Appendix C: Cytogenetic Assessments

Introduction to Chromosomes

A basic knowledge of chromosome abbreviations / terms is required to interpret cytogenetic test results. Typical human cells contain 23 chromosome pairs (46 total chromosomes). Twenty-two of these pairs are autosomal (non-sex) chromosomes. Each autosomal chromosome is referred to by its number, one through 22. The remaining two chromosomes (the 23rd pair) are referred to as sex chromosomes and are identified as either X (female) or Y (male). Females have two X chromosomes while males have one X and one Y chromosome.

Chromosomal abnormalities refer to changes in the amount or location of chromosomal material. Definitions of general categories of chromosomal abnormalities are provided below:

Addition: extra chromosomal material is present. This includes extra material within a specific region of a chromosome and entire extra chromosomes. Extra material is described by the location while extra whole chromosomes are described based on the quantity present. Trisomy refers to three chromosomes present (one extra) while tetrasomy refers to four chromosomes present (two extra).
Deletion: loss of chromosomal material. This includes loss of material within a specific region of a chromosome and entire missing chromosomes. Loss of material is described by location while entire missing chromosomes are described based on the quantity present. Monosomy refers to one chromosome present (one lost) while nullisomy refers to no chromosomes present (both lost).
Translocation: an exchange of chromosomal material between two or more chromosomes.
Inversion: the base pair order is reversed for a specific region of a chromosome.
Hyperdiploidy: the total number of chromosomes present is higher than normal. The definition of hyperdiploidy is typically further specified on the form being completed. For example, a form may require greater than 50 chromosomes be present to report hyperdiploidy.
Hypodiploidy: the total number of chromosomes present is lower than normal.

Abnormalities are described by identifying the involved chromosomes and specific locations, when applicable. The location is described when an abnormality involves only a specific section of a chromosome or when a translocation has occurred. The location is defined by two pieces of information, the chromosome arm and the arm region. The arm refers to the short (p arm) or long (q arm) end of the chromosome on opposite sides of the centromere. The arm region describes the distance from the centromere. See Figure 1 below for a depiction of the chromosome arm and arm regions. Definitions of common cytogenetic abbreviations and terms are provided in Table 1.

