1.1 Components of a standard cytogenetics report

All cytogenetic reports should have specific information which helps to standardize that each laboratory is performing a minimum standard of competency and accuracy of results. Clinical laboratory improvement amendments (CLIA), College of American Pathologists (CAP) and various US states have placed requirements on each report. The information below is required for CLIA, CAP, NY State and CA State for regulatory compliance.

- Specimen type
- Indication for testing
- Number of cells counted
- Number of cells analyzed
- Number of cells karyotyped
- Banding technique
- ISCN nomenclature
- Interpretation

1.1.1 Specimen type

Specimen type refers to the source of tissue that is being analyzed for cytogenetic testing. The most common specimen types are:

- amniotic fluid and chorionic villus sampling (CVS) for prenatal studies
- peripheral blood for studies of liveborn individuals
- fetal tissue for products of conception (fetal demise) studies
- bone marrow, bone core or peripheral blood for leukemias
- bone marrow or lymph nodes for lymphomas
- muscle or skin biopsies for possible mosaic studies
- tumor biopsies for acquired or inherited malignancies.
1.1.2 Indication for testing

Obtaining relevant clinical information about the patient is important in order to correlate cytogenetic results with the diagnosis. It sometimes becomes necessary for the laboratory to determine the appropriate set-up conditions of the specimen and the types of testing to perform, due to the various possibilities that exist. Therefore, in order for the laboratory to know what specific testing to perform, it needs all relevant patient information. Without the necessary patient and family clinical information, it may become a guessing game for the laboratory on the correct processing step to take. This is especially significant when it applies to cancer cytogenetics. Since certain cancer cells, including acute leukemias and myeloid disorders, divide continuously and do not require a B-cell or T-cell mitogen stimulant for cells to go through mitosis, the cultures that are initiated should be unstimulated 24-hour and 48-hour cultures. This is in contrast to chronic leukemias and other lymphoproliferative disorders, which do better with a B- or T-cell mitogen (e.g. IL4, TPA) to stimulate the cells to divide to have enough metaphases for analysis and which contain the abnormal cell type rather than normal lymphocytes. Also, knowing if acute lymphoblastic leukemia (ALL) is an indication for a patient will require only direct, overnight or 24-hour unstimulated cultures for analysis. Otherwise, there will be an overgrowth of normal cells dividing by the second day, and the abnormal lymphoblasts that are indicative of ALL will die off and not be present for analysis.

Culture initiation or set-up is also specific for the tumor type in question. No one culture medium is sufficient for all tumor types and so the culture medium should be specifically tailored for the proper growth of the abnormal tumor cells. For a guide on cancer cell culture media and growth factors for neoplastic cell growth, see the bibliography for detailed information.

1.1.3 Number of cells counted and analyzed

Counted cells refer to identifying a single cell and counting the number of chromosomes present plus identifying the sex chromosomes of that cell. Analyzed cells refer to identifying each chromosome homolog, band for band, to determine if any abnormalities exist within any of the chromosomes present.

Colonies refer to amniotic fluid cells that are cultured in situ on a small culture vessel, such as a coverslip. Colonies originate from single amniotic fluid cells that will grow and divide near each other in a colony, visibly separated from other originating amniotic fluid colonies. This type of culture allows for a greater distinction of progenitor cells in analysis versus allowing cells to congregate, grow and divide without spatial distinction, in which there is no knowledge of which cells are progenitor cells and which are the result of cell division and clones of progenitor cells. Without colonies, the cells in culture may be growing and dividing from only a very few hardy cells, and could possibly result in only a small number of original cells being analyzed, excluding possible mosaicism at a lower level.

The standard number of cells to be counted and analyzed depends on the specimen type. See Table 1.1 for a guide to the most common guidelines for cells counted and analyzed.

<table>
<thead>
<tr>
<th></th>
<th>Postnatal peripheral blood</th>
<th>Prenatal amniotic fluid</th>
<th>Prenatal chorionic villus sampling</th>
<th>Neoplastic bone marrow and blood</th>
<th>Fetal demise and liveborn tissues</th>
<th>Neoplastic tumors</th>
<th>Mosaic studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells counted</td>
<td>20</td>
<td>15 colonies or 20 cells</td>
<td>20</td>
<td>20</td>
<td>20–30</td>
<td>30–50</td>
<td></td>
</tr>
<tr>
<td>Cells analyzed</td>
<td>5</td>
<td>5 colonies or 5 cells</td>
<td>5</td>
<td>20</td>
<td>5</td>
<td>20–30</td>
<td>5</td>
</tr>
</tbody>
</table>
1.1.4 Number of cells karyotyped

The number of cells to be karyotyped is generally two per cell line. Exceptions to this rule include karyotyping only one cell of sideline clones in a neoplastic study, which will be discussed in greater detail in the cancer section of the book. More than two cells may be karyotyped if an abnormality is subtle and requires more than two cells to clarify the abnormality present.

1.1.5 Banding techniques

The standard banding techniques include those that clearly distinguish the significant bands identified by the International System for Human Cytogenetic Nomenclature (ISCN). The most common banding techniques which show the best banding patterns include G-banding, R-banding and Q-banding. Each technique uses different staining procedures to visualize the differential staining of cytosine/guanine (CG)-rich and adenosine/thymine (AT)-rich DNA. In each staining procedure, the bands observed are the same, but are visualized by AT with dark bands and CG with light bands or vice versa.

Other banding techniques are used to enhance specific regions of the chromosome, such as the centromere with C-banding, satellite regions of acrocentric chromosomes with nuclear organizer region (NOR) staining or telomeric regions with T-banding.

