical procedures to be followed.

E6.4.1 (b) Lymphoma may initially present as a mediastinal or other mass. Therefore, pathologic information should be pursued at the time of receipt of the tissue in the laboratory. If lymphoma is in the differential diagnosis, the cultures initiated should include at least one non-stimulated suspension culture as follows:

In general, short-term (to include a 24-hour unstimulated culture) are preferred, with the number of cultures established being consistent with the apparent cellularity of the tissue specimen.

Of note, in suspected lymphoma cases, bone marrow aspirate is frequently sent for staging purposes, but if it shows no or limited involvement by lymphoma, conventional cytogenetics will most often yield normal chromosome findings. Therefore the laboratory should be sure to discuss with the clinicians the need to get a biopsy
specimen from the involved lymph node or mass.

E.6.4.2 Analytical Standards

E.6.4.2 (a) Selection of cells to analyze: As with bone marrow and blood specimens, cells selected for analysis should not be chosen based on good chromosome morphology. After an area of a slide is chosen to begin analysis, the technologist should examine cells as they appear consecutively in the microscope field.

E.6.4.2 (b) Analysis
Analyze 10-20 G-banded metaphase cells. (Fewer than 20 metaphases is acceptable as long as the abnormal clone has been identified and characterized.)

Note: Some lymphomas have highly complex karyotypes. In such cases, it may not be practical to analyze 20 metaphases. Therefore, in such studies, it is recommended that a sufficient number of metaphases (generally at least 10) be analyzed to permit characterization of the abnormal clone(s). The laboratory director should ensure that in the case of performing an abbreviated study, the CPT codes billed are consistent with the work performed as described in Section E3.2 of the Standards and Guidelines for Clinical Genetics Laboratories.

Rationale for the "Note": Unlike a study for aplastic anemia or myelodysplastic syndrome wherein the proportion of abnormal versus normal metaphase cells may have clinical significance, in the analysis of a lymph node or solid tumor, the sole clinical importance is the presence or absence of an abnormal clone, and the characterization of the chromosomal abnormalities involved. In a tissue in which the laboratory finds only abnormal cells, all with highly complex karyotypes, the goal should be to analyze a sufficient number to permit characterization of the clone. Extending the study to 20 cells could be very time- and resource-consuming, without any real added value to the diagnosis of the patient.

Documentation
Follow guidelines for initial diagnostic workup of blood and bone marrow (Section E6.2).

If there is confirmed pathologic diagnosis of lymphoma, and analysis of 20 metaphases yields only normal cells, additional screening for specific lymphoma-associated chromosomal rearrangements (e.g., the 2;5, 8;14, 11;14, or 14;18 translocations) can be performed by G-banding or FISH.

E6.4.3 Turnaround Time

E6.4.3 (a) It is recommended that 90% of specimens have final reports completed within 21 calendar days.

E6.4.3 (b) Certain patient cases may need preliminary and/or STAT verbal or written reports. The laboratory should obtain sufficient information at the time of specimen receipt to permit prioritization in the laboratory in accord with clinical need.

E6.5 Chromosome Studies for Solid Tumor Abnormalities
Cytogenetic analysis of tumor tissue is performed to detect and characterize chromosomal abnormalities for purposes of diagnosis, prognosis, and patient management.

E6.5.1 General Considerations

E6.5.1 (a) A patient with a solid tumor may have conventional or molecular cytogenetic fluorescence in situ hybridization (FISH) analysis of the tumor tissue at the time of biopsy or resection (to aid in differential diagnosis), at a time of disease recurrence (to confirm recurrence and to investigate disease progression), or to identify metastatic tumor or a tumor of uncertain origin. The solid tumor specimen may be accompanied by or followed by collection of other tissue samples (e.g. bone marrow, cerebrospinal fluid) for disease staging.

E6.5.1 (b) The Laboratory Director and staff should be familiar with the chromosomal and molecular abnormalities associated with tumor types/subtypes and their clinical
E6.5.1 (c) The majority of pediatric tumors should be cytogenetically analyzed whenever sufficient fresh tissue is available since cytogenetic abnormalities are commonly disease or disease subtype specific and have prognostic significance. Cytogenetic analysis of selected adult tumors is indicated whenever such analysis may have diagnostic or prognostic value.

E6.5.1 (d) Methods for processing of tumor material will be determined by the cytogenetic laboratory based on available clinical and pathological findings. The cytogenetic laboratory should obtain as much information as possible about the suspected diagnosis and the tissue type at the time of sample receipt to choose the most appropriate tissue culture method(s). To the degree possible, the cytogenetic laboratory should communicate with the Pathologist to gain information regarding tumor type by frozen section and/or permanent section analysis of the tissue.

E6.5.1 (e) Molecular cytogenetic FISH analysis may be used as a primary or secondary method of evaluation of the tumor tissue. The availability of fresh tissue, the differential diagnosis, a need for rapid diagnostic information, and the type of information needed should be used to prioritize FISH relative to conventional cytogenetic analysis.

E6.5.1 (f) Cytogenetic analysis results must be interpreted within the context of the pathological and clinical findings.

E6.5.1 (g) For quality assurance, the laboratory should monitor the number and types of tumors received, the percentage of tumors with abnormal results, the cell culture success rate, and the success rate for FISH studies.

E6.5.1 (h) The presence or absence of specific abnormalities should be available to the physician as soon as is feasible to contribute to the patient’s plan of care.

E6.5.1.2 Specimen collection

E6.5.1.2 (a) Solid tumor samples should be collected in a sterile manner. For conventional cytogenetic analysis, the tissue sample must be fresh. The sample selected for cytogenetic analysis should be pure tumor if possible, without necrosis. The sample must not be placed in fixative or frozen. (Specimens that will be evaluated solely by FISH analysis may be fixed, frozen, or paraffin-embedded.)

E6.5.1.2 (b) The laboratory should request a sample size of 0.5 - 1cm³; if less tissue is available, the laboratory should accept as much as can be provided. If the sample size is very limited, e.g., fine needle aspirate or fine needle core biopsy, coverslip cultures are often successful. If, however, the sample size precludes cell culture and conventional cytogenetic evaluation, the sample may be amenable to interphase FISH analysis using touch-preparations or paraffin-embedded tissue sections; see Section E6.5.2.1(b) below.

E6.5.1.2 (c) The tumor sample should be transported in culture medium to the cytogenetics laboratory as soon as possible for immediate processing.

E6.5.1.3 Specimen processing

E6.5.1.3 (a) The cytogenetic laboratory should process the tumor sample as soon as it is received.

E6.5.1.3 (b) The tumor sample should be inspected and details of the sample size, color, and attributes recorded.

E6.5.1.3 (c) If obviously normal tissues are present, the tumor should be separated from non-tumor tissue for processing.

E6.5.1.3 (d) Disaggregation of tumor samples is needed for most tumor types. Mechanical and/or enzymatic methods may be employed. If sufficient tumor material is submitted, both methods of disaggregation are recommended. For some tumor types, different growth characteristics can be seen with exposure to collagenase versus no exposure to collagenase. If sufficient material is available, cultures should be initiated with and without enzyme exposure.

E6.5.1.3 (e) Culture methods, culture media, and culture conditions should be chosen to best
support the type of tumor received. In general, tumors can be divided into SRCTs (small round cell tumors) and non-SRCT-types (Appendix A). In general, SRCTs can be successfully grown in suspension and non-SRCTs are best grown with monolayer (flask or coverslip) culture methods. Most, but not all SRCTs will also grow in monolayer culture. If adequate tissue is obtained, both culture types should be initiated for SRCTs.

E6.5.1.3 (f) The culture vessels used are chosen by the laboratory. Coverslips cultures may be used to successfully culture very small tumor samples. Duplicate cultures should be established whenever possible.

E6.5.1.3 (g) Experience with tumor culture will provide the laboratory with information regarding optimal growth conditions for different tumor types. It can be helpful for the laboratory to maintain a database that documents how the different tumor types have grown and which culture and harvest conditions yield the abnormal clones. This data base can then be searched for optimal processing and harvesting methods for any new tumor received in the laboratory.

E6.5.1.3 (h) Frequent (daily) observation of cells in culture is needed to determine cell growth rate and optimal time to harvest. Tumor cells should be harvested as soon as possible upon adequate growth to capture early dividing tumor cells and prevent overgrowth by chromosomally normal cells.

E6.5.1.3 (i) Conditions used for cell harvest will vary among tissue types, e.g., mitotic inhibitors used (Colcemid, Velban, Ethidium Bromide, etc.), their concentration and exposure duration.

E6.5.1.4 Analytical methods

E6.5.1.4 (a) Analysis of metaphase chromosomes should include cells with both good and poor chromosome morphology in attempting to identify an abnormal clone. Once identified, the clonal cells with the best chromosome morphology should be analyzed, karyotyped or imaged to provide the most accurate breakpoint assignments.

E6.5.1.4 (b) Clonal abnormalities should be documented in two independent cultures, if possible, to ensure that in vitro culture artifact is not mistakenly identified as a clinically significant abnormality.

E6.5.1.4 (c) Cells that cannot be completely analyzed due to poor morphology should be scanned for obvious structurally abnormal chromosomes and abnormal chromosome counts.

E6.5.2 Analytical Standards

E6.5.2.1 Initial diagnostic studies

E6.5.2.1 (a) G-band analysis and documentation

Analyze 20 metaphase cells and/or a sufficient number of cells to characterize all abnormal clones and subclones.

For abnormal cells:
*If only one abnormal clone:* two karyotypes.
*If more than one related abnormal clone:* two karyotypes of the stemline and one of each sideline.
*If unrelated clones:* two karyotypes for each stemline and one for each associated pertinent sideline.

For normal cells:
*If only normal cells:* two karyotypes.
*If normal and abnormal cells:* one karyotype of a normal cell plus karyotypes for abnormal clone(s) as above.

E6.5.2.1 (b) Molecular cytogenetic FISH analysis
**Tissue types**

Sample types that may be used for FISH include (1) paraffin-embedded tissue sections, (2) touch preparations, (3) cytospin preparations, (4) cultured or direct harvest tumor cells, (5) fixed cytogenetically prepared cells, or (6) fresh-frozen tumor tissues.

**Paraffin-embedded tissue**

i. Before scoring a paraffin-embedded FISH slide, it is crucial that a Pathologist review a hematoxylin and eosin (H&E) stained slide and delineate the region of tumor cells that should be scored, as it can be difficult to differentiate normal from malignant cells using only DAPI counterstain. The technologist should be clear, before scoring the slide, where the malignant cells of interest are located on the slide.

ii. Formalin fixed paraffin-embedded tissue is acceptable for FISH analysis. Tissues preserved in B5 fixative or decalcified are usually not suitable for FISH.

iii. Tumor sections cut 3-4u thick and mounted on positively charged organosilane coated (silanized) slides work well. Request several (2-4) unstained sections and one H&E stained slide from the submitting laboratory.

