**Review Criteria for Assessment of Cytogenetic Analysis Using Automated and Semi-Automated Chromosome Analyzers (Text Only)**

This guidance was written prior to the February 27, 1997 implementation of FDA’s Good Guidance Practices, GGP’s. It does not create or confer rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both. This guidance will be updated in the next revision to include the standard elements of GGP’s.

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REVIEW CRITERIA FOR ASSESSMENT OF CYTOGENETIC ANALYSIS

USING AUTOMATED AND SEMI-AUTOMATED CHROMOSOME ANALYZERS.

This is a flexible draft document representing the current major

concerns and suggestions regarding in vitro automated and

semi-automated chromosome analyzers for cytogenetic analysis. It

is based on 1) current basic science, 2) clinical experience, 3)

current standard laboratory practice, and 4) previous submissions

by manufacturers to the FDA. As advances are made in science and

medicine, these review criteria will be re-evaluated and revised

as necessary to accommodate new knowledge.

PURPOSE: The purpose of this document is to provide

guidance on information to present to the

Food and Drug Administration (FDA) before a

device for chromosome analysis may be cleared

for marketing. This information enables FDA

to make better informed decisions based on a

uniform data base.

DEFINITION: The generic type device is intended for use

in the cytogenetics laboratory to aid

laboratory personnel in performing certain

procedures used in karyotyping human

metaphase/prometaphase cells for in vitro

cytogenetic analysis.

PRODUCT CODE: LNJ - 88

REGULATION

NUMBER: CFR ~ 864.5260 Automated cell-locating

device.

PANEL: PATHOLOGY

CLASS: II

REVIEW REQUIRED: 510(k)

REGULATORY ISSUES:

Manufacturers are encouraged to develop devices that will be

compatible with guidelines of laboratory regulatory

organizations.

Most cytogenetics laboratories subscribe to certain proficiency

testing and accreditation agencies or are state regulated. The

College oœ American Pathologist (CAP) and the Council of Regional

Genetics Networks (CORN) offer voluntary proficiency testing. In

New York State, the Department of Health is responsible for

laboratory testing and certification; the State of Oregon

licenses cytogenetics laboratories.

In New York State, cytogenetics laboratories that use image

analysis computer systems must develop a system for record

retention and retrieval that meets laboratory licensure quality

control standards.

I. Background

A. Cytogenetics in Clinical Practice

Cytogenetic analysis is an in vitro clinical laboratory

procedure that evaluates the chromosomes of a cell.

Clinical cytogenetics is the study of chromosomes and

their correlation to the phenotype (observable clinical

characteristics). Certain clinical characteristics

occur consistently in association with a particular

chromosome abnormality. This phenotype-karyotype

correlation is useful to the clinician in making a

clinical genetic diagnosis and prognosis.

There are two basic classes of chromosome aberrations:

numeric and structural. Common abnormalities of

chromosome number include aneuploidy, mosaicism, and

polyploidy. Structural abnormalities include

duplications, deletions, inversions, shifts, fragile

sites, etc.

Chromosome aberrations are found in association with

many anomalies of sexual development such as the Turner

syndrome (XO) and the Kleinfelter syndrome (XXY);

mental retardation (Trisomy 21 in the Down syndrome and

fragile X in the Fragile X syndrome); complex

malformation syndromes (Trisomy 13); spontaneous

abortions; and malignant disorders (chromosome

translocation between chromosome 9 and 22 in chronic

myeloid leukemia).

In addition to studies for detection of classic

chromosome abnormalities, more recent biotechnologies

add a new dimension to traditional cytogenetic

analysis. Cytogenetic procedures may be used to study

cell cycle associated phenomenon, gene amplification

(homogeneous staining regions and double minutes),

clastogen challenge, chromosome breakage syndromes,

chromosome fragile sites, and polymorphisms to monitor

organ/tissue transplantation. DNA probes that are site

specific are used for identification of chromosome

abnormalities by in situ hybridization using chromosome

specific DNA probes and for purposes of gene mapping.