Figure 1. Chromosome Structure

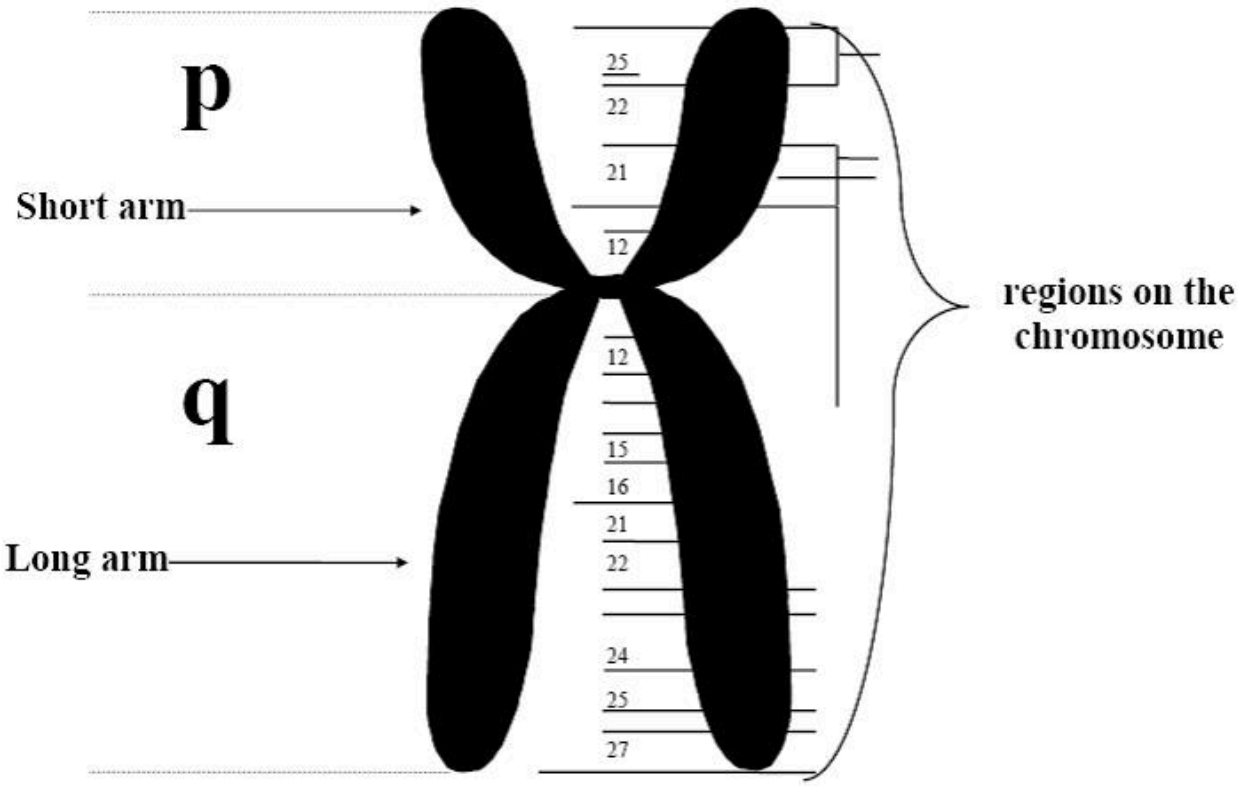


Table 1. Cytogenetic Abbreviations and Terms

Abbreviation/ Term	Definition
p	short arm of a chromosome
q	long arm of a chromosome
p+ / add(p)	addition of chromosomal material to the short arm of a chromosome
q+ / add(q)	addition of chromosomal material to the long arm of a chromosome
p- / del(p)	loss of chromosomal material to the short arm of a chromosome
q- / del(q)	loss of chromosomal material to the long arm of a chromosome
t	translocation of chromosomes; e.g., t(1;19)
+	addition of an entire chromosome (trisomy); e.g., +21
-	deletion of an entire chromosome (monosomy); e.g., -7
Ph+	Philadelphia chromosome, arises from translocation t(9;22)
inv	inversion of chromosomal material; e.g., inv(1)(p36q21)
der	Derivative
metaphase	cell phase at which chromosomes may be examined

karyotype	designation of results of chromosome analysis; karyotype may be defined at the cell level, cell line or clone level, or at the level of the individual

Cytogenetic Assessment Methods

Cytogenetic analysis is the study of chromosomes. Cytogenetic assessment involves testing a sample of cells for the presence of chromosomal abnormalities. Cytogenetic assessment can also be done to determine chimerism following an allogeneic infusion when there is a sex mismatch between the donor and recipient. Specific methods of assessment include karyotyping and fluorescence in situ hybridization (FISH).

Karyotyping, also referred to as conventional cytogenetics, is performed by culturing cells (growing cells under controlled conditions) until they reach the dividing phase. Techniques are performed to visualize chromosomes during cell division so various bands and reconfigurations are seen. Karyotype assessments typically examine around 20 cells. Figure 2 below shows an example of a karyotype. The chromosomes are arranged in numerical order with sex chromosomes included last.