**Touch preparations (TP)**

A Pathologist should make the TP or be involved in selecting the tissue for TP. Touch preparations are helpful when tissue architecture is not crucial. TPs should be made by lightly touching the tumor piece to a glass slide without smearing. Air dry or fix in alcohol.

**Cytospin preparations**

Cytospin preparations are useful for concentration of samples with very low cellularity, e.g., CSF.

**Fixed cytogenetically prepared cells**

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

**Fresh-frozen tumor tissues**

Such tissues may be useful in sequential analysis of recurring tumors or in the evaluation of archived specimens.

**Supplemental FISH analysis**

As a supplemental test, FISH may be indicated to (1) document a specific molecular event, e.g., gene rearrangement that is diagnostic, (2) provide a rapid result to aid in the differential diagnosis or planning of therapy, or (3) to assess gene amplification. Characterization of the initial diagnostic FISH abnormality and signal pattern will provide a method for future assessment and monitoring of disease status.

**Primary FISH analysis**

FISH may be used as a primary method for tumor evaluation (1) when fresh tumor tissue is not available, (2) when rapid diagnostic information is needed to narrow the differential diagnosis, (3) to determine whether there is gene amplification for prognostic and/or therapeutic purposes, (4) when no metaphase cells are obtained by culture of tumor material, or (5) when conventional cytogenetic analysis yields a normal result.

Examples of such cases include, but are not limited to:

**Small round cell tumors (SCRTs):**

i. FISH with a probe for the EWSR1 gene to identify tumors in the Ewing’s sarcoma family of tumors (EWS, pPNET, Askin tumor, esthioneuroblastoma), or other tumors with EWSR1 gene rearrangement, e.g., clear cell sarcoma, desmoplastic small round cell tumor, extraskeletal myxoid chondrosarcoma, myxoid round cell liposarcoma.

ii. FISH with a probe for the FOXO1A (FKHR) gene to identify alveolar-type rhabdomyosarcoma.

iii. FISH with a probe for the SS18 (SYT) gene to identify synovial sarcoma

**Gene amplification**

i. FISH with a probe for the MYCN gene to assess the presence or absence of gene
amplification in neuroblastoma

ii. FISH with a probe for the ERBB2 gene to assess amplification in invasive breast cancer.

Differentiation of tumors with similar histopathology

i. FISH with a probe for the BCR gene to detect monosomy 22 or deletion 22q. The BCR gene probe may be used as a surrogate for the INI1 gene to differentiate atypical teratoid/rhabdoid tumors of infancy from medulloblastoma or extrarenal rhabdoid tumors from sarcomas.

Other applications of FISH will be determined on an individual tumor/patient basis to facilitate the diagnostic evaluation and monitoring of disease status.

**Documentation**

Documentation of FISH results should be in accord with Sections E9 and E10 of these Standards and Guidelines for Clinical Genetics Laboratories.

<table>
<thead>
<tr>
<th>E6.5.2.2</th>
<th>Follow-up studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. May be indicated to assess recurrent disease or disease progression.</td>
<td></td>
</tr>
<tr>
<td>ii. May be indicated to differentiate recurrence of a tumor from a new disease process.</td>
<td></td>
</tr>
<tr>
<td>iii. Are indicated if the initial study failed.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E6.5.2.2 (a)</th>
<th>G-band analysis and documentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>i. Analysis should include a minimum of 20 metaphase cells. Additional cells may be scored by G-banding or FISH for a specific abnormality identified at initial diagnosis.</td>
<td></td>
</tr>
<tr>
<td>ii. Analysis should be performed with awareness of the possibility of a new clonal process, i.e., therapy-related malignancy.</td>
<td></td>
</tr>
<tr>
<td>iii. FISH analysis may be recommended for diagnoses characterized by an abnormality for which FISH testing is available.</td>
<td></td>
</tr>
</tbody>
</table>

*If both normal and abnormal cells or only abnormal cells:*

One or two karyotypes from each abnormal clone with a minimum of two karyotypes.

One karyotype of a normal cell, if a normal karyotype was not documented in a previous study; otherwise, one normal metaphase spread.

*If only normal cells: Two karyotypes.*

<table>
<thead>
<tr>
<th>E6.5.3</th>
<th>Turnaround Time (TAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAT should be appropriate for clinical utility. The cytogenetics laboratory may want to have a written policy describing how solid tumor cases are prioritized (with respect to each other and with respect to other sample types).</td>
<td></td>
</tr>
</tbody>
</table>

| E6.5.3.1 | Due to the multiplicity of tumor types and to variability of growth in culture, turnaround times will vary. However, the TAT for each individual tumor should be as rapid as possible given such factors. Final results should be available within 28 calendar days. |

| E6.5.3.2 | FISH analysis results should be available within 1-3 days for most tumors and 7 days for paraffin-embedded tissues. |

| E6.5.3.3 | Preliminary verbal reports should ideally be given in 7-10 days, and the date of such results should be documented in the final report. The content of the preliminary report should be documented if it differs significantly from that of the final report. |

| E7.1 | The indirect nature of sex chromatin analysis has rendered the test obsolete. Any patient in whom the question of sex chromosome abnormality is being considered should have complete chromosome analysis. |

<table>
<thead>
<tr>
<th>E8</th>
<th>Reporting Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final written reports of the results of diagnostic testing should include the following information:</td>
<td></td>
</tr>
</tbody>
</table>

| E8.1 | Case identification includes name (or other first identifier), date of birth of patient, date of collection and/or receipt of specimen, laboratory accession number(s), tissue |
type and name(s) of physician(s) or authorized person who ordered the test and to whom report is sent.

E8.2 Specific details of the study to be reported should include:
- Indication for study.
- Numbers of cells in which chromosomes were counted, analyzed and karyotyped.
- Cell culture times and conditions and banding methods employed, when they bear on the cytogenetic interpretation.
- Banding method, level of resolution and current ISCN karyotype designation(s) of cells analyzed.
- A statement of additional work done to resolve questions of mosaicism.
- Correlation with previous studies. When parallel controls are used for comparative purposes in a study, the results of those controls.
- Interpretation of results to include: correlation with clinical information, indication of an abnormal result where applicable, recommendations for additional laboratory genetic studies for the patient and/or family, and a discussion of the significance of the findings, when appropriate. When appropriate, recommendations for genetic counseling should be made. The interpretation should be clear to a nongeneticist physician.
- When investigational procedures are employed, the investigational nature of the testing.
- Cautions as to possible inaccuracies and test limitations.
- Individuals qualified as under B3.1 must sign all final reports. Password protected electronic signatures can be used to fulfill this requirement.
- Specifics of any preliminary results given including what the preliminary result was, the date and the person to whom the report was given.
- Date of final report.

E8.3 Laboratory identification includes name, address, and phone number of the laboratory in which the study was performed.

E9 Metaphase Fluorescence in Situ Hybridization (FISH)

E9.1 General Considerations
Regulatory requirements may differ depending on the origin and FDA approval status of molecular probes used in FISH. The FDA regulates manufacturers who market medical devices, including in vitro diagnostic devices and certain reagents, such as molecular probes, used by laboratories to develop in-house tests. CMS (formerly called HCFA), through CLIA '88, regulates laboratories and their practices.

E9.1.1 Molecular probes are of three general types:
Those probes regulated by FDA as analyte specific reagents (ASRs) for use in clinical settings. They are of two types:

Class II and III ASRs which are sold as components of tests or kits (in vitro diagnostics) that have been cleared or approved by the FDA in conjunction with review of the Class II or Class III ASRs ("ASR kits"). The clinical laboratory is responsible for ensuring that the test system is operating within the performance specifications stated in the product insert. Assays reported as "FDA approved" must be performed exactly according to the manufacturer’s instructions.

Class I ASRs are exempt from FDA premarket approval or notification, and Class II and Class III ASRs are marketed or used as independent components, separate from an approved test or kit ("ASRs"). ASRs may be sold to clinical laboratories regulated under CLIA ’88 as qualified to perform high complexity testing (or clinical laboratories regulated under Veterans Health Administration directive 1106). 21 CFR §809.30(a)(2). FDA regulations require the inclusion of a disclaimer on all reports of results of in-house tests using ASRs, 21 CFR §809.30(e), and restrict the ordering of such tests, 21 CFR §809.30(f). See Section E9.7.
Probes developed and used exclusively in-house, and not sold to other laboratories ("home-brew probes"), are not currently regulated by the FDA. Clinical laboratories using such probes must verify or establish, for each specific use of each probe, the performance specifications for applicable performance characteristics, e.g., accuracy, precision, analytical sensitivity and specificity, etc. (42 CFR §493.1213). A laboratory making its own probes should meet the standards set forth under Section G (Clinical Molecular Genetics).

Probes labeled "for research use only" (RUO) or "for investigational use only" (IUO) are subject to FDA approval but have not been approved by the FDA for clinical use. Laboratories may consider whether such probes may be used under the practice of medicine exemption or an investigative device exemption (IDE). Clinical laboratories using such probes must verify or establish performance standards as provided above. (42 CFR §493.1213). In overview, both probe validation, including sensitivity and specificity, and assay analytical validation, including sensitivity and specificity, must be documented for the use of any molecular probe (42 CFR §493.1213).

E9.1 General Considerations
Regulatory requirements may differ depending on the origin and FDA approval status of molecular probes used in FISH. The FDA regulates manufacturers who market medical devices, including in vitro diagnostic devices and certain reagents, such as molecular probes, used by laboratories to develop in-house tests. CMS (formerly called HCFA), through CLIA '88, regulates laboratories and their practices.

E9.1.2 Indications for use of metaphase FISH include evaluation of:
- marker chromosomes
- unknown material attached to a chromosome
- rearranged chromosomes (including cryptic translocations)
- suspected gain or loss of a chromosome segment
- mosaicism

E9.1.2.1 Under most circumstances, metaphase FISH should be considered an adjunct to conventional cytogenetic studies.

E9.2 Documentation of test or analytic validation is required under CLIA '88 for any new test being placed into clinical service after September 1994. In the present context, a "test" is defined by the specific use of a probe, rather than by the generic "FISH" technology. Subsequent biannual calibration verification of the test system is also required under CLIA '88. (See 42 CFR §493.1217 and Section E9.3.)

E9.2.1 Validation requirements vary with the regulatory status of the test/device (see above). Note that there may be alternative approaches to validation and the following is presented as a consensus method.

E9.2.1.1 Unique sequence FISH probes not approved as Class II or III ASR kits (ASRs, home-brews, IUOs and RUOs) must be validated in two ways:

Probe validation/localization should be confirmed by:

Scoring of a minimum of 5 metaphase cells to verify that each probe hybridizes to the appropriate chromosome target(s) and to no other chromosomes. Care should be taken in evaluation of potential probe contamination, as the contaminating probe may be present in a dilute concentration, thus hybridizing more weakly than the probe of interest.