For a comprehensive discussion of banding techniques, refer to the bibliography at the end of the chapter.

1.1.6 Band levels

The banding level refers to an estimated total number of black, gray and white bands throughout the genome as it would appear in an ideogram of each chromosome. In the ISCN 2013 edition, on pages 16–31, ideograms of the chromosomes are described by band levels. There are a few reports in the literature of standardizing approaches to count the total number of bands in a karyotype. One approach is to count bands including the telomere, centromere and all the dark and light bands on chromosome 10. Table 1.2 details the correlation between the number of bands with the band level, using chromosome 10 as a reference.

Another approach for estimating band level is to count segments of specific chromosomes. For two different approaches, see a summary of these band estimations in Tables 1.3 and 1.4. Examples of cells with their corresponding karyotypes of each band level are depicted in Figure 1.1.

In a cytogenetics report, recording band level is generally a requirement. There is some debate on whether the highest band level observed in the best cell should be recorded in the report, or whether the band level of the best karyotype should be reported, or an average of the cells or karyotypes. Many laboratories record the best band level seen in a karyotype, which is easily documented for regulatory purposes and which may be corroborated if that karyotypic image is placed in the report itself.

Typically, the band level of a normal prenatal specimen of amniotic fluid and chorionic villus sampling is approximately 450 bands. For peripheral blood on liveborns, the typical band level is 500–550 bands. Hematological malignancies and solid tumors typically have fewer bands, generally in the range of 300–400 bands, reflecting the difficulty in analyzing dividing cells from abnormal cell types in malignancies.

When performing a high-resolution study, in which the minimum band level is 550–650 bands, a comment in the interpretation may be useful in order for the reader to know at what level chromosome analysis was achieved. It is also useful to report when the banding level did not reach the minimum requirement established by the laboratory or regulatory agency.
### Table 1.2  Band level by counting the bands on chromosome 10 (adapted from Welborn and Welborn 1993)

<table>
<thead>
<tr>
<th>Number of bands on chromosome 10</th>
<th>Estimated band level</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>375</td>
</tr>
<tr>
<td>13–14</td>
<td>400</td>
</tr>
<tr>
<td>15–16</td>
<td>425</td>
</tr>
<tr>
<td>17–18</td>
<td>450</td>
</tr>
<tr>
<td>19–21</td>
<td>475</td>
</tr>
<tr>
<td>22–23</td>
<td>500</td>
</tr>
<tr>
<td>24–25</td>
<td>525</td>
</tr>
<tr>
<td>26–28</td>
<td>550</td>
</tr>
<tr>
<td>29–30</td>
<td>600</td>
</tr>
<tr>
<td>31–32</td>
<td>650</td>
</tr>
<tr>
<td>33–34</td>
<td>700</td>
</tr>
<tr>
<td>35–36</td>
<td>750</td>
</tr>
<tr>
<td>37–38</td>
<td>800</td>
</tr>
<tr>
<td>39–40</td>
<td>850</td>
</tr>
</tbody>
</table>

### Table 1.3a  Tabulated band resolution of chromosomal segments (adapted from Josifek et al. 1991)

<table>
<thead>
<tr>
<th>Chromosome region</th>
<th>Total bands counted for each band level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Band level 350–400</td>
</tr>
<tr>
<td>Chromosome region 1 from p31-p32</td>
<td>1</td>
</tr>
<tr>
<td>Whole chromosome 10</td>
<td>5</td>
</tr>
<tr>
<td>Short arm of chromosome 11</td>
<td>2</td>
</tr>
<tr>
<td>Long arm of chromosome 12</td>
<td>4–5</td>
</tr>
<tr>
<td>Whole chromosome X</td>
<td>6–8</td>
</tr>
<tr>
<td>Total bands counted</td>
<td>18–21</td>
</tr>
</tbody>
</table>

### Table 1.3b  Correlation of total bands with band level

<table>
<thead>
<tr>
<th>Total bands</th>
<th>Band level</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>350</td>
</tr>
<tr>
<td>21</td>
<td>400</td>
</tr>
<tr>
<td>28</td>
<td>450</td>
</tr>
<tr>
<td>34</td>
<td>500</td>
</tr>
<tr>
<td>40</td>
<td>550</td>
</tr>
<tr>
<td>47</td>
<td>650</td>
</tr>
<tr>
<td>54</td>
<td>750</td>
</tr>
<tr>
<td>60</td>
<td>850</td>
</tr>
</tbody>
</table>
Table 1.4 Counting gray G-positive bands on chromosomes 10, 18q and 19

<table>
<thead>
<tr>
<th>Band level</th>
<th>Chromosomes with the number of G-positive gray bands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>150–200</td>
<td>6</td>
</tr>
<tr>
<td>200–425</td>
<td>7–9</td>
</tr>
<tr>
<td>425–700</td>
<td>10–12</td>
</tr>
<tr>
<td>&gt;700</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 1.1 Examples of metaphase cells with their corresponding karyotypes of each band level. (a) 46,XY estimated at a 350 band level. (b) 46,XX estimated at a 400 band level.
Figure 1.1 (Continued) (c) 46,XX estimated at a 450 band level. (d) 46,XY estimated at a 550 band level. (e) 46,XY estimated at a 750 band level. Courtesy of Sarah South PhD, ARUP Laboratories.
1.1.7 Summary of ISCN nomenclature

The International System for Human Cytogenetic Nomenclature (Shaffer et al. 2013) is the best resource for understanding how to write cytogenetic nomenclature. There should be little to no variation in the way laboratories write the ISCN nomenclature of an abnormality; however, it is not always easy to figure out how to write or understand ISCN nomenclature without some tutoring. This book will explain how to write each type of abnormality that exists and give examples of reports on how it is interpreted.