Figure 2. Karyotype

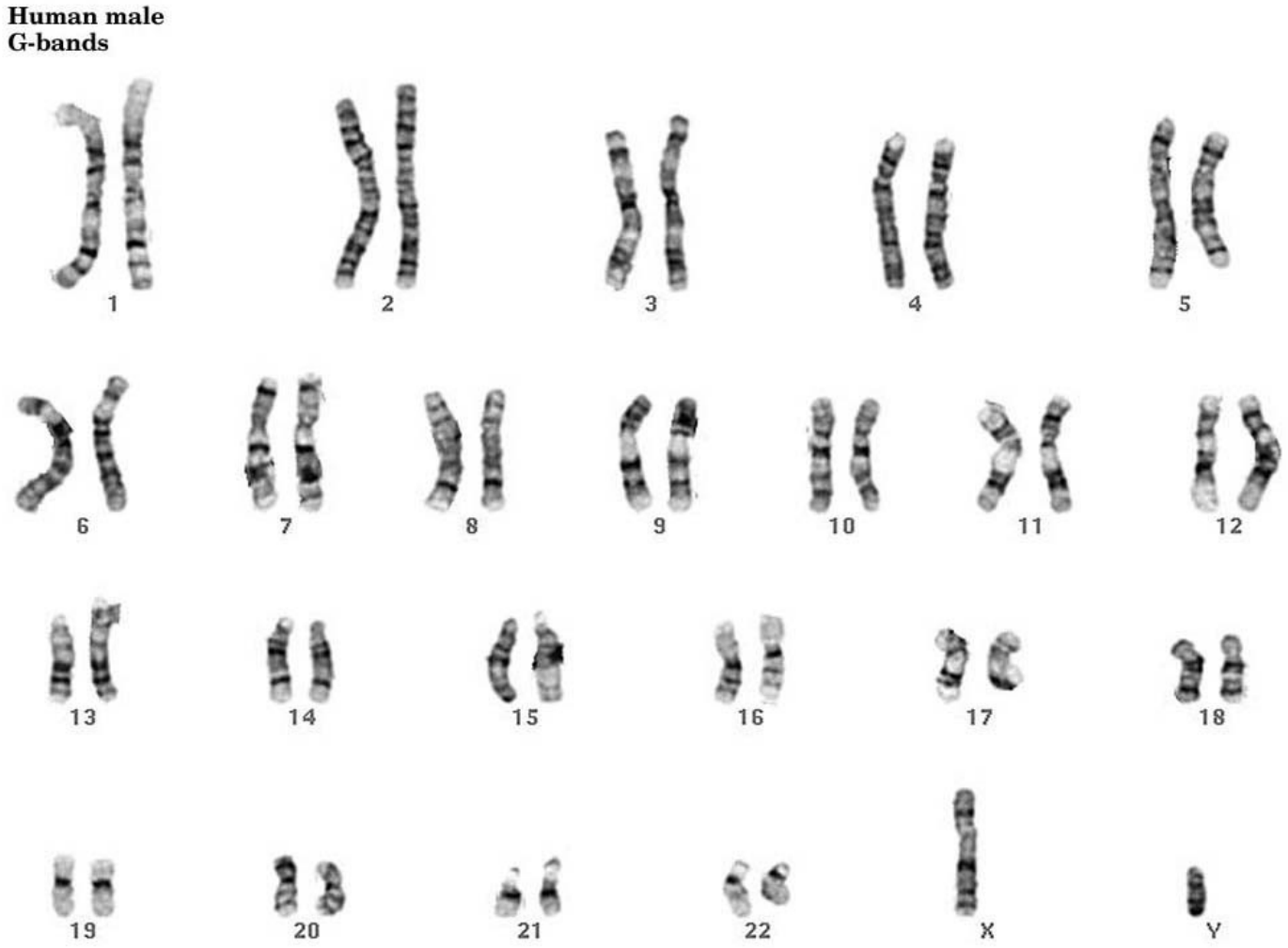


Image source: Department of Pathology. Cytogenetics Gallery, University of Washington, www.pathology.washington.edu/galleries/cytogallery/main.php?file.

Karyotype results are provided in a unique format which is demonstrated in Figure 3 below. Commas separate each finding within a karyotype. A slash separates different karyotypes identified in the sample. Different karyotypes are identified when findings are detected in some, but not all cells. In Figure 3, a slash is used to indicate two different karyotypes were identified from the examined cells. The number of cells demonstrating each karyotype is denoted in brackets following each karyotype description.

When reporting karyotype results, a data manager must distinguish between clonal and non-clonal findings. Clonal abnormalities are present in multiple cells and indicate a separate cell line, such as a malignant population, is present. If an abnormality is only detected in a single cell (or two cells in the case of deletions), it should not be reported. In this case, the finding could represent an isolated non-clonal abnormality or an inaccurate observation by the examiner. Refer to the general reporting guidelines below when determining which abnormalities to report.

Additions: must be present in at least two cells.
Deletions: must be present in at least three cells.
Translocations: must be present in at least two cells.
Inversions: must be present in at least two cells.

Karyotyping may also detect constitutional abnormalities. These are abnormalities present since birth. Examples include, but are not limited to, trisomy 21 and Klinefelter's syndrome. It is not necessary to report constitutional abnormalities when reporting karyotyping results.