One of the following methods should be used to determine chromosomal localization:
- inverted DAPI, sequential G-/R-/or Q- to FISH or other banding method;
- use of a cell line containing the region of chromosome of interest as an independently identifiable target on a solid stained chromosome (e.g., structural rearrangements, trisomy, etc.);
- other methods that localize the probe at a level of resolution appropriate to the intended chromosome target.

The source of the metaphase nuclei preparation may be any tissue type as the substrate (matrix) in question is the chromosome.
Localization should ensure that:

- the tested probe is the intended probe;
- no unknown probe is contaminating that lot

Localization should also identify any cross-hybridization inherent to that probe. Probes with significant cross-hybridization should not be used.

**Analytical validation: sensitivity** (percentage of scorable metaphase cells with appropriate number of distinct signals) and specificity (percentage of signals from nontarget sites) must be established for each new probe.

Assays using commercially available probes approved as ASR kits must meet the sensitivity and specificity parameters stated in package inserts provided by the manufacturer.

For probes that are not FDA approved for "in vitro diagnostic use," the laboratory must establish analytical sensitivity, specificity and reportable ranges of results.

Analytical sensitivity and specificity should be established by analysis of the hybridization of the probe to the chromosomes of interest (genomic target). Sensitivity is defined as the percentage of metaphases with the expected signal pattern at the correct chromosomal location. Specificity is defined by the percentage of signals that hybridize to the correct locus and no other location. An adequate number of cells and loci should be scored to ensure that the probe is sensitive and specific for the clinical testing being performed.

**Analytical sensitivity and specificity** should be established by analysis of the hybridization of the probe to chromosomes representing at least 200 distinct genomic targets. For instance, a target sequence for which the hybridization signals from each of the chromatids is separable would require analysis of 50 cells (4 targets per metaphase). If the target sequence is at or near the centromere such that the hybridization signals are not clearly separable, the analysis would require 100 cells (2 targets per metaphase). Cells should be from 5 chromosomally characterized individuals (aneuploid cell lines can maximize target number). Pooling of cells from these 5 individuals (presuming comparable mitotic indices) is acceptable. However, all cells should have the same number of potential targets, if pooled. Discordance may require that the individual cell lines be tested separately. Greater discriminatory power may be needed to distinguish mosaicism.

Comparable analytic sensitivity and specificity must be established for each new lot of probe. This may be accomplished by analyzing a patient sample simultaneously with old and new lots to document consistency.

---

**E9.2.2 Repeat Sequence or Whole Chromosome Probes**

**E9.2.2.1 Probe localization validation** should be as described in Section E9.2.1.1a.

**E9.2.2.2**

- **Analytical sensitivity of:**
  - **whole chromosome probes** is internally controlled and depends on the detection of hybridization to the normal chromosome target(s) in the cell.
  - **repeated sequence probes** is a function of the target size on the chromosome and therefore is both an individual and population based determination. Therefore, care in the interpretation of negative results is required. Hybridization to normal chromosome target sequences provides internal controls.

**repeated sequence probes** should hybridize to nontarget sequences (background signals of intensity greater than or equal to signals at actual targets) in less than 2% of cells. Because specificity is a function of homology to other sequences and the stringency of hybridization, test conditions may need to be modified.
<table>
<thead>
<tr>
<th>Section</th>
<th>Text</th>
</tr>
</thead>
</table>
| E9.2.2.3 | **Analytical specificity** is determined by calculating the proportion of probe bound to the target vs. the proportion bound to other chromosome regions. For analytical specificity, whole chromosome probes should hybridize to nontarget sequences (background signals of greater than or equal intensity to signals at actual targets) in less than 2% of cells.

**Repeated sequence probes** should hybridize to nontarget sequences (background signals of intensity greater than or equal to signals at actual targets) in less than 2% of cells. Because specificity is a function of homology to other sequences and the stringency of hybridization, test conditions may need to be modified. |
| E9.3 | **Biannual (twice per year) calibration or continuous quality monitoring verification** is required (42 CFR §493.1217) in the use of all FISH probes. This can be accomplished through a method of continuous monitoring of test results. For example, important test characteristics to monitor might include: (1) correct number of signals (i.e., no contamination of probe, no degradation of probe) and (2) no excess background or other technical problem that would preclude interpretation. Most likely, these factors are monitored for every FISH assay. However, documentation of this monitoring process is required. |
| E9.4 | **Target tissues** include any tissue from which adequate numbers of dividing cells can be obtained. Although the above analytical validation considerations should be specific to the intended tissue of use, in tests that target metaphase chromosomes as the test matrix, the considerations are no different than those of standard cytogenetic analysis. Particular tissues and/or disease states may lead to chromosomes of poor morphologic quality and possibly to reduced signal intensity. |
| E9.5 | **Processing standards appropriate to particular tissues** are as specified earlier. The impact of slide/cell age on FISH probe hybridization efficiency should be considered. |
| E9.6 | **Analytical Standards**

It is recommended that hybridization test systems (excluding whole chromosome probes) include control probes to tag the chromosome of interest. Such probes also afford a limited level of quality control by providing an internal control of the hybridization efficiency. However, the target sequence on a normal chromosome serves as a better control of hybridization efficiency.

If a probe is used that does not have an inherent control signal (i.e., an X or Y chromosome probe in a male), another sample that is known to have the probe target should be run in parallel with the patient sample.

The following analytical standards for testing presume that sensitivity and specificity are at least 98%. If lower, a corresponding increase in the number of cells scored to attain comparable confidence levels is required. (See Table 1 at end of Section E12.) |
| E9.6.1 | **Cell selection** for analysis should be based on the observed hybridization of the control probe(s) and the target-specific probe to metaphase chromosome(s). Cells showing chromosome-bound background (hybridization signals from nontarget sites) should not be scored. |
| E9.6.2 | Although a single slide is sufficient for analysis, any **suspicion of mosaicism** should be confirmed in cells from an additional slide(s). Additional tissue/cell culture-specific considerations apply. |
| E9.6.3 | For **characterization of nonmosaic marker chromosomes or unknown chromosome regions in derivative chromosomes**, a laboratory that has attained ³ 98% sensitivity of the assay during its internal test validation must analyze a minimum of 5 metaphase cells. |
| E9.6.4 | For **nonmosaic microdeletion analyses**, a minimum of 10 metaphase cells should be analyzed. If any cells are discordant, additional cells from a second slide should be analyzed to attain confidence limits of mosaicism exclusion comparable to that of routine cytogenetic analysis. |
| E9.6.5 | Results should be confirmed by at least two experienced individuals, one of whom may be the laboratory director. |
A minimum of two images (either photographic or digital) should be preserved in the FISH records of the case file.

E9.7 Ordering and Reporting

E9.7.1 Tests using ASRs may be ordered only by physicians and other persons authorized by applicable state law. 21 CFR §809.30(f).

E9.7.2 The manufacturing source and identification of the probe(s) used, (either gene symbol or locus symbol), the number of cells evaluated, and detailed hybridization results must be reported.

E9.7.3 Limitations of the assay must be stated, some of which may be described in the manufacturer's package insert.

E9.7.3.1 Pursuant to 21 CFR §809.30(e), the following specific disclaimer must be included in test reports of all FISH testing using ASRs:

"This test was developed and its performance characteristics determined by [laboratory name] as required by CLIA '88 regulations. It has not been cleared or approved for specific uses by the U.S. Food and Drug Administration."

The wording of the above statement is mandatory and should not be changed. However, because the statement may cause some confusion regarding whether such tests are clinically necessary and reimbursable, laboratories may wish to add clarifying language, such as the following, after the disclaimer:

"The FDA has determined that such clearance or approval is not necessary. This test is used for clinical diagnostic purposes. It should not be regarded as investigational or for research."

Laboratories also may wish to add language such as the following, if accurate:

"Pursuant to the requirements of CLIA '88, this laboratory has established and verified the test's accuracy and precision."

E9.7.4 Metaphase FISH analysis provides information only about the probe locus in question. It does not substitute for complete karyotypic analysis.

E9.7.5 Care must be taken in the interpretation of results from derivative chromosome regions of small size, because current whole chromosome probe cocktails may not be uniformly distributed across the full length of a target chromosome.

E9.7.6 Care must be taken in the interpretation of negative results from studies based on repeated sequence probes, due to rare individuals with small numbers of the repeated sequence target.

E9.7.7 When using metaphase FISH to document a microdeletion in which the probe is not the gene in question, care must be taken in interpreting results.

E9.7.8 Dependent on the robustness of the reference ranges established during analytical validation and the number of cells scored, appropriate comments as to the possibility of mosaicism should be considered.

E9.7.9 If a microduplication is suspected, clinical interpretation must be based, at least in part, on the analysis of interphase nuclei (see E10 and E10.5.8 specifically). In addition, results should be confirmed by alternative methods (e.g., clinical information, molecular analysis) whenever possible.

E10 Interphase/Nuclear Fluorescence In Situ Hybridization

E10.1 General Considerations (see E9.1)

E10.1.1 These Standards and Guidelines are designed for use with standard clinical diagnostic tests using interphase in situ hybridization. These guidelines do not address all possible applications. Applications involving rare tissues (some solid tumors, etc.) should be considered as investigational tests until either more specific guidelines are
established or sufficient experience is acquired to meet these guidelines. These guidelines are not intended to address interphase FISH used in preimplantation genetics or in some fixed tissue section assays.