The following are the main concepts in writing any cytogenetic result. First is the number of chromosomes present in any person’s cells or genome. The usual number of chromosomes (modal chromosome number) for the human species is 46, so all normal karyotypes (another word for cytogenetic designation) have a graphic organization of chromosomes by number, and so the nomenclature begins with 46 in humans.

The second part of karyotypic designation is the sex chromosome complement. Normal human females contain two X chromosomes and normal human males contain one X and one Y chromosome.

Notice that punctuation is very important in cytogenetic nomenclature. All chromosome numbers are followed by a comma and no spaces are used to separate chromosome number from sex chromosome content.

Is the ISCN rules for normal results

First write the modal chromosome number followed by a comma.
Then write the sex chromosome complement.

Therefore, normal results would be either 46,XX or 46,XY for a female or male, respectively.
constitutional, neoplastic disorders, metaphase and interphase fluorescence in situ hybridization (FISH) analyses and array comparative genomic hybridization analyses. As each abnormality is described in this book, the correct ISCN designation will be discussed, so as to help with writing the correct nomenclature.

### 1.1.8 Report formatting

Various regulatory agencies require certain information on each report, including the following.

- Patient name
- Ordering physician
- Collection date
- Report date
- Unique patient identifier (usually the unique laboratory case number or specimen identification number), and the date of birth is suggested, not required
- Indication for study
- Specimen type
- Number of cells counted
- Number of cells analyzed
- Number of cells karyotyped
- Chromosome banding level
- Type of banding performed
- Types of cultures used for analysis
- ISCN result
- Interpretation
- Name and signature of reporting personnel
- Name of the medical director
- Laboratory identification, which may include the name of the laboratory and regulatory agency number (such as a CLIA ID number)
- Location of the laboratory and locations of each part of the analysis if there is more than one location involved

One example reporting format includes three sections: one for the demographic information of the patient, one for the clinical information associated with testing, and one for the results and interpretation. A fourth area of the report may include an image or images of the karyotype if desired.

### EXAMPLE REPORTING OF A NORMAL RESULT

**Laboratory Name**

**Cytogenetics Report**

<table>
<thead>
<tr>
<th>Patient Name: Jane Doe</th>
<th>Collection Date: 1/1/2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordering Physician: Dr Smith</td>
<td>Report Date: 1/10/2014</td>
</tr>
<tr>
<td>Date of Birth: 1/1/2000</td>
<td>Laboratory Number: A14-000021</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen Type: Peripheral blood</th>
<th>Indication for Study: Down syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cells counted: 20</td>
<td>Banding: G-banding</td>
</tr>
<tr>
<td>Number of cells analyzed: 5</td>
<td>Banding resolution: 550</td>
</tr>
<tr>
<td>Number of cells karyotyped: 2</td>
<td>Cultures performed: 72-hour PHA stimulated</td>
</tr>
</tbody>
</table>
1.2 Prenatal normal results

Prenatal studies differ from postnatal studies in the type of cells that are analyzed. Prenatal specimens are composed of fibroblast cells, epithelial cells and other cells sloughed off from the growing fetus in amniotic fluid. These cells, when put in a culture vessel, will adhere to the bottom of the vessel and grow until confluent and there is no more room for growth expansion. The two specimen types currently used for prenatal diagnosis in the first or second trimester are CVS and amniotic fluid. These two specimen types differ in the timing of collection and analysis, the clinical procedure in obtaining a specimen, and how the cells are grown and analyzed in the laboratory.

Prenatal samples may be obtained from percutaneous umbilical cord sampling (PUBS), generally obtained in the third trimester, if a fetal karyotype is needed, usually due to abnormal ultrasound findings. Testing with PUBS is the same as for postnatal peripheral blood chromosome analysis. Details for this testing will be described subsequently in Section 1.3.

1.2.1 Prenatal cell analysis

For CVS, a minimum of 20 cells are counted and a minimum of five cells are analyzed for each specimen. This analysis is usually divided among at least two primary cultures to ensure a minimum of two independent sources of cells for evaluation. The number of 20 cells was derived due to the possibility of mosaicism — that is, more than one cell line present in culture. Twenty cells will rule out the possibility of 14% mosaicism at a 95% confidence interval or 21% mosaicism with 99% confidence. This is now a standard procedure in most laboratories worldwide.

For amniotic fluid (AF), a minimum of 15 colonies are counted with a minimum of five cells analyzed for each patient. Also, a minimum of two primary cultures are analyzed when possible. Since clinicians generally divide an amniotic fluid specimen into two 15 mL conical tubes, the convention is to grow each tube of cells independently and analyze at least some cells from each of the tubes received in the laboratory. A total of a 15 colony analysis has been designated as a standard procedure, since each of the 15 colonies will account for known independent cells, which is a better method for ruling out mosaicism than by culturing cells indiscriminately.

When AF specimens need to be subcultured, a total of 20 cells are counted and the process is analogous to CVS analysis.
Therefore, results are reported somewhat differently between CVS and AF; namely, by the cell count and by the possible presence of maternal cell contamination in CVS. Example reporting of results usually requires some variation between the specimen types.