Figure 3. Karyotype Results

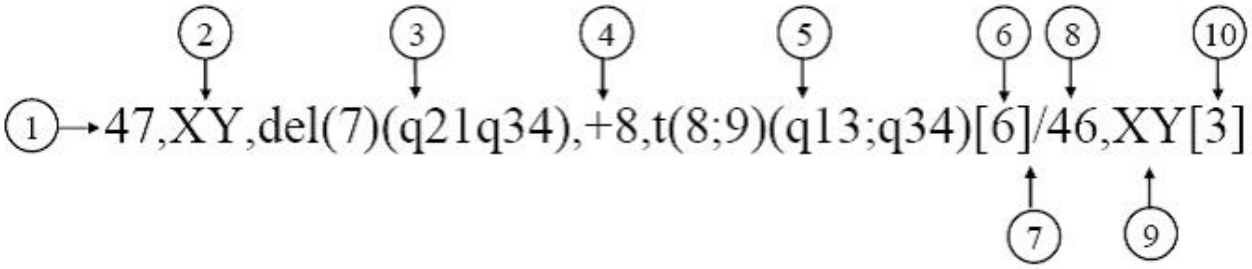


Table 2. Karyotype Results

Number	Definition
1	Number of chromosomes detected.
2	Sex chromosomes.

3	Deletion of chromosomal material on the long arm of chromosome 7 between regions 21 and 34.
4	Trisomy 8; extra chromosome 8.
5	Translocation of chromosomal material on the long arm of chromosome 8 and the long arm of chromosome 9.
6	Number of cells (metaphases) examined with these abnormalities.
7	Separates information about differing karyotypes.
8	Number of chromosomes detected.
9	Sex chromosomes.
10	Number of cells examined with this normal karyotype.

FISH is a molecular cytogenetic technique using fluorescent probes that bind to a **specific** part of a chromosome (i.e., the probes recognize and bind to fragments of DNA). It is a sensitive technique that can assess hundreds of cells per test. Theprobes are mixed with cells from the tissue sample. A fluorescent "tag" is then used to visualize the binding of the probe to the cells. Probes can identify the number of chromosomes or gene copies within a cell as well as the relative locations of specific genes or chromosome regions. Unlike karyotype assessments, FISH can be done on non-dividing, or interphase, cells. A FISH assessment typically examines between 200 and 500 cells.

Each probe has a specific target or set of targets and is therefore only capable of detecting abnormalities associated with that area. Additionally, the type of probe used affects the interpretation of the results. See below for descriptions of common categories of FISH probes.

Centromere Enumeration Probe (CEP): targets the centromere and is used to count the number of a specific chromosome in a cell (e.g., trisomy 8 or +8).
Locus Specific: targets a single locus other than the centromere and is used to detect additions, deletions, or rearrangements.
Dual Fusion: targets two loci and is used to detect translocations.
Break-apart: used to confirm gene rearrangements. The 3' portion of the gene or region is in one color and the 5' is in another color. If rearranged, colors are separate.

It is important to know what a FISH assessment is testing for before trying to interpret the results. For instance, a probe specific to the p arm of chromosome 9 would not be capable of detecting a deletion anywhere on chromosome 4. In Figure 4 below, two cells were exposed to centromere enumeration probes (CEP) specific to chromosomes 6 and 8 in order to determine the number of each chromosome present. Both cells have two copies of chromosome 6 (green probes) and three copies of chromosome 8 (red probes). This FISH result indicates the presence of a trisomy of chromosome 8.