<table>
<thead>
<tr>
<th>E10.1.2</th>
<th>Applications for use of interphase FISH include evaluation of: • numerical abnormalities • duplication • deletion • rearrangements • sex chromosome constitution • mosaicism • gene amplification (not specifically addressed in this guideline) • Detection of a given chromosomal abnormality is dependent upon availability of an appropriate probe(s).</th>
</tr>
</thead>
<tbody>
<tr>
<td>E10.1.3</td>
<td>Interphase FISH analysis provides information regarding only the specific probe locus used. It does not substitute for complete karyotypic analysis.</td>
</tr>
<tr>
<td>E10.1.4</td>
<td>Abnormalities detected by interphase FISH that are also considered reliably detectable by chromosome analysis should be <strong>confirmed by conventional cytogenetic analysis.</strong></td>
</tr>
<tr>
<td>E10.1.4.1</td>
<td>For disease monitoring purposes, repeated adjunctive chromosomal analyses may not be necessary.</td>
</tr>
<tr>
<td>E10.1.5</td>
<td>Whole chromosome painting probes or whole p arm or q arm probes should not be used for interphase FISH analysis.</td>
</tr>
<tr>
<td>E10.1.6</td>
<td>It is the laboratory director's responsibility to ensure and <strong>document that technologists who perform interphase FISH tests are appropriately trained</strong> and have demonstrated consistent ability to score and interpret these cases. Color blindness testing is recommended.</td>
</tr>
<tr>
<td>E10.1.7</td>
<td>Prior to use of interphase FISH technology in clinical diagnostic cases, the following validation stages must be completed: Probe localization validation (E9.2.1.1a and E10.2) Assay analytical validation through establishment of a database of reportable reference ranges (E10.3). <strong>Assay analytical validation must be completed for each new probe (including control probes within a multiprobe mix).</strong> The extent of validation differs between the various types of FDA-approved probes (analyte specific reagents (ASRs); the traditional Class II and III devices (ASR kits) obtained from commercial companies operating under good manufacturing practices; and the non-FDA cleared probes, those labeled RUO (research use only) and IUO (investigational use only) and home-brew probes. (See E9.1.1.) Data collection for documenting analytical validation and establishing a database of reportable reference ranges is required only prior to the initial transfer of the test into clinical diagnostic use for each specific intended use and cell type. Incompletely validated probes may be used in exceptional clinical situations, but this must be stated in the final report.</td>
</tr>
<tr>
<td>E10.2</td>
<td><strong>Probe Validation (See E9.2)</strong></td>
</tr>
<tr>
<td>E10.2.1</td>
<td>Localization confirmation should follow guidelines for metaphase FISH probe validation described in Section E9.2.1.1a.</td>
</tr>
<tr>
<td>E10.2.2</td>
<td><strong>Probe Sensitivity and Specificity (see E9.2.1.1b)</strong></td>
</tr>
</tbody>
</table>
| E10.2.3 | Probe validation assessments should be based on each probe's performance separately (within the resolution of the different probe signals). The values obtained from...
different probes that are provided together in a cocktail (e.g., probes to 18, X, and Y) should not be averaged to determine probe validation values.

**E10.3** Assay Analytical Validation and Establishment of a Database of Reportable Reference Ranges.

Assay sensitivity and reportable ranges must be set in each laboratory based on the following database collection and analyses and/or statistical analyses. Results from samples used to establish reportable ranges should not be reported as test results.

Validation requirements vary among FDA-approved Class II and III ASR kits in which extensive analytical (and clinical) validation has been performed by the manufacturer. (See E9.1.1a and E9.2.1.)

**E10.3.1** Database collection must be specific for an intended tissue type or cell population. For instance, database collection must distinguish between cultured versus uncultured amniocytes due to known differences of cell populations. This differentiation between tissue types and cell populations is at the discretion of the laboratory director.

The normal database should consist of an adequate number of cells from a group of control individuals (as determined by the director) who do not have abnormalities involving the target (and control) probes. Acceptable normal databases should include at least 500 nuclei each from 20 control samples or 200 nuclei each from 30 control samples. When possible, an abnormal database should be established. This database should be limited to include only samples from individuals who have abnormality(ies) involving the specific target probe(s).

**E10.3.2** E10.3.2 Reportable Reference Ranges [8,9]

Reportable ranges for normal and abnormal results should be based on at least a 95% confidence interval achieved from the appropriate database.

A normal result can be considered valid if the percentage of cells that are normal is within the 95% confidence interval based on the database of normal individuals, or outside the 95% confidence interval (when available) for individuals with the abnormality.

An abnormal result can be considered valid if the percentage of nuclei that are abnormal (e.g., monosomy, trisomy) is outside of the 95% confidence interval based on the database of normal individuals, or within the 95% confidence interval (when available) for individuals with the abnormality.

It should be noted that, while assays intended to detect nonmosaic abnormalities should have an analytical sensitivity equal to or exceeding 95%, as assay sensitivity increases, fewer cells may be evaluated (see Table 1).

**E10.3.2.1** Detection of mosaicism should be based on results from a normal database. Quantitation of mosaicism can be determined by mixing of cells at known proportions from individuals with abnormalities and those without, and this may be informative as to the limitations of mosaicism identification. However, proportions of abnormal cells falling between the 95% confidence levels for normal and abnormal likely reflect true mosaicism.

To detect mosaicism >20%, probe analytical sensitivity should equal or exceed 95%. If detection of lower levels of mosaicism is desired, a higher analytical sensitivity percentage may be necessary to reach a comparable confidence interval (see Table 1).

**E10.4** Biannual (twice per year) calibration or continuous quality monitoring is required to ensure that assay analytical sensitivity and specificity remain at the levels established during initial validation. This can be accomplished through a method of continuous monitoring of test results (i.e., no contamination of probe, no excess background, results falling in reportable ranges). Most likely, these factors are monitored for every FISH assay (see Section E9.3); however, the process must be documented at least biannually.

Any test results that fall outside of the reportable range should be repeated. Continual test results that are outside of reportable ranges should initiate re-assessment of the test
system. Documentation of this monitoring process is required.

E10.5 Analytical Standards

The following analytical standards for testing presume that the probe analysis sensitivity is at least 95% for those probes not intended to be used for mosaicism detection. See Table 1 to determine the numbers of cells needed to reach a 95% confidence level based on the analytical sensitivity of the assay in the laboratory.

E10.5.1 **Selection of cell nuclei** for analysis should be based on the observed hybridization of the probe(s). If utilizing a control probe, score only nuclei with the expected number of control probe signals.

Nuclei that are broken, overlapped, or have significant background "noise" should not be scored.

E10.5.2 If more than one probe is used simultaneously, different fluorochrome colors must be used to allow differentiation.

E10.5.3 As noted in section E9.2.2.2, care must be exercised in the interpretation of results from studies based on repeated sequence probes. Although rare, individuals exist who have a low copy number of a repeat on one homolog. This could result in misleading results due to reduced hybridization and/or signal intensity.

E10.5.4 Interphase FISH analyses attempting to detect mosaicism should be interpreted cautiously at the discretion of the laboratory director.

E10.5.5 The presence of contamination by maternal cells (in prenatal cases), bacteria, or fungus can lead to false positive or negative results. Routine processes to identify these contaminants are recommended, such as evaluating spun pellet for visible blood, which can indicate maternal cell contamination, or evaluating slides for nonspecific background signals that could indicate fungal or bacterial contamination.

E10.5.6 All analyses must be performed and/or evaluated by at least two qualified individuals (one of whom can be the laboratory director). The degree to which the scoring should be split between these individuals is at the discretion of the laboratory director.

E10.5.7 For analysis of constitutional numerical abnormalities or microduplications, a minimum of 50 total nuclei (25 per reader) should be scored.

If scores from the two individuals are significantly discrepant, a third analysis by another qualified individual should be pursued or the test repeated.

If a result does not meet laboratory established reporting ranges, the study should be repeated. If no additional material is available, a third analysis (at least 50 nuclei) by a qualified individual can be performed in an attempt to account for questionable results (e.g., potential poor hybridization/background on a portion of the slide).

If mosaicism is suspected for a constitutional abnormality, the number of nuclei scored should be sufficient to have ruled out with 95% confidence a result of nonmosaic normal, or abnormal, if clinically relevant (see Table 1).

E10.5.8 Interphase FISH may be used as an adjunctive test to assess levels of mosaicism/chimerism of cell lines with abnormalities previously established by standard banded chromosome and/or metaphase FISH analysis.

For analysis of acquired numerical abnormalities or microdeletions/duplications, the total number of nuclei scored should be sufficient to have excluded with 95% confidence either nonmosaic normal or abnormal (see Table 1).

For a given study, if presence of the abnormality is clearly detectable within the analysis of a total of 50 nuclei by two independent readers, it is up to the director's discretion to limit the study and report the results provided the reportable range used is based on a database of a similar number of analyses.

If repetitive sequence probes are used, an initial scoring of 10 metaphase cells for intensity of signal in the individual to be tested is recommended (see E10.5.4).
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E10.5.9</td>
<td>For analysis of acquired abnormalities, a minimum of 50 total nuclei (25 per reader) should be scored per probe. If an abnormal cell line is suspected, an appropriate number of nuclei should be scored (see Table 1).</td>
</tr>
<tr>
<td>E10.6</td>
<td>A minimum of two images (either photographic or digital) should be preserved for the records of each case.</td>
</tr>
<tr>
<td>E10.7</td>
<td>Ordering and Reporting</td>
</tr>
<tr>
<td>E10.7.1</td>
<td>Interphase FISH ordering and reporting standards and guidelines should follow those articulated in Section E9.7 for metaphase FISH. In addition, the following require special consideration.</td>
</tr>
<tr>
<td>E10.7.2</td>
<td>When using interphase FISH to detect a microdeletion or microduplication in which the probe is not the gene in question, in cases where a normal result is found, a disclaimer should be included in the written report stating the limitation of the test. Such a disclaimer may include information as given in the following example: &quot;The probe used, however, may give a normal result in cases that are due to very small deletions, point mutations or other genetic etiologies.&quot;</td>
</tr>
<tr>
<td>E10.7.3</td>
<td>Interphase FISH results that require confirmation by conventional chromosome analysis can be released prior to the cytogenetic analysis, although the results should be considered preliminary (see E10.1.4.1).</td>
</tr>
<tr>
<td>E10.8</td>
<td>Special Case Interphase Analysis: It is recognized that technology and probe development may proceed at such a rapid pace that the Standards and Guidelines may not specifically address all situations in entirety. It is the laboratory director's responsibility to ensure quality assurance and proper pre- and post-analytical practices that are consistent with the general guidelines presented here.</td>
</tr>
<tr>
<td>E10.8.1</td>
<td>Paraffin-embedded samples: Pre-hybridization specimen handling, including fixation, block cutting, deparaffination and protein digestion, are critical parameters that affect results and results interpretation. Each of these factors must be optimized for tissue type and preparation method.</td>
</tr>
<tr>
<td>E10.8.1.1</td>
<td>Selection of nuclei for analysis should be based on location of cells of interest (e.g., if there are cancer cells and normal stroma on the same section, caution must be taken to score the appropriate cell type) and optimal hybridization of the probe. When the tissue to be scored is &quot;tumor tissue&quot; (to rule out the presence of a malignancy-associated rearrangement) a Pathologist must review the slides and identify the appropriate tissue to score for the Cytogenetics Laboratory.</td>
</tr>
<tr>
<td>E10.8.1.2</td>
<td>Signal scoring should involve focusing through the entire section to detect signals in different planes. The number and location of nuclei to be scored may vary due to the number of cells available in each tissue section. Scoring criteria for FDA-approved devices supercede those presented here.</td>
</tr>
<tr>
<td>E10.8.1.3</td>
<td>Appropriate control probes may not be readily available (e.g., amplification controls). In such cases, a negative (e.g., no amplification) and a positive (e.g., known amplification) control sample should be included in the analytical process.</td>
</tr>
<tr>
<td>E11</td>
<td>Multi-Target FISH Tests</td>
</tr>
<tr>
<td>E11.1</td>
<td>General Considerations</td>
</tr>
</tbody>
</table>
| E11.1.1 | Multi-target FISH tests include assays developed to analyze multiple chromosome loci (>2) in a single test format. Multi-target FISH tests provide information regarding only the specific probe loci used. This testing does not substitute for complete karyotype analysis. Abnormalities detected by multi-target FISH should be confirmed by another methodology whenever possible (e.g., cytogenetic G-banding, locus-specific FISH). It is the laboratory director's responsibility to ensure and document that technologists
who perform multi-target FISH tests are appropriately trained and have demonstrated consistent ability to score and interpret these tests.