### 1.2.2 Amniotic fluid

Amniotic fluid specimens are generally obtained at 14–18 weeks’ gestation, with some clinicians performing early amniocentesis at 12–14 weeks’ gestation. AF is generally easier to obtain than CVS and does not have the risk of maternal cell contamination unless it is a very bloody sample, implying that either placental decidua or maternal tissue is present.

Most amniotic fluid analyses are performed with *in situ* cultures, which allow for a discrete number of original progenitor cells to be cultured. This will ensure that a known number of independent cells will be evaluated in the cytogenetic analysis. This process is accomplished ideally by seeding the amniotic cells on coverslips in a small culture vessel, usually petri dishes, that allow for cell growth separately from neighboring cells. The original progenitor cells will then grow and divide through mitotic division approximately once each day and form a colony of identical cells. Each progenitor cell will form its own colony of cells independently from the other colonies, thus ensuring the growth of different cells to be analyzed. Cells, once confluent, are then processed *in situ* for cytogenetic analysis, keeping each colony of cells intact for the chromosomal evaluation.

In some instances, there are an inadequate number of colonies available for analysis. When this happens, the cells need to be subcultured whereby all the colonies are collected and distributed evenly in the culture vessel to promote growth. In this case, the cells are then treated as a normal cell culture and not a colony culture.

---

**EXAMPLE REPORTING OF NORMAL FEMALE AF RESULTS**

**ISCN Result:** 46,XX  Normal Female Karyotype

**Interpretation**

Chromosome analysis revealed a normal female chromosome complement in all 15 colonies examined from multiple cultures of amniocytes.  
There was no evidence of a chromosome abnormality within the limits of the current technology.

**EXAMPLE REPORTING OF NORMAL MALE AF RESULTS**

**ISCN Result:** 46,XY  Normal Male Karyotype

**Interpretation**

Chromosome analysis revealed a normal male chromosome complement in all 15 colonies examined from multiple cultures of amniocytes.  
There was no evidence of a chromosome abnormality within the limits of the current technology.
When only one tube of amniotic fluid is received, some laboratories may add the following comment to the interpretation.

**EXAMPLE REPORTING OF RESULTS FROM ONLY ONE TUBE OF AF**

**ISCN Result:** 46,XY Normal Male Karyotype

**Interpretation**

Chromosome analysis revealed a normal male chromosome complement in all 15 colonies examined from multiple cultures of amniocytes. However, all cells analyzed were obtained from a single tube of amniotic fluid, which is below our laboratory standard of analyzing cells from at least two tubes received.

There was no evidence of a chromosome abnormality within the limits of the current technology.

### 1.2.3 Chorionic villus sampling

Chorionic villus sampling specimens are usually obtained clinically between weeks 10 and 14 of gestation. This was developed in order to do prenatal diagnosis in the first trimester of pregnancy as an earlier diagnostic genetic test rather than second trimester amniotic fluid testing. Though CVS has a slightly higher risk of miscarriage and fetal defects compared to amniotic fluid testing, it is a preferred test for some individuals who want results sooner.

The cells obtained from CVS are villi from the trophoblast of the growing fetus and may at times include decidua, which may be maternal or placental in origin, and must be carefully processed in the laboratory to ensure that the fetal cells and not maternal cells are analyzed. The villi need to be carefully disseminated into small pieces and placed in a culturing vessel. The cells obtained are fibroblasts, which will adhere to the surface of the vessel in which the cells are placed. These cells will then grow and divide and, when cells are confluent, will be lifted off the bottom of the vessel and collected for the process of cytogenetic analysis. Since there is a possibility of maternal cell contamination, depending on the quality of the specimen received, some reports may want to include information regarding the quality of that specimen.

**EXAMPLE REPORTING OF NORMAL FEMALE CVS RESULTS – GOOD QUALITY OF FETAL VILLI RECEIVED**

**ISCN Result:** 46,XX Normal Female Karyotype

**Interpretation**

Chromosome analysis revealed a normal female chromosome complement in all 20 cells examined from multiple cultures of chorionic villus sampling.

There was no evidence of a chromosome abnormality within the limits of the current technology. These results most likely reflect the fetal rather than the maternal karyotype. However, due to the nature of the tissue submitted, the possibility of maternal cell contamination cannot be excluded.
EXAMPLE REPORTING OF NORMAL FEMALE CVS RESULTS – PREDOMINANTLY MATERNAL DECIDUA RECEIVED

ISCN Result: 46,XX Normal Female Karyotype

Interpretation

Chromosome analysis revealed a normal female chromosome complement in all 20 cells examined from multiple cultures of chorionic villus sampling.

There was no evidence of a chromosome abnormality within the limits of the current technology. The sample appeared to contain only maternal decidua. Therefore, this result may represent maternal cell contamination rather than the fetal karyotype.

EXAMPLE REPORTING OF NORMAL FEMALE CVS RESULTS – POOR QUALITY OF FETAL VILLI RECEIVED

ISCN Result: 46,XX Normal Female Karyotype

Interpretation

Chromosome analysis revealed a normal female chromosome complement in all 20 cells examined from multiple cultures of chorionic villus sampling.

The specimen consisted entirely of membranous tissue. While this tissue is most likely fetal in origin, the results could reflect maternal cell contamination rather than the fetal karyotype.

Some laboratories like to report the amount of villi received. This information could be added in the interpretation, including the following possible comments.

FOR ADEQUATE MATERIAL RECEIVED (MINIMUM 10mg)

This amount of chorionic villi received was approximately _ mg of cleaned sample. This is considered adequate sampling for cytogenetic analysis.