Figure 4. FISH

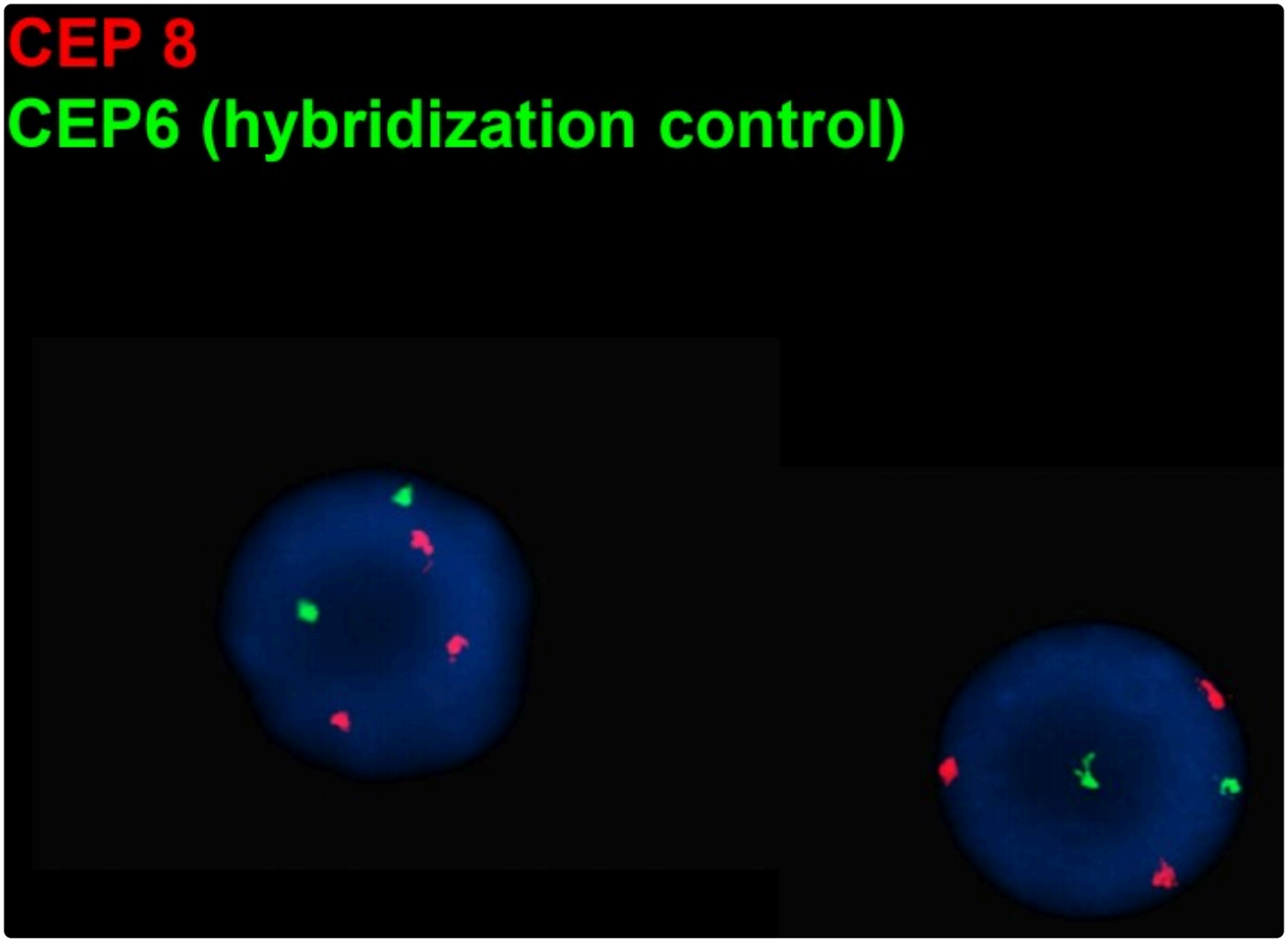
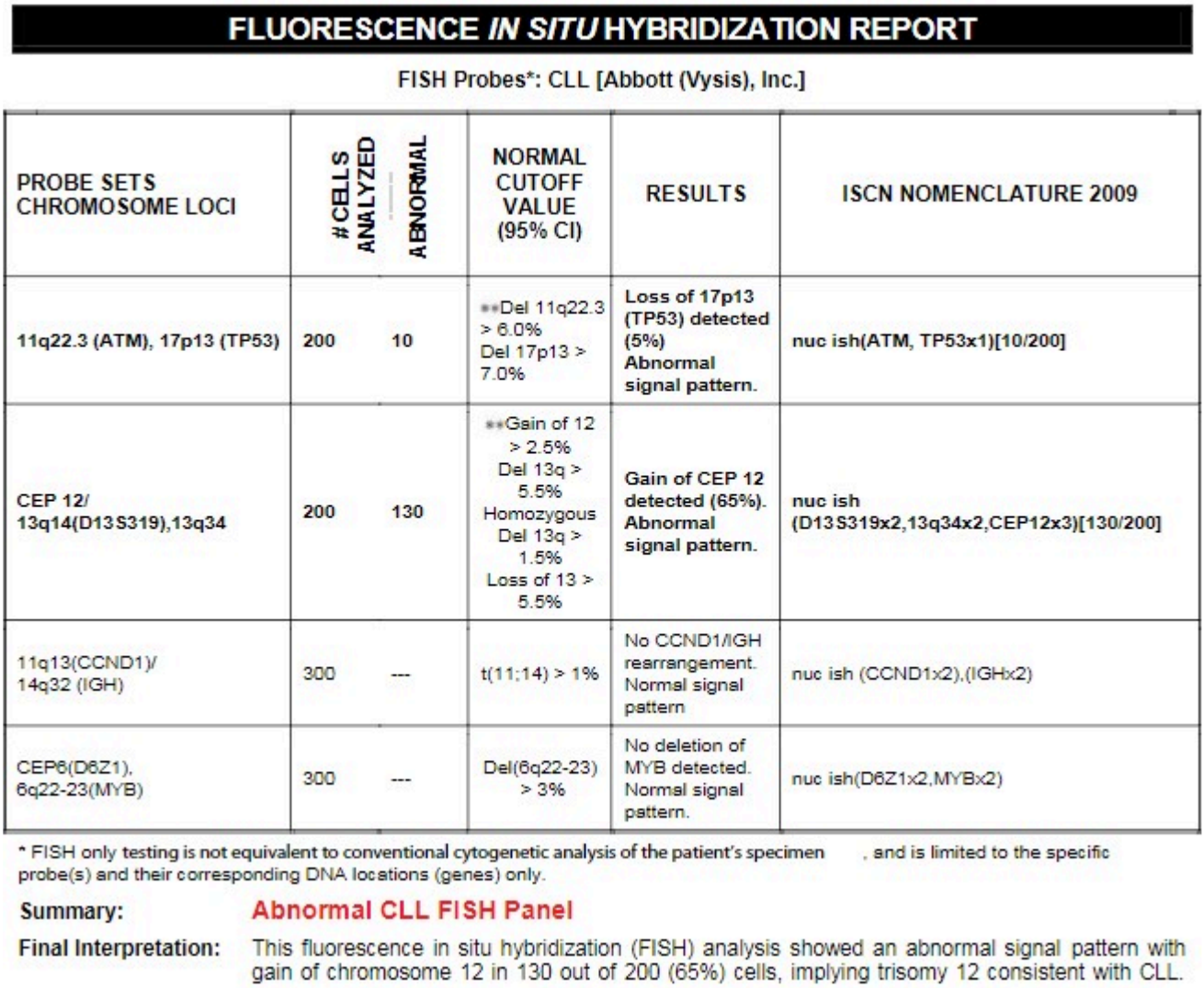