E11.2  Probe Validation

Probes used in these tests should be validated for localization, sensitivity and specificity as described in Section E9.2.1.a.

Multi-target FISH tests that involve interphase FISH analysis should follow the previous guidelines given for interphase FISH probe validation, including establishing databases and reportable reference ranges (E10.2.2-E10.4), as well as biannual calibration.

Multi-target FISH testing on metaphase cells should generally follow the validation requirements outlined in E9.2.1.a.

E11.3  Analytical Standards

Probes used for interphase multi-target analysis will require database generation to establish reportable ranges. Refer to the guidelines on interphase FISH for these tests (E10.3 and E10.5).

Probes used for metaphase analysis should follow metaphase FISH analytical standards (E9.6):

For nonmosaic metaphase analysis, a total of 5 cells should be evaluated for repetitive and painting probes.

For nonmosaic metaphase analysis, at least 5 cells should be evaluated for the majority of the unique sequence probes, whenever possible.

At least 30 metaphase cells should be studied for evaluation of mosaicism.

Image documentation should consist of at least one image for each locus or combination of loci (either photographic or digital) to be preserved for the records of the case. All abnormalities detected with one or more of the probes should be documented by at least one additional image.

E11.4  Ordering and Reporting

Follow the previous FISH reporting guidelines (including the mandatory FDA statement). For situations in which nomenclature has not been specifically addressed by ISCN 2005, and in which the reporting of all normal loci could be confusing for clinicians (e.g., subtelomere analysis), the director may establish a consistent and clinically meaningful reporting mechanism. This shorthand nomenclature or other reporting format should be documented in the laboratory standard operating procedures.

E12  (See references)

E13  Microarray Analysis for Constitutional Cytogenetic Abnormalities

E13.1  General considerations

Constitutional cytogenetic abnormalities include aneuploidy (extra or missing chromosomes) and structural aberrations (deletions, duplications, translocations, inversions, marker chromosomes). Microarray analysis by CGH-based or SNP-based arrays can detect DNA copy number gains and losses, thereby identifying unbalanced chromosome abnormalities, including unbalanced microscopic and submicroscopic abnormalities.

This technique cannot detect balanced rearrangements, some ploidy changes, and mutations (nucleotide base pair changes). Uniparental disomy cannot be detected by CGH-based arrays but may be uncovered using SNP-based arrays.

Low-level mosaicism for unbalanced rearrangements and aneuploidy may not be detected by array CGH. Each laboratory must determine the sensitivity of the
microarray for detection of mosaicism. Genomic microarrays typically consist of pieces of DNA attached to a solid support (e.g., glass microscope slide). Depending on the design of the array, the extent to which individual clones or probes on the array can be validated, and the clinical indication for testing, an array may be used either as an adjunct to more established testing methods, such as routine chromosome analysis and targeted FISH assays, or as a primary diagnostic tool for detecting chromosomal abnormalities.

The sensitivity of this test depends in part on the number, distribution, and size of the clones/probes on the array and the algorithmic parameters used in the data analysis.

### E13.2 Validation

Various types of microarrays may be used for the diagnosis of chromosome abnormalities in a clinical laboratory: those that are U.S. Food and Drug Administration (FDA) approved and available through a commercial source, IUO (investigational use only) or RUO (research use only) microarrays, which are commercially available and validated within the laboratory that intends to use them as a clinical tool, and those that are “home-brew,” developed within the laboratory that intends to use them as a clinical tool. Depending on the intended clinical application of the array, each of the types of arrays may have different levels of validation.

#### E13.2.1 FDA-approved commercially available microarrays to be used as adjunctive or as a replacement to established cytogenetics and FISH

[Note: At the time of the publication of these Guidelines, there are no commercially available FDA-approved microarrays for this application. However, laboratories are advised to keep abreast of new developments in this rapidly developing technology.]

For FDA-approved arrays that are used to replace established methodologies (e.g., traditional cytogenetic analysis, FISH), the laboratory should run a series of normal and abnormal controls to establish performance parameters of the array (E13.2.4.1). To the extent possible, the laboratory should use abnormal controls that represent abnormalities that the array is designed to detect.

For FDA-approved arrays that are to be used as adjuncts to traditional cytogenetic analysis and clinically targeted FISH assays, validation may be accomplished by running a series of normal controls and abnormal controls that in combination address the regions of the genome represented on the array.

The method and scope of validation must be documented within the laboratory and the concomitant limitations of the analysis must be described in the laboratory report.

#### E13.2.2 IUO or RUO commercially available microarrays to be used as adjunctive or as a replacement to established cytogenetics and FISH.

For IUO/RUO arrays that are used to replace established methodologies (e.g., traditional cytogenetic analysis, FISH), the laboratory should aim to validate each clone or region on the microarray using at least one reference DNA with a known abnormality for each disorder represented on the array.

For IUO/RUO arrays that are to be used as adjuncts to traditional cytogenetic analysis and clinically targeted FISH assays, validation may be accomplished by running a series of normal controls and abnormal controls that in combination address the regions of the genome represented on the array.

The method and scope of validation must be documented within the laboratory and the concomitant limitations of the analysis must be described in the laboratory report.

#### E13.2.3 Home-brew microarrays to be used as adjunctive or as a replacement to established cytogenetics and FISH
For home-brew arrays that are used to replace established methodologies (e.g., traditional cytogenetic analysis or FISH), the laboratory should ideally FISH map each clone to verify cytogenetic map location before constructing the array. Alternatively, the laboratory may provide additional evidence to verify clones through end-sequencing or PCR amplification of genes of interest.

The laboratory should attempt to validate each clone or region on the microarray using at least one reference DNA with a known abnormality for each disorder represented on the array.

The method and scope of validation must be documented within the laboratory and the concomitant limitations of the analysis must be described in the laboratory report.

### E13.2.4 Demonstration of expertise and validation of a new technology in the laboratory

In addition to validation of the various types of microarrays, the laboratory must demonstrate expertise in array performance and analysis.

The following steps must be conducted by the laboratory before offering diagnostic testing with any of the types of microarray (e.g., FDA approved, IUO/RUO, home-brew). This process is necessary whether the laboratory is using microarrays for the first time, changing microarray platforms (e.g., BAC arrays, oligonucleotide arrays), or changing microarray manufacturers.

#### E13.2.4.1 Validation of new microarrays (new platform or new manufacturer)

New microarrays to the laboratory should be validated with DNA representing as many chromosomal abnormalities as possible to obtain the experience and confidence needed to perform microarray testing. A minimum of 30 different chromosomally abnormal specimens should be tested.

In addition, the laboratory should exchange five samples (normal or abnormal in a blinded fashion) with another reference laboratory providing microarray testing. After this initial validation period, new microarray lots can be validated as described in E13.2.4.3.

#### E13.2.4.2 Validation of a new (version of a) microarray established in the laboratory

This validation does not apply to new platforms from the same manufacturer (see E13.2.4.1). The intent of this validation is to validate an enhanced (new version) of a microarray that has been previously validated for diagnostic use in the laboratory. The laboratory should validate a new microarray (e.g., enhanced or new version) using five abnormal specimens to assess accuracy of performance. If possible, the abnormal specimens should reflect the additional regions of the genome or syndromes on the new microarray under validation.

#### E13.2.4.3 Validation of a new lot of the same microarray established in the laboratory

The intent of this validation is to reasonably assess that the new lot performs as the previous lot. The manufacturer should supply documentation of the quality control comparison between lots. In addition, each new lot should have documentation by the manufacturer that printing of each clone was successful (visualization of spots using a DNA-specific stain) for BAC arrays or synthesis of each oligonucleotide was successful (synthesis verification) for oligonucleotide arrays. For new lots, one (preferably) abnormal specimen is repeated on the new lot and compared with the result from the old lot to establish equivalency.

In addition, it is advisable that each new lot of a microarray be validated with two samples from chromosomally normal individuals (these may be the control samples) to identify: problems in the printing (poor spots) of clones or synthesis of oligonucleotides, problems in reagents (poor target DNA quality, suboptimal reagents), or problems in the analysis.

#### E13.2.5 Proficiency testing
The laboratory should establish internal proficiency testing of normal and abnormal specimens for microarray analysis as part of the laboratory’s internal quality assurance program and ongoing quality improvement program. In the absence of the availability of an external proficiency program for microarray analysis by a deemed entity (e.g., CAP), the laboratory should establish external proficiency testing of normal and abnormal specimens for microarray analysis as part of its quality assurance program and ongoing quality improvement program. Deidentified normal and abnormal DNA specimens should be exchanged (in a blinded fashion) with another reference laboratory performing microarray testing. Alternatively, DNA specimens could be received from an outside cytogenetics laboratory and correlation between the cytogenetic or FISH results and the microarray results may be sufficient to provide ongoing proficiency. Proficiency testing should include both normal and abnormal specimens and should be performed according to CLIA ’88 guidelines. When an external proficiency testing program becomes available through an appropriate deemed organization, the laboratory should be encouraged to enroll and participate in all relevant challenges.

Documentation of participation and the performance results of all internal and external proficiency tests must be retained by the laboratory and made available to all accreditation agency inspectors.

**E13.3 Analytic standards**

Monitoring preanalytical variables, analytical variables, and postanalytical variables should be part of the laboratory’s quality assurance and quality improvement programs. Such variables may include quality of the specimen received, number of hybridization failures, and number of cases requiring parental studies.

**E13.3.1 Array design**

The performance of microarray analysis in detecting chromosome aberrations is dependent on the resolution of the array, which is limited by the size of the DNA targets and the distance between those sequences naturally located on the chromosome. BAC microarrays typically have cloned DNA targets with an average size of 160 Kb, whereas oligonucleotide probes range in size from 25mers to 75mers.

Microarrays may be developed for specific regions of the genome (targeted genomemicroarrays) for detection of unbalanced subtelomeric rearrangements, aneuploidy, particular microdeletion/microduplication syndromes, or a genomewide array with a specified distribution/spacing of clones/ probes (for example, 1Mb array; one oligonucleotide/SNP per 300 kb array), or microarrays may be developed for the entire genome using overlapping (contiguous) clones (whole genome microarrays) or high-density oligonucleotides. The coverage of the various commercial oligonucleotide arrays currently range in size from one probe per 6 kb to one probe per 70 kb.