Clinicians request cytogenetic analysis for individuals

with clinical findings or a medical or family history

indicative of a chromosome abnormality. Requests for

cytogenetic studies have increased in the last decade

due to the demand for prenatal chromosome testing and

because of the widespread use of chromosome analysis in

cancel diagnosis and monitoring.

An accurate cytogenetics analysis is essential for the

clinician to make an accurate clinical genetic

diagnosis and prognosis for patient management,

pregnancy planning and prenatal diagnosis.

An incorrect diagnosis, both false positive and false

negative, will have far reaching medical and legal

implications. Therefore it is imperative that any

device used for purposes of cytogenetic analysis is

sensitive, specific, safe, and effective.

B. Historical Background

The correct chromosome number in humans, 46, was determined

in 1956 by Tjio and Levan2. Prior to this date the normal

human chromosome number was considered to be 48. With this

new knowledge came the recognition that certain chromosome

abnormalities were associated with specific congenital

defects.

Improved techniques for handling mitotic chromosomes

awakened interest in human cytogenetics. In 1959 Lejeune,

et al., described the first chromosome abnormality

associated with a clinical syndrome, trisomy 21 in the Down

syndrome3. With the advent of chromosome banding techniques

in the 1970s, it was possible to identify with certainty all

chromosome pairs and to characterize more accurately

abnormalities of chromosome number and structure. For a

given banding technique, each chromosome pair exhibits a

unique pattern of differential staining along the length of

the chromosome.

The traditional method for analyzing chromosomes is labor

intensive. By the early 1960s, development of instruments

for 4 automated analysis of metaphase chromosomes was well

under way. The primary objective was to develop automated

systems which perform as well as a cytogeneticist using

conventional techniques and are faster and more cost

effective.

Because of the rapid increase in the work load of the

cytogenetics laboratory and the improved computer

capabilities for image processing, these devices are being

used with increased frequency to automate parts of the

manual procedure.5,6 Various computer hardware and software

features are available which are designed to assist in one

or more steps of the process outlined in II.C.

C. Basic Steps in Routine Cytogenetic Analysis7,8,9

1. Specimen Types and Cell Preparation. (Automated

chromosome analyzers are not involved in this step.)

a. Obtain a specimen with a large population of

cycling (dividing) cells

Specimens that contain rapidly dividing cells (e g ,

bone marrow, solid tumors and chorionic villus) may be

harvested without culturing (direct method). Other

types of issue (e g., peripheral blood lymphocytes,

skin fibroblasts and amniotic fluid cells) must be

cultured in a nutritive media and controlled

environment before harvesting.

b. Harvest the cells

Arrest cell division at the metaphase stage of the cell

cycle by adding colcemid or a similar agent that

inhibits spindle fiber formation. Treat the cells with

a hypotonic agent to swell the cells and to facilitate

a better spread of the metaphase chromosomes when they

are dropped onto a microscope slide or are grown on

coverslips. Treat them with a fixative to kill the

cells, clarify the chromosome morphology, and enhance

the basophilic property of the chromosomes.

c. Prepare the slides and stain the cells

2. Selection and Analysis of cell with Metaphase

Chromosomes (Automated Chromosome Analyzers may modify

or aid in one or more of these processes.)

a. Select a predetermined number of metaphase spreads

of suitable quality for study and count the

chromosomes to determine the modal chromosome

number.

b. Select several representative spreads for detailed

analysis. The number of cells selected varies

depending on individual laboratory practices and

the clinical indication for testing.

c. Photograph the representative metaphase spreads

and make an appropriate number of photographic

prints.

d. Cut individual chromosomes from the prints. Pair

and arrange them in a standard format following

the International System for Human Cytogenetic

Nomenclature (ISCN), 1985 guidelines10.

e. Prepare a final report using standard nomenclature

that includes a summary and interpretation of the

reservations and the number of cells counted and

analyzed. Send the report to the referring

clinician. Keep permanent records including a

copy of the final report, the original metaphase

images and karyotypes, and the microscope slides

on file for a period of time determined by the

individual institution or other regulatory agency.