Image source: Weisdorf, Daniel J., MD. “Cytogenetics.” 2017 Clinical Research Professionals / Data Management Conference. Orlando. 21 Feb. 2017. Cibmtr.org. Web. 6 Dec. 2017.

FISH results are usually provided as a percentage or ratio of cells, for which, an abnormality was detected. The result may also be accompanied by a normal range to define when the test is considered positive for the abnormality being assessed. An interpretation / impression section of the report is also common and should be referenced when reporting testing results. Figure 5 is an example FISH report. It includes the identity of each probe, the number of abnormal cells, the normal range, a result for each probe, and a final interpretation. The report confirms the TP53 and CEP12 probes detected abnormalities; however, the TP53 probe did not detect an abnormality at a rate above the normal cut off value (7%). The final interpretation indicates these findings represent a gain of chromosome 12 (trisomy).

Figure 5. FISH Results



FLUORESCENCE *IN SITU* HYBRIDIZATION REPORT

FISH Probes*: CLL [Abbott (Vysis), Inc.]

PROBE SETS CHROMOSOME LOCI	# CELLS ANALYZED	# CELLS ABNORMAL	NORMAL CUTOFF VALUE (95% CI)	RESULTS	ISCN NOMENCLATURE 2009
11q22.3 (ATM), 17p13 (TP53)	200	10	**Del 11q22.3 > 6.0% Del 17p13 > 7.0%	Loss of 17p13 (TP53) detected (5%) Abnormal signal pattern.	nuc ish(ATM, TP53x1)[10/200]
CEP 12/ 13q14(D13S319),13q34	200	130	**Gain of 12 > 2.5% Del 13q > 5.5% Homozygous Del 13q > 1.5% Loss of 13 > 5.5%	Gain of CEP 12 detected (65%). Abnormal signal pattern.	nuc ish (D13S319x2,13q34x2,CEP12x3)[130/200]
11q13(CCND1)/ 14q32 (IGH)	300	---	t(11;14) > 1%	No CCND1/IGH rearrangement. Normal signal pattern	nuc ish (CCND1x2),(IGHx2)
CEP6(D6Z1), 6q22-23(MYB)	300	---	Del(6q22-23) > 3%	No deletion of MYB detected. Normal signal pattern.	nuc ish(D6Z1x2,MYBx2)

* FISH only testing is not equivalent to conventional cytogenetic analysis of the patient's specimen , and is limited to the specific probe(s) and their corresponding DNA locations (genes) only.