The microarray targets may include large insert clones [BACs, P1-derived artificial chromosomes (PACs)], smaller insert clones (cosmids, fosmids, plasmids), or smaller DNA segments (oligonucleotides, cDNA). Yeast artificial chromosomes are generally not recommended because they are often chimeric and would have suboptimal utility in diagnostic applications. Multiple clones/probes representing any single clinically relevant locus provide additional diagnostic information and should be included on the array. Comparison of the microarray results among these clones/probes at a single locus may provide a more sensitive assay. Contiguous clones/probes spanning a large region provide supplementary diagnostic information regarding size of deletions or duplications as an adjunct to clinical and other laboratory findings. The laboratory should establish the number of clones/probes used to determine an abnormal threshold.

**E13.3.1.1 Considerations for BAC array design**

If possible, each clone should be represented multiple times on the microarray (minimum of two to four times) and preferably in more than one location on the microarray to increase test reliability and reduce uninformative or false-positive/-negative results due to technical artifact. The array should contain a set of normalization clones for which a normal ratio is established after each microarray analysis. The laboratory should determine and document the method for normalization. In some instances, this may be provided by the manufacturer. Clones may be selected...
from available public databases and obtained through a public or private source. Regardless of whether the microarray is a home-brew or manufactured by a commercial entity, the identity of each clone should be verified through end-sequencing, FISH localization, or some other comparable method. This verification should be documented within the laboratory quality control plan for home-brew arrays or provided to the end user by the manufacturer for commercially manufactured arrays. The laboratory director should make every effort to obtain documentation of clone validation from the manufacturer.

Clones not mapping to the proper/intended locus should be discarded from the array or omitted from the final laboratory analysis.

E13.3.1.2 Considerations for oligonucleotide array design

Oligonucleotide arrays should be designed with consideration of the statistical algorithms that will subsequently be used for determining abnormal thresholds. The number of probes within a given region of interest (e.g., within a region known to be associated with a clinical syndrome) should be sufficient such that the assay will have the sensitivity necessary for detection of an abnormality. For example, if applying an algorithm that requires 10 oligonucleotides within a 0.3 Mb region to fall outside of the normal ratio as a cutoff, the laboratory director needs to ensure that there is a minimum of 10 oligonucleotides within the critical region to detect a deletion or duplication therein. For oligonucleotide arrays that are not customized for the laboratory, it is essential that the director familiarize himself/herself with the coverage on the array; even arrays that have an “average” spacing of as little as 10 kb may have significant gaps that are clinically relevant. There should also be redundancy of a subset of probes on the array, with the redundant probe sequences being represented at more than one location on the microarray. The oligonucleotide CGH arrays should contain a set of normalization probes (e.g., negative spots that should not hybridize to human DNA and positive saturated spots) for which a normal ratio is established for each analysis. The laboratory should determine and document the method for normalization. In some instances, this may be provided by the manufacturer. For SNP arrays, a set of control probes should be included on the array for the purpose of determining a threshold for abnormal results.

Probes selected from the public domain should be listed with their physical and cytogenetic positions on the human genome. All probe descriptions and annotations should be openly accessible. Details regarding design and synthesis verification as well as all quality control steps taken to validate and assess the performance/reproducibility of the array should be documented and provided by the manufacturer.

E13.3.2 Specimen requirements

E13.3.2.2 DNA requirements and processing

The laboratory should establish the minimum DNA requirements to perform the test. Considerations include whether the test is performed once per specimen or, for array CGH, performed twice in a “dye-swap” or “dye-reversal” strategy. The laboratory should have written procedures in the laboratory procedure manual and/or quality management program for DNA extraction and labeling, DNA quantification (e.g., fluorometer, spectrophotometer), obtaining adequate quality and concentration of DNA (e.g., examination by gel electrophoresis), proper fragmentation (e.g., via sonication or digestion), and adequate fluorescent labeling (e.g., examination by gel electrophoresis, visual inspection, ultraviolet/visible spectroscopy).

The laboratory should have documentation of these parameters in each patient record.

E13.3.2.3 Suboptimal samples

If a sample does not meet requirements of the laboratory and is deemed suboptimal, the recommended action is to reject the specimen and request a repeat. If obtaining a repeat specimen is not possible, whole genome amplification could be considered if the laboratory is experienced in this technique and if the potential biases inherent in the technique are detailed in the report so that the physician and patient are informed of the limitations of this technique. Written standards describing when and how the whole genome amplification procedure is performed should be incorporated into the laboratory manual.
<table>
<thead>
<tr>
<th>E13.3.3</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13.3.3.2</td>
<td>Establishing controls</td>
</tr>
<tr>
<td>It is recommended that the laboratory establish both male and female control samples and guidelines that detail whether they are to be used in mismatched (opposite sex) or same-sex comparisons.</td>
<td></td>
</tr>
<tr>
<td>The laboratory should establish whether single male and single female specimens will serve as controls or whether combined pools of multiple male and/or multiple female DNAs will be used as controls. The limitations of both approaches should be understood by the laboratory and taken into consideration in the interpretation of results.</td>
<td></td>
</tr>
<tr>
<td>In most instances, controls with presumably normal karyotypes are used. However, there may be instances when use of a control with a particular abnormal karyotype might be appropriate.</td>
<td></td>
</tr>
<tr>
<td>E13.3.3.3</td>
<td>Validating new lots of controls</td>
</tr>
<tr>
<td>Each new control DNA or new lot of prepared control DNA should be compared by microarray analysis to the previous lot of control DNA.</td>
<td></td>
</tr>
<tr>
<td>E13.3.3.4</td>
<td>Controls for specific clinical situations</td>
</tr>
<tr>
<td>The laboratory must establish whether the same controls will be used for all hybridizations or whether different controls will be used for each hybridization. At this time, some laboratories prefer to use individual male and female controls, and some prefer to use pooled male and pooled female DNA as controls. Some laboratories prefer same-sex controls, and some prefer opposite-sex controls for microarray hybridizations. Any of the above strategies would be acceptable. The laboratory should document the specific rationale for choosing certain strategies and have provisions to accommodate the various disadvantages for one strategy versus another for certain situations. Considerations include CNVs carried by the individuals used as controls and whether opposite-sex or same-sex controls are used. Opposite-sex controls provide a built-in “abnormal” result, which may be helpful in assessing the quality of the microarray results through assessment of dose differences between the X and Y chromosomes. Same-sex controls may offer better sensitivity for detecting gains or losses of regions on the X and Y chromosomes. However, for some suspected sex chromosome and ploidy abnormalities that may be difficult to distinguish by microarray analysis, the laboratory may choose to perform both same-sex and opposite-sex control hybridizations or may choose to confirm the results by FISH or cytogenetic analysis.</td>
<td></td>
</tr>
<tr>
<td>E13.3.4</td>
<td>Microarray data analysis</td>
</tr>
<tr>
<td>E13.3.4.1</td>
<td>BAC arrays</td>
</tr>
<tr>
<td>The laboratory should apply statistical methods to assess the ratio of the control DNA specimen to the patient DNA specimen for each clone. When the clones are spotted more than once on the array, the ratios of the control DNA specimen to the patient DNA specimen can be averaged across identical spots. The statistical methods used should be described in the laboratory’s procedure manual.</td>
<td></td>
</tr>
<tr>
<td>Normalization should be used to establish the baseline ratio of control DNA specimen to the patient DNA specimen before the analysis of the microarray.</td>
<td></td>
</tr>
<tr>
<td>The laboratory must establish the normal and abnormal reference ranges (nullisomy, single-copy loss, single-copy gain, and amplification) for each region of the genome represented on the microarray using normal control DNA specimens for validation. The abnormal ranges should be tested using representative known DNA from chromosomally abnormal individuals or cell lines that carry aberrations for gains and losses anticipated in the construction of each particular microarray. The laboratory must establish methods to present the data obtained from the ratios of the control DNA specimen and the patient DNA specimen. Array data are often depicted as numerical values or graphically represented. Low-level mosaicism for unbalanced rearrangements and aneuploidy may not be detected by array CGH. Thus, each laboratory should either determine the sensitivity of the microarray for detection of mosaicism or should</td>
<td></td>
</tr>
</tbody>
</table>
identify this limitation in the patient report.

**E13.3.4.2 SNP arrays**

The laboratory should apply statistical methods to assess the likelihood that a given region of SNP oligonucleotide probes is gained or lost. Signal intensity values from replicate probes may be combined and reported as a single value for each probe sequence. Various algorithms and software exist for determining DNA copy number changes, and a single algorithm/software package should consistently be used for all microarray validations. When switching to an alternate algorithm/software package, reanalysis of the data used for initial validation should be performed to demonstrate comparable performance. Normalization of the data should be considered as it may potentially eliminate technical biases attributable to factors such as differences in scanners, operators, and replicates. The laboratory must establish the normal and abnormal values that indicate the copy number state (nullisomy, single copy loss, single copy gain, and amplification) for each probe/region of the genome represented on the microarray using normal control DNA specimens for validation. The laboratory should establish the number of clones/probes used to establish a result and determine an abnormal threshold. The abnormal values should be tested using representative known DNA from chromosomally abnormal individuals or cell lines that carry aberrations for gains and losses anticipated based on the design and construction of each particular microarray.

The laboratory must establish methods to present the data obtained from the comparison of the intensity values of the normal control reference set and the patient DNA specimen. Array data are often depicted as numerical values or graphically represented.

Low-level mosaicism for unbalanced rearrangements and aneuploidy may not be detected by microarray analysis. Thus, each laboratory should either determine the sensitivity of the microarray for detection of mosaicism or should identify this limitation in the patient report.

**E13.3.4.3 Oligonucleotide arrays**

The laboratory should apply statistical methods to assess the ratio of the control DNA specimen(s) to the patient DNA specimen for each oligonucleotide/probe. The laboratory director must be familiar with the principles of the software program being applied. Specifically, the director must know how an outlying value on one oligonucleotide/probe affects the ratio generated for a neighboring oligonucleotide/probe. Critical to the analysis will be the rule (sometimes called “filters”) that are used in the analysis to determine that there is a significant gain or loss of a chromosomal region. Some manufacturers provide standard rules or filters to set the cutoff points. However, the laboratory should rigorously test the rules or filters as part of the validation of the assay. For example, one standard filter that might be used as a criterion for an abnormal result could be 10 oligonucleotides within a 0.5-Mb region with a minimum absolute ratio value (based on a log 2 ratio) of 0.3. Depending on the density of the array across each region, that rule may be too stringent or too lenient. Thus, it may be necessary to adjust depending on the region being analyzed. Importantly, the moving average across each chromosome should be carefully examined. This is particularly true for cases that are mosaic, which will typically generate absolute ratio values less than that expected for a nonmosaic case and thus may fall below the standard cutoff value. However, such mosaicism may be apparent upon examination of the running averages. Each laboratory should determine the sensitivity of the microarray for detection of mosaicism or should identify this limitation in the patient report.

