D5683
(Rev. 140, Issued: 05-29-15, Effective: 05-29-15, Implementation: 05-29-15)

§493.1276 Standard: Clinical cytogenetics

(b) The laboratory must have records that document the following:

(b)(1) The media used, reactions observed, number of cells counted, number of cells karyotyped, number of chromosomes counted for each metaphase spread, and the quality of the banding.

(b)(2) The resolution is appropriate for the type of tissue or specimen and the type of study required based on the clinical information provided to the laboratory.

(b)(3) An adequate number of karyotypes are prepared for each patient.

Interpretive Guidelines §493.1276(b)(1)-(b)(3)Culture Type	**Minimum Number of Spreads Counted per Patient**	**Minimum Number of Cells Analyzed per Patient**
Amniotic Fluid		
Flasks	15 cells from at least 2 independent primary cultures	5 cells from at least 2 independent primary cultures
in situ	15 cells from at least 10 colonies from 2 independent primary cultures	5 cells from different colonies and split between different primary cultures

Many laboratories use a combination of the flask and in situ culture methods or use the flask method as a backup for the in situ method.

Chorionic Villus		
Direct	15 cells	5 cells
Culture	as in amniotic fluid, flask technique	
Peripheral Blood		
Constitutional	20 cells	5 cells
Possible sex chromosome abnormality	30 cells (total count)	5 cells

Culture Type	**Minimum Number of Spreads Counted per Patient**	**Minimum Number of Cells Analyzed per Patient**
Blood (cancer)	20 cells	20 cells

Culture Type	Minimum Number of Spreads Counted per Patient	Minimum Number of Cells Analyzed per Patient
Bone Marrow (cancer)	20 cells	20 cells
Tissue Fibroblasts	15 cells from 2 independent cultures	5 cells split between 2 independent cell cultures

For confirmation of chromosomally abnormal amniotic fluid results, or familial chromosome abnormality, examination of fewer cells is permitted.

A number of factors may influence the quality of the metaphase spreading (e.g., humidity, air flow, cell concentration, and cell storage conditions).
An analysis of at least 50 cells is recommended when:

- Single trisomic cells are found during a study;
- Mosaicism is suspected on the basis of a phenotype not correlating with the karyotype during the study; or
- Sex chromosome abnormalities are suspected.

Additionally, when mosaicism is suspected, ensure that an adequate number of cells or nuclei are scored.

- Follow manufacturer's instructions for the probe in accordance with the FDA requirements for "Analyte Specific Reagents (ASR)."
- Establish or verify test system performance using each new probe and each new lot of probe in accordance with D5421 or D5423; thereafter the laboratory must ensure test methodology performance in accordance with D5411.
- Establish criteria for scoring the number of probe signals and the number of cells to be examined. Use D5425.

For fragile X analysis:

- Males - at least 50-100 cells should be scored for negative analysis.
- Females - at least 100-150 cells should be scored for negative analysis.

The presence of the Xq27.3 fragile site should be confirmed with chromosome banding.

Fragile X studies require low folate medium and media which includes treatment with an antimetabolite such as fluorodeoxyuridine (FUdR), methotrexate, excess thymidine,

fluordeoxycytidine (FdC) or other proven induction systems.

General guidance

Examine the karyotypes and a slide from among the laboratory cases and determine if the quality of banding and resolution was sufficient to render the reported interpretation. Examination of the long arm on the 18th chromosome should demonstrate at least two distinct dark staining G-bands at the 400 band level.

Verify that the laboratory's policy establishes a specific band level of resolution that would be dependent upon the study requested.

High resolution chromosome analysis should refer to studies done above the 550 band stage. (Above 650 band stage for an unfocused study. A focused study should be done at a level of resolution at which the band in question is clearly separated from surrounding bands in one member of the homologous pair in question.) Use D5683.

Probes §493.1276(b)(1)-(b)(3)

For fragile X analysis, if a folate deficient medium is not used as described above, how does the laboratory ensure the validity of the test system and the accuracy of results? Use D5411 or D5413, as applicable.

How many photographic and/or computerized karyotypes are prepared from each cell line? (A minimum of 2 is recommended.)

What band level of resolution is used by the laboratory to rule out structural defects (i.e., routine or 400-500 band stage, or high resolution or 650-850 band stage)?