FOR INADEQUATE MATERIAL RECEIVED (LESS THAN 10mg)

This amount of chorionic villi received was approximately _ mg of cleaned sample. This is considered less than adequate sampling for cytogenetic analysis, i.e. less than our general minimum requirement of 10 mg.

FOR INADEQUATE MATERIAL RECEIVED (LESS THAN 10mg) AND A NORMAL FEMALE RESULT

This amount of chorionic villi received was approximately _ mg of cleaned sample. This is considered less than adequate sampling for cytogenetic analysis, i.e. less than our general minimum requirement of 10 mg. Due to this result showing a normal female karyotype, maternal cell contamination cannot be excluded.
1.2.4 Maternal cell contamination

Maternal cell contamination (MCC) is defined as a co-mixture of maternal cells with those of the fetus. This only becomes apparent when the fetus is either male or has a cytogenetic abnormality. Even with a cytogenetic abnormality of a female, one cannot exclude maternal cells from true fetal mosaicism. Therefore, minimizing MCC is critical in prenatal specimens, or there is a risk of analyzing the mother rather than the fetus. In CVS, diligently separating out the chorionic villi from decidua, which may be maternally derived, is critical. Approximately 1–2% of CVS specimens show MCC. One can estimate this amount to be 2–4%, considering the equal likelihood of having MCC in female fetuses that are not distinguishable.

In CVS, the incidence of MCC is higher in cultured specimens than in direct preparations, making direct preparations an advantage for this purpose alone. However, for complete chromosome analysis, cultured specimens are preferred due to the better quality of chromosome morphology. The literature also reports a higher incidence of MCC in transcervical CVS versus the transabdominal method.

For amniotic fluid specimens, there is a very low rate of MCC (approximately 0.2–0.4%), which may be present in bloody samples or those with a posterior placenta. Posterior placentas may interfere with the amniocentesis procedure, increasing the likelihood of puncturing and obtaining placental material with the fluid.

For fetal demises when only placental tissue with no known fetal parts is obtained for cytogenetic analysis, the risk of MCC is substantial. Products of conception (POC) tissue in which no fetal parts are distinguishable may also yield a high degree of MCC. To best diagnose a POC for MCC, FISH analysis may be performed, which has a better outcome with interphase analysis to visualize even a small degree of XX versus XY cells (to be discussed further in Chapter 14, Interphase analysis).

For peripheral blood chromosome studies, MCC is very rare and not usually considered as a high probability when mosaicism is seen.

EXAMPLE REPORTING OF MATERNAL CELL CONTAMINATION WITH NORMAL MALE RESULTS IN AF

ISCN Result: 46,XY[12]/46,XX[3]

Interpretation

Chromosome analysis showed two cell lines present in multiple cultures of amniotic fluid. One cell line showed a normal male chromosome complement in 12 cells. The remaining three cells showed a normal female chromosome complement. The normal female cells most likely represent maternal cell contamination.

Maternal cell contamination occurs in less than 1% of amniotic fluid specimens. Other explanations may include a reabsorbed twin or true chimerism.

Recommendations:

1. Genetic counseling.
2. Ultrasound may be useful in visualizing male genitalia as well as detecting a viable or reabsorbed twin.
3. A repeat amniocentesis could be considered if clinically indicated.
EXAMPLE REPORTING OF MATERNAL CELL CONTAMINATION WITH NORMAL MALE RESULTS IN CVS

ISCN Result: 46,XY[15]/46,XX[5]

Interpretation

Chromosome analysis showed two cell lines present in multiple cultures of chorionic villus sampling. One cell line showed a normal male chromosome complement in 15 cells. The remaining five cells showed a normal female chromosome complement. The normal female cells most likely represent maternal cell contamination; however, a twin pregnancy or chimerism cannot be excluded.

EXAMPLE REPORTING OF RESULTS OF A TWIN PREGNANCY WHEN BOTH TWINS HAVE THE SAME KARYOTYPE

ISCN Result: 46,XX or 46,XY (for each reported twin) Normal Female/Male Karyotype

Interpretation

Chromosome analysis revealed a normal female/male chromosome complement in all 20 cells examined from multiple cultures of amniocytes/chorionic villus sampling.
There was no evidence of a chromosome abnormality within the limits of the current technology.

Note: Accurate cytogenetic results from twin pregnancies depend on the successful sampling of both fetal sacs. Although accurate in most cases, it is possible that the same results for twin pregnancies represent the sampling of only one of the two fetuses.

1.3 Neonatal normal results

Cytogenetic studies of newborns and liveborns in general are performed on peripheral blood specimens and at times, if a very fast result is needed on a newborn, bone marrow may be obtained. Bone marrow studies are being phased out more and more, since the only recommended need for a fast turnaround time on newborns is for cases of possible cri-du-chat syndrome or Down syndrome, in which a heart defect is present and a physician needs to decide whether or not to perform surgery within hours to save the baby. If it is known that the baby has a genetic defect where surgery will not ameliorate the problem, surgery is usually not performed. However, if the baby has an isolated heart defect unrelated to a chromosomal abnormality, then surgery may save the baby’s life. Consequently, a quick cytogenetic study may be useful, if not critical, in these cases. However, FISH analysis for the disorder, if available, may be a better approach than cytogenetic analysis, since cells would not need to be cultured, but rather analysis may be performed on a direct preparation with interphase cells.