**E13.3.5 Interpretation: copy number variants (CNVs)**

The laboratory director and staff should be familiar with benign CNVs or CNVs of unknown clinical significance on the array as well as the expanding current literature and databases on the more commonly reported CNVs and should interpret the microarray analysis in this context. The laboratory director should also consider the possibility that a clinically significant abnormality can occur in known variant regions. These may include deletions and duplications encompassing a known CNV or may represent a homozygous alteration of a CNV that would otherwise be benign when
heterozygous. CNV databases have been established to assist the laboratory director in making decisions about gains and losses detected by microarrays. Examples of such databases are the Database of Genomic Variants (http://projects.tcag.ca/variation/) and DECIPHER (http://www.sanger.ac.uk/PostGenomics/decipher/)

The laboratory must have a written procedure to determine whether any regions covered by clones or probes on the array represent known regions of benign CNVs within the genome. This may include documentation of established polymorphic databases from the laboratory and/or other institutions. It is important that, with the exception of purported benign CNVs, all regions showing abnormal copy number findings be characterized by either FISH, parental studies, quantitative polymerase chain reaction (PCR), or one of the methods noted above, and the laboratory director be familiar with the rapidly changing literature on the location and frequency of these CNV sites.

<table>
<thead>
<tr>
<th>E13.3.6</th>
<th>Confirmation of abnormal microarray results</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13.3.6.1</td>
<td>Clone identity</td>
</tr>
<tr>
<td>A justified concern about microarray analysis is confirmation that each spot represents the correct clone. It is nearly impossible to validate each clone once the array is printed. Validation can be achieved if the laboratory has DNA from samples with abnormalities with copy number changes for each clone or locus (region) represented on the microarray. The laboratory director and staff should be acutely aware of any discrepancies in array data potentially attributable to incorrect identification of a clone on a microarray. Discrepancies can be addressed through FISH with the clone in question to metaphase cells of the patient showing the potentially anomalous result.</td>
<td></td>
</tr>
</tbody>
</table>

| E13.3.6.2 | Probe identity |
| Oligonucleotide probes are synthesized by using information from the public domain. The loci used need to be well documented by Human Gene Mapping Workshop, Geneatlas, the Genome Database, or publication in the peer-reviewed scientific literature. Given that many thousands of probes are printed on the array, it is virtually impossible to validate each probe once the array is printed. The manufacturer should have vigorous protocols for verifying that synthesis was successful. Although very unlikely, the laboratory director and staff should be acutely aware of any discrepancies in array data potentially attributable to incorrect physical assignment of a probe sequence on the human genome and thus on the microarray. Because current analysis strategies typically consider more than one oligonucleotide for determination of true gains and losses, the effects of an incorrectly assigned oligonucleotide may be negligible. However, should a discrepancy become apparent, the oligonucleotide in question should be excluded from the analysis. |

| E13.3.6.3 | Confirmation of abnormal or ambiguous results |
| The laboratory should establish a protocol that allows for confirmation of abnormal or ambiguous microarray results. This may include cytogenetic analysis using banding techniques, FISH, PCR, or other comparable methods. |

| E13.3.6.3.1 | BAC arrays |
| Most cytogenetic laboratories may find the use of FISH, with the abnormal clones identified from microarray analysis, as the method of choice for confirmation. The laboratory director must ensure that confirmation can be established and, if confirmation is to be performed by FISH, that clones are available to the laboratory in a timely manner to maintain clinically relevant turnaround times. The laboratory should have a written policy in place that deals with discrepant results, i.e., when an array shows an abnormality that cannot be confirmed by FISH (the sensitivities of these two tests may differ). BACs (or other microarray targets) used to confirm microarray results should be validated in the same way that comparable FISH probes or other DNA reagents are validated for clinical use. See E9.2.1.1 of these Standards and Guidelines. This validation can be conducted simultaneously with analysis of the patient’s metaphase or interphase cells. |
The director may choose to use a probe for confirmation analysis that has been previously validated in the laboratory, if available for the particular region on the microarray showing the abnormality.

During the FISH confirmation of an abnormal microarray result, a control probe may be used in another region on the same chromosome involved in the abnormality. However, if a control probe is not used, another method for the unequivocal identification of the chromosomes may be used (e.g., inverted 4',6-diamidino-2-phenylindole images).

E13.3.6.3.2 Oligonucleotide arrays

Most cytogenetic laboratories may find the use of FISH as the method of choice for confirmation of abnormal regions. Clones that span the region to be confirmed can be chosen by using publicly available databases. The laboratory director must ensure that confirmation can be established and, if confirmation is to be performed by FISH, that clones are available to the laboratory in a timely manner to maintain clinically relevant turnaround times. The laboratory should have a written policy in place that deals with discrepant results, i.e., when an array shows an abnormality that cannot be confirmed by FISH (the sensitivities of these two tests may differ). BACs (or other microarray targets) used to confirm microarray results must be validated in the same way that comparable FISH probes are validated for clinical use. See E9.2.1.1 of these Standards and Guidelines. This validation can be conducted simultaneously with analysis of the patient’s metaphase or interphase cells.

The director may choose to use a probe for confirmation analysis that has been previously validated in the laboratory, if available for the particular region on the microarray showing the abnormality.

During the FISH confirmation of an abnormal microarray result, a control probe may be used at another region on the same chromosome involved in the abnormality. However, if a control probe is not used, another method for the unequivocal identification of the chromosomes may be used (e.g., inverted 4',6-diamidino-2-phenylindole images).

Alternate molecular methods such as quantitative PCR and multiplex ligation-dependent probe amplification may be used for confirmation, and the use of these techniques is also subject to clinical validation (See G5).

E13.3.6.4 Parental/family studies

Parental studies or studies of other family members may be indicated after the identification of some chromosome abnormalities. The laboratory director must evaluate the microarray analysis results and confirmatory test results and determine the best method for parental studies (chromosome analysis, FISH, or microarray analysis). See E13.4.2.4 for general recommendations about when parental studies should be performed.

It will be critical to have relevant clinical data on the patient and family members for interpretation of these follow-up studies.

E13.4 Ordering and reporting

E13.4.1 Test ordering

It is at the discretion of the clinician in consultation with the laboratory director as to whether microarray analysis is appropriate for any particular patient. Whether any specific array should be used as a primary diagnostic tool or as an adjunct to other types of testing including traditional cytogenetic analysis, FISH, or molecular analysis is dependent on the extent to which the loci on the array have been validated and the clinical situation. When the clinical suspicion is high that a patient has a disorder that is presumably diagnosable by the array, and that locus on the array has not been validated, and an established clinical assay is readily available, the array should not be used as a primary diagnostic tool.

The laboratory director is responsible for describing both the advantages and limitations of testing with any particular array so that the clinician can make an
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13.4.2</td>
<td>Reporting standards</td>
</tr>
<tr>
<td>E13.4.2.1</td>
<td>Turnaround times</td>
</tr>
<tr>
<td></td>
<td>The laboratory should have written standards for microarray analysis test prioritization and turnaround times that are based on the indication for referral (such as newborn with congenital anomalies, child with mental retardation). These turnaround times should be clinically appropriate.</td>
</tr>
<tr>
<td>E13.4.2.2</td>
<td>Clinical relevance of results</td>
</tr>
<tr>
<td></td>
<td>All results should, to the extent possible, be interpreted in the context of other clinical and laboratory findings.</td>
</tr>
<tr>
<td>E13.4.2.3</td>
<td>Limitations of testing</td>
</tr>
<tr>
<td></td>
<td>Current microarray analysis technologies will detect only gains and losses of chromosomal segments. Thus, a normal microarray result does not exclude mutations (nucleotide base pair changes) in any gene represented on the microarray and does not exclude a balanced rearrangement or epigenetic events (e.g., uniparental disomy or imprinting mutations). Additional testing may be appropriate for certain syndromes or conditions when the microarray analysis yields normal results.</td>
</tr>
<tr>
<td>E13.4.2.4</td>
<td>Follow-up studies</td>
</tr>
<tr>
<td></td>
<td>FISH or microarray studies of parents or other family members should be recommended when an abnormality is identified by microarray analysis. In some cases, such follow-up testing is performed to exclude a balanced parental rearrangement or the possibility that a parent has a deletion or duplication. In other cases, parental testing is needed when gains or losses are detected by microarray analysis in regions of the genome with unclear clinical significance. In these cases, parental studies may be indicated to distinguish between a familial variant/ population polymorphism and a de novo, possibly clinically relevant alteration.</td>
</tr>
<tr>
<td>E13.4.2.5</td>
<td>Reporting microarray analysis results</td>
</tr>
<tr>
<td></td>
<td>The laboratory must determine normal and abnormal ranges for reporting microarray analysis results. The report should include a description of these ranges or the criteria that have been applied to determine normal versus normal findings. The laboratory director should express the microarray results in a meaningful manner using International System for Human Cytogenetics Nomenclature approved nomenclature (ISCN 2005) when possible, including any FISH and karyotype results. The microarray results may be expressed using clone designations or in terms of the nucleotide boundaries for any gains or losses in copy number or may list genes partially or completely within the altered region. If this format is used, a specific genome browser nucleotide numbering must be specified (e.g., March 2006 assembly) in the report interpretation, and some indication of the breakpoints should be given to the extent that the technology permits. Whether International System for Human Cytogenetics Nomenclature or nucleotide positions or both are used to specify results, a written interpretation must be provided explaining the findings in a manner understandable to the average health care professional. Results of confirmation studies should also be reported. Reporting of any questionable variant regions should include a comment noting that CNVs exist, and interpretation of clinical significance should be made with caution. The laboratory report should provide recommendations regarding parental/family studies including the optimal method of testing for relatives and that genetic counseling should be sought.</td>
</tr>
<tr>
<td></td>
<td>If the array is of the variety that can only be applied as an adjunct to, but not a replacement for, traditional cytogenetic analysis and targeted FISH assays, this limitation of use must be clearly stated on the final laboratory report.</td>
</tr>
<tr>
<td></td>
<td>Technical information about the microarray being used (e.g., commercial source, number and types of clones and loci represented, and lot number) should be included on the report.</td>
</tr>
</tbody>
</table>
Additionally, the report should provide the limitations of the test in a disclaimer. An example disclaimer is as follows: This microarray was constructed by (insert name of company) for the sole purpose of identifying DNA copy number gains and losses associated with chromosomal imbalances. This microarray will detect (list detection capabilities, which may include aneuploidy, deletions, and duplications) of the loci represented on the microarray. It will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and balanced insertions), point mutations, or imbalances of regions not represented on the microarray and may not detect low levels of mosaicism. The failure to detect an alteration at any locus does not exclude the diagnosis of any of the disorders represented on the microarray. This test was developed and its performance characteristics determined by (your laboratory name here) as required by CLIA ’88 regulations. It (has/has not) been cleared or approved for specific uses by the U.S. Food and Drug Administration. Pursuant to the requirements of CLIA ’88, this laboratory has established and verified the test’s accuracy and precision.