Summary: **Abnormal CLL FISH Panel**

Final Interpretation: This fluorescence in situ hybridization (FISH) analysis showed an abnormal signal pattern with gain of chromosome 12 in 130 out of 200 (65%) cells, implying trisomy 12 consistent with CLL.

Image source: Cancer Genetics, INC. "CGI Sample Reports." Issuu. N.p., 16 Nov. 2013. Web. 11 Dec. 2017. https://issuu.com/cgi201/docs/cgi_sample_reports_booklet/37>.

FISH reports may only refer to a probe by a gene name without indicating the chromosome number / region. For example, the report in Figure 5 could have only specified an ATM probe was used without also indicating the gene location was 11q22.3. It may be necessary, depending on the CIBMTR form, to know the gene location to accurately report the test results. The laboratory performing the study is the best resource for more information about the test that was done. A probe search can also be done using the HUGO Gene Nomenclature Committee's website genenames.org. This website provides gene symbols, approved names, associated names, and chromosomal locations for many of the probes in current use.

Chimerism and Disease Assessment

Cytogenetic assessments can be performed to identify markers of disease, determine chimerism following an allogeneic cellular infusion, or both. Cytogenetic assessment of chimerism is usually only done for sex mismatched pairs of recipients and donors. In these cases, a karyotype or FISH study can determine the ratio of cells containing female vs. male sex chromosomes. A unique form of karyotype assessment, Q

banding, can also be used to assess chimerism for sex matched recipient and donor pairs; however, molecular techniques involving PCR amplification are much more common.

Disease assessment by cytogenetic methods involves the identification of disease-specific markers (e.g., -7, del(5q), Philadelphia chromosome). Once a marker is identified, cytogenetic assessments can be repeated to determine whether the marker, and therefore the disease, is still detectable. The types of markers identified can affect the disease classification and inform the treatment plan. A cytogenetic assessment cannot be considered a disease assessment until this method has detected a marker of disease. In other words, if cytogenetic studies have always been negative, the recipient's disease is not considered to be assessed by this method because there are no known cytogenetic abnormalities to evaluate. Pay attention to the wording of the question on the CIBMTR form being completed. "Was testing performed?" may be answered differently than "Was the disease assessed?"

CIBMTR forms generally capture chimerism data separately from disease assessment data. Therefore, it is important to know what information can be reported based on the assessment performed.

Example: Consider a recipient of an allogeneic product obtained from a sex mismatched donor as part of treatment for AML. The cytogenetic abnormality t(8;21) was identified as a marker of this recipient's disease on previous cytogenetic assessments. Would the following cytogenetic assessments be reported in chimerism data fields, disease assessment data fields, or both?

> **Karyotype:** report this assessment in both chimerism and disease assessment data fields. A karyotype is capable of detecting autosomal and sex chromosomes. The test would confirm whether the t(8;21) abnormality was still present and also provide a ratio of female to male cells.
> **FISH [X / Y probe(s) only]:** only report this assessment in chimerism data fields. The probes are able to provide a ratio of female to male cells, but are not capable of detecting the t(8;21) abnormality.
> **FISH [t(8;21) probes only]:** only report this assessment in disease assessment data fields. The probes are able to detect the t(8;21) abnormality, but are not capable of providing a ratio of female to male cells.
> **FISH [X / Y probe(s) and t(8;21) break apart probe]:** report this assessment in both chimerism and disease assessment data fields. The X / Y probe(s) will provide chimerism data while the t(8;21) probe results will be captured as a disease assessment.

Manual Updates:
Sections of the Forms Instruction Manual are frequently updated. The most recent updates to the manual can be found below. For additional information, select the manual section and review the updated text.

Date	Manual Section	Add/ Remove/ Modify	Description
2/ 20/ 18	Appendix C: Cytogenetic Assessments	Add	Added table below figure 3 to explain karyotype findings.

2/12/18	Appendix C: Cytogenetic Assessments	Add	Added the following description of constitutional abnormalities. *Karyotyping may also detect constitutional abnormalities. These are abnormalities present since birth. Examples include, but are not limited to, trisomy 21 and Klinefelter's syndrome. It is not necessary to report constitutional abnormalities when reporting karyotyping results.*
2/9/18	Appendix C: Cytogenetic Assessments	Modify	Version 2 of Appendix C: Cytogenetic Assessments of the Forms Instruction Manual released.

Last modified: Mar 20, 2018