Therefore, for the most part, cytogenetic analysis on peripheral blood is the preferred sample type for postnatal chromosome analysis and yields better chromosome morphology and banding level than other specimen types. Peripheral blood, like fibroblasts, goes through cell division approximately once every 24 hours, and cells can be synchronized to divide simultaneously to yield a large number of metaphase cells for analysis. Peripheral blood samples have the advantage over fibroblasts as it is easier to obtain cells in metaphase (harvested cells) and samples may be subjected to high-resolution techniques more easily than fibroblasts, thus yielding not only a large number of cells, but also cells
with longer chromosomes. One other advantage of peripheral blood is the ability to collect (harvest) cells in metaphase, days sooner than fibroblasts, thus achieving a far better culture turnaround time of 2–3 days versus 5–10 days for fibroblast cells.

Whole blood samples and even harvested cells may be saved easily for further studies such as microarray or FISH analyses when a clinical indication for further testing is useful or even preferred. For example, with a clinical indication of mental impairment, developmental delay and autistic features, genomic microarrays are now considered a first-tier test for cytogenetic analysis rather than metaphase chromosome studies by the American College of Medical Genetics (ACMG). In such cases, when only chromosome studies are requested, a comment may be added in the cytogenetic report of a normal chromosome study to suggest microarray analysis, which may give further information at a higher level of chromosome resolution.

Below is an example of a recommended comment to add microarray analysis after a normal chromosome study when enough material is left for further analysis.

| Chromosomal microarray analysis (CMA) may prove informative in this case. The current specimen, if available, may be used for this analysis. |

1.4 Normal variants in the population

The definition of a normal genetic variation is one that occurs at 1% or greater in the general population. This is true for chromosomal variants as well as molecular genetic variation changes. These variants are generally inherited but can occur de novo.

1.4.1 Large heterochromatic regions (Figure 1.2)

One of the most common chromosome genetic variants is a variable amount of heterochromatin below the centromere in the proximal long arm of specific chromosomes: namely, chromosomes 1, 9 and 16. These variants have no clinical consequence and, therefore, laboratories do not even include them in clinical reports, unless desired.

<table>
<thead>
<tr>
<th>ISCN RULES FOR REPORTING A LARGE HETEROCHROMATIC VARIANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ First write the modal chromosome number of 46, followed by the sex designation.</td>
</tr>
<tr>
<td>■ Then write the chromosome involved followed by the abbreviation “qh+.”</td>
</tr>
</tbody>
</table>

Note: there are no parentheses around the chromosome or the qh + designation.

For example: 46,XY,9qh+ represents a large heterochromatic region on the proximal long arm of chromosome 9.

<table>
<thead>
<tr>
<th>Figure 1.2 Large heterochromatic region of chromosome 9 – 9qh+.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Courtesy of Sarah South PhD, ARUP Laboratories.</td>
</tr>
</tbody>
</table>
One of the most common chromosome genetic variants is the inversion around the centromere of chromosome 9: inv(9)(p12q13). An inversion of this region is a change in the heterochromatin to the proximal short arm. Similarly, an inversion of the centromeric region of chromosomes 1, 2 and 16 is also seen. These variants generally have no clinical consequence and, therefore, some laboratories do not even include them in clinical reports, although some do add a comment in the interpretation of the report.

**ISCN RULES FOR REPORTING A NORMAL VARIANT PERICENTRIC INVERSION**

- First write the modal chromosome number of 46, followed by the sex designation.
- Then write the abbreviation “inv” followed by the chromosome involved, enclosed by parentheses.
- Then write the breakpoints of the inversion, starting with breakpoints of the short arm (p arm) followed by breakpoints of the long arm (q arm) enclosed by parentheses.

For example: 46,XY,inv(9)(p12q13) for a pericentric inversion around the centromere of chromosome 9.

When both an inversion and a large heterochromatic region are observed on a single chromosome, for example on chromosome 9, then the ISCN nomenclature is written as: 46,XY,inv(9)(p12q13)9qh++. (Figure 1.4).

**EXAMPLE REPORTING OF A VARIANT PERICENTRIC INVERSION**

**ISCN Result:** 46,XX,inv(9)(p12q13) Normal Female Karyotype

**Interpretation**

Chromosome analysis revealed a normal female chromosome complement in all 20 cells examined. However, a pericentric inversion of chromosome 9 was present in all cells. This rearrangement is considered a normal variant in the population with no known clinical significance.
1.4.3 Variant satellite regions

Acrocentric chromosomes are chromosomes that contain a long arm and a short arm containing no euchromatic region and that have stalks and satellite regions on the short arm (Figure 1.5). The stalk region contains DNA that codes for ribosomal RNA (rRNA) and is described as the nuclear organizer region (NOR). When these regions are expressing the genes for rRNA, the NOR may be visualized with specialized staining, called silver staining (Figure 1.6). These will be seen as large dark regions in the short arms. When seen by R- or G-banding, the stalk regions appear as an unstained area. The region above, or distal to, the stalk is the satellite region, seen as a black band at the top. Variant stalk and satellite regions may look much larger or possibly smaller than normal, or the satellites may appear as double satellites (Figure 1.7), but are still considered as a normal variant if no euchromatic DNA material is present. A shortened stalk region, seen by the distance from the centromere to the satellite, is generally not mentioned in a report, because lack of a stalk would refer to rRNA that is not currently being expressed in the cell and would not have any noticeable distance from the centromere to the satellite.

Figure 1.5 Depiction of an acrocentric chromosome with satellite and stalk regions.

Figure 1.6 Silver staining – AgNOR stain.
However, these variants may be added to a report to distinguish these regions if they appear too large or different to ignore, but are known by various banding techniques not to have any euchromatic material that may be of clinical significance.