References
(Sections E1 – E4)


References
Sections E.1 through E.12 (*excluding section E.6 though E.6.5.4*)


References
Sections E6 through E6.5.3.3


References
Sections E13 through E13.4.2.5


Ballif BC, Kashork CD, Saleki R, Rorem E, et al. Detecting sex chromosome


### Table 1

<table>
<thead>
<tr>
<th>Frequency (%) of 2nd Cell Line &gt; &amp; Analytical Sensitivity</th>
<th>95% Power</th>
<th>99% Power</th>
<th>Analytical Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90%</td>
<td>95%</td>
<td>99%</td>
</tr>
<tr>
<td>50</td>
<td>11</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
<td>19</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Appendix A

**Selected Solid Tumors According to Culture Method**

Tumors may be divided into small round cell tumors (SRCTs) or non-small round cell tumors (NSRCTs) based on histopathology and whether the tumor is expected to grow in suspension (SRCTs) or as a monolayer culture (NSRCTs). Some tumors may grow with either method. Since the histopathology of a tumor is generally unknown at the time of receipt, this guide can help in deciding how to culture a tumor. If sufficient material is provided for a SRCT, culture with both methods; if only a small amount of tumor is received it is safer to initiate the tumor culture as a monolayer particularly if coverslip culture is used. Suspension or direct harvest may provide material for FISH if culture growth fails.

**Small round cell tumors**

- *Suspension only tumors*
  - Lymphoma
  - Plasmacytoma
  - Histiocytosis

- *Suspension and/or monolayer*
  - Neuroblastoma
  - Retinoblastoma
  - Central primitive neuroectodermal tumor (PNET) or medulloblastoma
  - Ewing’s sarcoma, peripheral primitive neuroectodermal (pPNET)
  - Rhabdomyosarcoma
  - Osteosarcoma

**Non-small round cell tumors**

- *Brain tumors*
  - Ependymoma
  - Glial tumors, glioblastoma, ganglioglioma
  - Astrocytoma
  - Oligodendroglioma
  - Choroid plexus tumors
  - Meningioma

- *Mesenchymal tumors or sarcomas or ‘spindle cell’ tumors*
  - Hepatoblastoma, hepatocellular carcinoma
  - Wilms tumor
  - Malignant fibrous histiocytoma (MFH), fibrosarcoma
  - Synovial sarcoma
  - Clear cell sarcoma
  - Desmoplastic small round cell tumor
  - Liposarcoma, lipoma
### Appendix B. Diagnostic/clinical significance of genetics of some solid tumors

<table>
<thead>
<tr>
<th>DISEASE ENTITY</th>
<th>CHROMOSOMAL ABERRATION</th>
<th>GENES INVOLVED FISH TARGETS</th>
<th>CLINICAL SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GENITOURINARY</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear cell RCC</td>
<td>-3 or del(3p)</td>
<td>3p, VHL, other unknown gene</td>
<td>Characterize non-papillary RCC</td>
</tr>
<tr>
<td></td>
<td>del(3p) with gain 5q</td>
<td>3p, 5q</td>
<td>Favorable prognosis</td>
</tr>
<tr>
<td></td>
<td>del(3p) with loss 5q</td>
<td>3p, 5q</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-14, del(14q)</td>
<td>14, IgH</td>
<td>Metastasis, unfavorable</td>
</tr>
<tr>
<td>Papillary RCC</td>
<td>t(2;16)(p13;q22)</td>
<td>PRCC/TFE3</td>
<td>Characterize adult papillary RCC</td>
</tr>
<tr>
<td>t(Kp11.2) RCC</td>
<td>t(1;16)(q21;p13)</td>
<td>ASPLCRS/TFE3</td>
<td>Characterized pediatric papillary RCC</td>
</tr>
<tr>
<td></td>
<td>t(3;17)(p12;q25)</td>
<td>CLTC/TFE3</td>
<td>Balanced translocation in RCC</td>
</tr>
<tr>
<td></td>
<td>t(3;16)(p12;q22)</td>
<td>PS5/TFE3</td>
<td>Characterized pediatric papillary RCC</td>
</tr>
<tr>
<td></td>
<td>t(11;14)(q13;p13)</td>
<td>ALPMA/TFEB</td>
<td>Characterized pediatric papillary RCC</td>
</tr>
<tr>
<td></td>
<td>-1p, t(11q13)</td>
<td>Chromosome enumeration</td>
<td>Subset of RCC, children, young adult</td>
</tr>
<tr>
<td></td>
<td>-22q22-q31</td>
<td>1p, CDKN1</td>
<td>Distinguish from oncocytoma</td>
</tr>
<tr>
<td></td>
<td>t(12;13)(p12;q25), t(11;22), t(11;14)</td>
<td>SMARCB1; BCR as surrogate</td>
<td>Distinguish from chromophobe</td>
</tr>
<tr>
<td></td>
<td>t(13;14)</td>
<td>ET16/17R3</td>
<td>Diagnostic</td>
</tr>
<tr>
<td></td>
<td>t(14;15)(p13;q21)</td>
<td></td>
<td>Diagnostic</td>
</tr>
<tr>
<td></td>
<td>t(16;17)(q22;p13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(19;22)(p13;q13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(19;22)(q13;p11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(19;22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder, papillary</td>
<td>del(9)(p21)</td>
<td>CDKN2A</td>
<td>Homozygous deletion higher grade, stage</td>
</tr>
<tr>
<td></td>
<td>8p; -7; +7; 17</td>
<td>8p, unknown gene</td>
<td>Higher recurrence rate, progression</td>
</tr>
<tr>
<td>Wilms Tumor</td>
<td>+6q, +1q, 1p, -22, 17p</td>
<td>1p, 1q, 16q, 17p, +17, WT1</td>
<td>Genetic instability</td>
</tr>
<tr>
<td>Prostate</td>
<td>+7q31, 8p22, -8q24, 17p13-17p16; +Rq24</td>
<td>7p1L, MYC, TPS3, TPS</td>
<td>Unfavorable histology; augmented chemotherapy if 1p, 16q-</td>
</tr>
<tr>
<td></td>
<td>10q</td>
<td>TPS3, MYC</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GIST</strong></td>
<td>-14/14q; -22/22q;</td>
<td>14, 22, KIT mutations</td>
<td>Distinguish from smooth muscle tumors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Response to TKis</td>
</tr>
<tr>
<td>Liver</td>
<td>+20; +2; +5; +1q22</td>
<td>Chr 2, 8, 20, 1q</td>
<td>Distinguish from HCC, HHM</td>
</tr>
<tr>
<td></td>
<td>+1p</td>
<td>19q</td>
<td>Distinguish from hemangiomia or malignant tumor</td>
</tr>
<tr>
<td></td>
<td>+22</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>Pleomorphic adenoma</td>
<td>PLAG1</td>
<td>Diagnostic benign</td>
</tr>
<tr>
<td></td>
<td>Mixed apocrine carcinoma</td>
<td>MECT1/1MAML2</td>
<td>Diagnostic malignant</td>
</tr>
<tr>
<td></td>
<td>Warthin’s tumor</td>
<td>MECT1/1MAML2</td>
<td>Benign tumor</td>
</tr>
<tr>
<td><strong>BREAST</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invasive intraductal</td>
<td>dmin, hsr</td>
<td>ERBB2 amp</td>
<td>Worst prognosis, response to TKis, Mab</td>
</tr>
<tr>
<td>Secretory Breast</td>
<td>t(12;15)(p13;q25)</td>
<td>ERRB2, TOP2A co-amp</td>
<td>Co-amplification, better response to FEC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETV6/17R3</td>
<td>Favorable outcome; distinguish from IDC</td>
</tr>
<tr>
<td><strong>CNS</strong></td>
<td>Astrocytic tumors</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glioblastoma</td>
<td>+7, -10q; 9p21; 19q</td>
<td>EGFR, PTEN</td>
</tr>
<tr>
<td></td>
<td>Oligodendrogial tumors</td>
<td>+7, 10q; 9p</td>
<td>CDKN2A</td>
</tr>
<tr>
<td></td>
<td>Anaplastic</td>
<td>+1p; -19q; del(1;19)(q10;p10)</td>
<td>19q, PTEN, EGFR, CDKN2A</td>
</tr>
<tr>
<td></td>
<td>Mixed oligoastrocytoma</td>
<td>+7, -10;10q; -15q</td>
<td>1p36, 19q13.3</td>
</tr>
<tr>
<td></td>
<td>Oligoastrocytoma</td>
<td>19q; 1p</td>
<td>EGFR, PTEN</td>
</tr>
<tr>
<td></td>
<td>Ependymoma</td>
<td>+7, -22q; 14q; +1q; 6q; +7; 9p; i(17q); 17p; -10/10q; -7</td>
<td>7p, 1q, NF2</td>
</tr>
<tr>
<td></td>
<td>Spinal</td>
<td>+1p; +1q; 1p</td>
<td>3q25, ps, 16; EGFR/CDKN2A</td>
</tr>
<tr>
<td></td>
<td>Intracranial</td>
<td>+1q; 1q</td>
<td>MYC, MYC</td>
</tr>
<tr>
<td>Medulloblastoma (MB)</td>
<td>+1q; 1p</td>
<td>SFRB2</td>
<td></td>
</tr>
<tr>
<td>Supratentorial PNET</td>
<td>-22 or del(22q11.23)</td>
<td>1q, 19</td>
<td>SMARCB1; BCR as surrogate</td>
</tr>
<tr>
<td>AT/RT</td>
<td>-22 or del(22q11.2)</td>
<td>22q, NF2</td>
<td></td>
</tr>
<tr>
<td>Meningioma</td>
<td>1p; -14/14q</td>
<td>1p, IgH</td>
<td></td>
</tr>
<tr>
<td>Choroid plexus tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significance**
- **Primary abnormality**: Increased risk of recurrence, anaplastic
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Chromosome Abnormalities</th>
<th>Prognostic Features</th>
</tr>
</thead>
</table>
| RCC, renal cell carcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Younger patients benefit from immunotherapy,
older patients may require combination therapy. |
| CMN, congenital mesoblastic nephroma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| PIN, prostate intraepithelial neoplasia | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| GIST, gastrointestinal stromal tumor | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| TKIs, tyrosine kinase inhibitors | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| MK, melanocytic nevus | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| BCC, basal cell carcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| SCC, squamous cell carcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| AEC, adenocarcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| SCC, squamous cell carcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| AEC, adenocarcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |
| SCC, squamous cell carcinoma | **Chromosome Abnormalities:**<br> *Chromosomes involved:*<br> 1, 3, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, 19, 22 | **Prognostic Features:**<br> Prognosis depends on stage and extent of disease. |