# NEW YORK STATE DEPARTMENT OF HEALTH
# CLINICAL LABORTORY EVALUATION PROGRAM

## COMMENTS and RESPONSES to PROPOSED CYTOGENETICS STANDARDS

The Proposed Standards in the areas of Cytogenetics were circulated for comment on March 1, 2016. The announcement was sent to NYS-permitted facilities that held or were in application for a permit (facilities). This distribution was by e-mail to the facility and laboratory contact person's e-mail address. The documents were posted to the CLEP website.

The comment period ended April 22, 2016. A total of four comments were received.

The standards are considered to be accepted and will be adopted and effective as of August 5, 2016.

| Standard | Guidance |
|---|---|
| **Cytogenetics Sustaining Standard of Practice 21 (CG S21): Reporting**<br><br>In addition to the requirements of Part 58, the final report shall include:<br><br>a) use of the current International System for Human Cytogenetic Nomenclature (ISCN);<br><br>b) the number of cells analyzed and, when applicable, the number from which karyotypes were prepared;<br><br>c) band resolution attained;<br><br>d) in cases of culture failure or where a definitive diagnosis is not possible, suggestions for additional testing;<br><br>e) an interpretation of findings;<br><br>f) a statement on limitations of the test, including possible inaccuracies;<br><br>g) suggestions as to whom the physician and/or patient may consult for discussion of prognosis implications of abnormal results (e.g., genetic counseling);<br><br>h) Reports that include FISH results must include:<br>1) number of cells analyzed<br>2) probe target and vendor<br>3) cutoff values for interphase FISH, and<br><br>i) Reports that include CMA must include:<br>1) platform description, including number and distribution of probes<br>2) genome build used for analysis and interpretation. | A summary and interpretation of the results are recommended.<br><br>a) Results may be reported in other formats in addition to ISCN |

**Comment 1:**

We do not currently report our cutoffs, as we are concerned that clinicians will make decisions that they should not make if the value is below our cutoffs. We currently put a comment on reports for values that are above, but close to our cutoff, to warn the clinician to carefully review before considering this truly positive:

"The decision to call this FISH result abnormal is based on our laboratory validation data for this probe that indicates >__% abnormal cells is considered a positive result. However, as the percentage of abnormal cells in this case is close to our cut-off value, correlation of this finding with other laboratory and clinical data is strongly recommended."

**RESPONSE 1:**

*The cutoff values provide information for clinicians regarding the limit of detection of the test.*

**Comment 2:**

What value is provided to the client by having the FISH probe vendor noted on the final report when the gene and target regions are on the report?

**RESPONSE 2:**

*Different vendors' probes may bind different regions at or near the same target(s). The precise probe location may be clinically relevant in some situations.*

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| **Cytogenetics Sustaining Standard of Practice 16 (CG S16): Metaphase Analysis**<br><br>The laboratory shall analyze a minimum number of metaphases as indicated below:<br><br>a) a minimum of 20 metaphases, except for prenatal, in situ, which requires 15 metaphases; and,<br>b) count cells from at least two cultures for all specimens except peripheral blood for constitutional chromosome abnormality analysis. | Analyzed means to establish the number of centric chromosomes in a metaphase AND evaluate individual chromosomes in their entirety, i.e., each metaphase is critically analyzed, including chromosome count, sex chromosome complement, cytogenetic aberrations and vernier location.<br><br>The minimum count will often be exceeded when multiple cell lines are observed. Based on a laboratory's pre-established criteria, cells from replicate cultures may be analyzed.<br><br>When mosaicism is suspected on the basis of a phenotype that does not fit with the karyotype or when sex chromosome abnormalities are suspected~~, or when single trisomic cells are found during a study,~~ an analysis of at least 50 cells is recommended. |

**Comment 1:**
This standard is more stringent than both ACMG and CAP (see below). With the exception of sex chromosomes, it is unclear what the added value is of analyzing 20 metaphases per case.

Section E5.1.2 of the ACMG Standards and Guidelines for Clinical Genetics Laboratories reads:

> 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.

Section CYG.41100 (Analysis – Non-neoplastic Samples) of the CAP Cytogenetics Checklist reads:

> A minimum of five cells, with the exception of abbreviated studies, are analyzed.

**RESPONSE 1:**
*Analysis of 20 metaphases provides greater power to detect mosaic aberrations. Analysis of five cells would not reliably detect mosaic copy number aberrations that are detectable by many CMA tests. It is not clear why lower sensitivity in chromosome analysis would be desirable.*

**Comment 2:**
*Our recommendation: Follow the ACMG guidelines stated below.*

Section E5.1.2.2 of the ACMG Standards and Guidelines for Clinical Genetics Laboratories (revised in 2010) reads:

> "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."

**RESPONSE 2:**

*Language similar to this guideline has been added to the guidance for CG S12: "When one cell with a sex chromosome aberration is identified by routine analysis, it is recommended that a minimum of 10 additional metaphase cells be analyzed."*