**ISCN RULES FOR THE VARIATION OF THE STALK REGION**

- First write the modal chromosome number of 46, followed by the sex designation, followed by a comma.
- Then write the chromosome number involved followed by the short arm designation “p” followed by “stk.”
- Then write the designation “+” for an enlarged stalk or “−” for a shortened stalk.

For example: 46,XY,14pstk+ for a large stalk region on chromosome 14.

**EXAMPLE REPORTING OF AN ENLARGED STALK REGION**

**ISCN Result:** 46,XY,14pstk+  Normal Male Karyotype

**Interpretation**

Chromosome analysis revealed a normal male chromosome complement in all 20 cells examined. However, a large stalk region on the short arm of chromosome 14 was present in all cells. Specific banding techniques were performed to verify that this DNA material is not euchromatin and, consequently, would not result in DNA expression. Therefore, this rearrangement is considered a normal variant in the population with no known clinical significance.

**ISCN RULES FOR THE VARIATION OF THE SATTELITE REGION**

- First write the modal chromosome number of 46, followed by the sex designation, followed by a comma.
- Then write the chromosome number involved followed by the short arm designation “p” followed by the designation “s+” for an enlarged stalk or “s−” for a shortened stalk.

For example: 46,XY,22ps+ for an enlarged satellite region on chromosome 22 (Figure 1.8).
1.4.4 Variant Yqh

The Y chromosome is significant due to the unique genes on its short arm and proximal long arm, but much of the DNA material in this small chromosome has only heterochromatin in the distal long arm (Yqh region). This Yqh region is quite variable in size, but as long as there is some noticeable heterochromatin, the chromosome is considered normal, regardless of the amount of the Yqh region. C-banding is a good method for detecting the presence or absence of heterochromatin on the long arm of the Y chromosome (Figure 1.9). However, there may be a need or desire to comment in the report regarding this region.

For example, when the Yqh region is very small or unnoticeable but known to be present via extra banding techniques, such as C-banding, the Yqh- designation may be used. Alternatively, when the Yqh region is exceptionally large, beyond the size of chromosome 18, a comment may be given in the report describing a Yqh+ region (Figure 1.10).
It may be possible to see satellites on a chromosome region, which is not normal. In these cases, the abnormality will be discussed in the corresponding chapters. For example, satellites on the Y chromosome are discussed in Chapter 10 on sex chromosome abnormalities. For satellites on unidentifiable chromosomes, see Chapter 7 on marker chromosomes.
1.5 Disclaimers and recommendations

Some laboratories include disclaimers and recommendations in their reports. Disclaimers are used to indicate potential issues which may affect the accuracy of a result, such as issues associated with the limits of the current technology. Possible disclaimers may contain the following information.

**EXAMPLE REPORTING OF DISCLAIMERS**

- Based on the chromosome morphology and banding resolution of this study, standard cytogenetic methodology does not routinely detect subtle or submicroscopic rearrangements, low-level mosaicism, and some artifacts including maternal cell contamination.
- Testing performed in the absence of an accurate clinical indication may not give conclusive results.
- Chromosome analysis will not detect genetic conditions with Mendelian, multifactorial or environmental etiologies.
- Any image that accompanies this report is a representative image only and should not be used for the diagnosis of a patient.

Recommendations are helpful in a report to give pertinent information to clinicians regarding further action or testing that is based on either the result itself or the result in combination with the indication.

**EXAMPLE REPORTING OF COMMON RECOMMENDATIONS**

- Genetic counseling.
- Examine parental chromosomes to determine a potential origin of this abnormality.
- If clinically indicated, a follow-up study may be considered.
- If an increased incidence of fetal loss has been observed in this couple, peripheral blood studies on the parents may give further information.
- Monitor subsequent pregnancies with prenatal diagnosis.
- Consider chromosome studies on at-risk family members.
- A confirmatory cytogenetic study should be performed at the time of delivery or termination.
- Chromosome analysis of the patient’s first-degree biological relatives is suggested to identify other carriers of this rearrangement who are also at risk.
- Due to the location of the breakpoints in this rearrangement and the nature of the unbalanced derivative chromosomes that could be inherited in future pregnancies, this patient may be at an increased risk for liveborn offspring with congenital anomalies and miscarriages.
- Familial chromosomal rearrangements are generally not associated with an increased risk of phenotypic and/or developmental abnormalities; however, submicroscopic deletions, duplications, or disruption of a gene or regulatory element at the breakpoints that may cause abnormalities cannot be excluded.
- Genetic counseling is recommended and prenatal diagnosis should be offered for future pregnancies.
1.6 Culture failures

Cultures are initiated differently for each specimen type, depending on the source of the cell type. For prenatal specimens and solid tissues, including products of conception, the cell type is generally fibroblastoid, thereby requiring long-term tissue culture in flasks or another sterile vessel for days to weeks in an incubator. These cells may be grown on coverslips, flasks (T-12 size) or flasks (T-25 size), depending on laboratory preference. In all cases, fibroblast cells adhere to the surface of the growth vessel within 24 hours and after this time require daily culture medium to be added or changed for cell growth and division. Cells are harvested in the growing stage when they are subconfluent.

Optimal specimen acquisition by the clinician is critical to cell growth in culture in the laboratory. Bloody prenatal specimens, inadequate material received, non-viable tissues obtained, non-sterile techniques used and contamination before the specimen is received in the laboratory all contribute to poor cell growth and possible culture failure. Transport of the specimen is also critical for cell viability in the laboratory. Transport for longer than 24 hours, in too hot or cold a temperature, or cells transported without proper containment in a sterile vessel all contribute to poor growth of cells in culture and possible culture failure.
Once a specimen is in the laboratory, other factors contribute to culture failures. Since fibroblast cells require constant changes in culture media and sterile culture techniques to maintain growth and prepare the cells for collection or harvest for chromosome analysis, culture failures may occur due to problems in laboratory technique. Many of these culture failures can be ameliorated by good training, sterile technique and proper standard operating procedures.

The other specimen type generally obtained for constitutional chromosome analysis is peripheral blood. Peripheral blood is usually grown in conical or round-bottom tubes in which the cells float in culture medium for 48–72 hours. The original culture medium placed in the tube with the cells is usually sufficient for cell growth and division for this time period before the cells are ready to be harvested for chromosome analysis. Culture failures are rare and predominantly due to the specimen itself, rather than being related to laboratory techniques. Culture failures may occur due to patient medication or radiation that inhibits cell growth, an indication of severe anemia causing a paucity of cells obtained or a low white blood cell count related to a hematological disease.

Culture failures caused by laboratory techniques may be due to poor quality control (QC) measures for monitoring culture media and other factors relating to the equipment in the laboratory, such as incubators, refrigerators, freezers and biological safety hoods for maintaining sterility. Once proper QC measures are taken in the laboratory, culture failures will be minimized. Table 1.5 shows approximate acceptable culture failure rates for each specimen type.

### Table 1.5 Typical acceptable failure rates by specimen type

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Accepted culture failure rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid and chorionic villus sampling</td>
<td>5–10%</td>
</tr>
<tr>
<td>Solid tissues (biopsies)</td>
<td>5–10%</td>
</tr>
<tr>
<td>Products of conception</td>
<td>Not estimated due to viability of specimen received</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1–5%</td>
</tr>
</tbody>
</table>

#### EXAMPLE REPORTING OF CULTURE FAILURES IN POCs

The sample received failed to grow in culture. It is not unusual for this type of specimen from a fetal demise to fail to grow in culture.

**Recommendation**

If an increased incidence of fetal loss has been observed in this couple, peripheral blood studies on the parents may prove informative.

#### EXAMPLE REPORTING OF CULTURE FAILURES IN PRENATAL SPECIMENS

The sample received failed to grow in culture. It is not unusual for bloody samples or specimens with suboptimal fetal material received to fail to grow in culture.

**Recommendation**

A repeat CVS specimen or amniotic fluid specimen for chromosome analysis may give further results.
1.7 Contamination

Contamination of cultures may be due to the presence of bacteria or fungi that occurs within the specimen itself, at the time of collection, during transportation or in the laboratory. If a specimen is contaminated within the individual or before it is received in the laboratory, standard laboratory procedures may eradicate or lessen the degree of contamination enough to perform cytogenetic analysis. However, this possibility decreases the longer the specimen remains in culture.

Even if the specimen appears free of contamination at the time it is received in the laboratory, it may subsequently acquire bacteria or fungi. Again, treatment protocols may reduce or eradicate the problem to the extent that chromosome analysis may be successful.

Standard laboratory techniques usually call for adding an antibacterial and antifungal reagent, such as gentamicin, to culture media to decrease the possibility of contamination in cell cultures. Usually a 1% reagent will forestall any contamination if the specimen is clean at the time it is received in the laboratory.

By far the worst type of contamination is fungal. Fungus tends to be hearty and grows very quickly, such that typical antifungal reagents will not be sufficient to eradicate the fungus from the specimen. Bacterial contamination may be slower to grow and may not kill the specimen as rapidly as fungus, but is still a danger to cell viability. The best approach for saving the specimen and the laboratory from widespread contamination is to remove the contaminated specimen from the others and isolate it in a holding incubator. Treating the specimen with double the amount of antibiotic/antimycotic reagent and treating daily are suggested. Also, harvesting the specimen as soon as possible rather than waiting for proper confluency will actually increase the probability of a successful number of viable cells for chromosome analysis.

The worst-case scenario is to leave the contaminated specimen in the same incubator as the other specimens, thereby compromising all the samples in the laboratory. Once contamination is widespread in one or more incubators, it becomes increasingly difficult to eradicate the source of contamination without a laborious, time-consuming process.

To proactively prevent contamination, use a biological safety hood with an ultraviolet (UV) light source. The hood should be used at all times when working with long-term cultures. Additionally, each day (or night), the UV light should remain on for a minimum of 15 minutes to destroy any possible contamination.
contaminants. After each use in the hood, wiping down the surface with isopropyl alcohol will kill any contaminant from the area. Bleach (10%) may be used daily to kill any remaining source of contaminant for hoods and tabletop counters in the laboratory to protect against contaminants.

**EXAMPLE REPORTING OF CONTAMINATION WITHIN 24 HOURS OF RECEIPT IN THE LABORATORY**

The sample received showed signs of bacterial/fungal contamination within 24 hours of receipt in the laboratory. This indicates that the sample had been contaminated prior to its arrival in the laboratory.

Repeated attempts to induce the cultures to grow were not successful; therefore, cytogenetic analysis was not possible. Send a repeat sample to attempt further cytogenetic analysis, if possible.

**EXAMPLE REPORTING OF CONTAMINATION OF UNKNOWN SOURCE**

The sample received failed to grow in culture due to the presence of bacterial/fungal contamination.

Repeated attempts to induce the cultures to grow were not successful; therefore, cytogenetic analysis was not possible. Send a repeat sample to attempt further cytogenetic analysis, if possible.

**References**


**Bibliography**