II. Device Description

A. General Principles and Features

Key issues in the review of these devices center on

specific intended use statements and claims dependent

on the type of device manufactured.

The following features/capabilities are representative

of devices currently marketed or being developed in the

United States for clinical use. Describe fully these

and any other features for which claims are made in the

labeling section of the 51OK submission.

Work Station

Hardware/Software

Electronic camera with automatic focusing

Monitor (color, monochrome)

Word Processor

Special Decision Making Features

Automatic karyotyping

Metaphase finding

Chromosome finding

Satellite analysis

in situ hybridization analysis

Sister chromatin exchange (SCE) analysis

Automatic cutting and/or separation of

chromosomes

Image manipulation

Positioning

Rotating

Moving

Eliminating artifact (dodging)

Enlarging selected metaphases, chromosome

pairs, or individual chromosomes

Enhancing

Contrasting

Image capturing process by a TV camera

Resolving gray scale (levels of grayness)

Printer interface

Microscope interface

Capacity for networking

Data storage mechanism and capacity

Ability to recognize and analyze prophase

chromosomes

Staining methods the instrument can utilize

Chromosome recognition capability

Training feature

B. Description of Specific Features:

1. Metaphase Finders/Scanners aid the

cytotechnologist to locate rapidly suitable

metaphases for analysis. These instruments

automatically scan the microscope slide to locate

likely metaphase spreads. The instrument may rank

the metaphase cells according to quality and store

their microscope slide coordinates in the

computer's data base. Metaphase finding/scanning

instruments are not always accurate. In some

cases their use is limited to specific types of

staining and they may not be appropriate for use

with certain types of disorders.

2. Chromosome Counters determine the chromosome

number by automatically counting the number of

chromosomes in a given metaphase spread.

3. Photomicroscopy and Photographic Dark Room

Processes eliminate the need for photomicroscopy,

photographic dark room work and cutting and

pasting chromosomes when performing karyotyping.

The process uses digital image processing to

digitize the metaphase images by dividing the

picture into a grid of pixels. The resolution and

detail is determined by the number of pixels in

the image and the range in the level of contrast

(grayness). The level of grayness may

theoretically range from 0 to 256. Optical

information about each pixel as well as its

location may be processed and stored in the

computer data base. The metaphase chromosomes are

manipulated (cut) and arranged (pasted) in pairs

on the karyotype card (projected onto the computer

monitor). Some instruments are designed to do the

"cutting and pasting" automatically (see section,

B.4.b., below) - others require the operator to

manipulate the images (see section B.4.a., below).

4. Interactive and Automatic Karyotyping

a. Interactive systems have no decision making

ability and depend on the operator to

classify the chromosomes from the computer

screen and arrange them on the computerized

karyotype sheet.

b. Automatic karyotyping systems exist with

varying amounts of decision making ability.

Chromosomes are classified on the basis of

chromosome dimension (e.g., ratio of short

arm to long arm) and banding pattern profile.

5. Enhancement, Alteration and Manipulative Features.

Some instruments have features that enhance or

contrast chromosome images in metaphases and/or

individual chromosomes to improve the banding

characteristics of the chromosome(s). Other

features alter or allow the operator to alter

chromosome morphology and/or other cellular

characteristics. These features include:

straightening, enlarging, trimming,

"mirror-image", "enhancement" (selectively

altering staining pattern within a metaphase

spread or within a chromosome region), and

dodging/lifting the cytoplasmic background.

6. Specialized Analysis (in development) A few

instruments have the ability to perform analysis

of specialized studies such as chromosome

satellites, in situ hybridization and sister

chromatid exchange (SCE). Some chromosome

analyzers automatically count the number of

satellites or SCEs per metaphase cell or the

number of hybridized probe sites per interphase or

metaphase cell. Although this feature is currently

being used for research purposes, it has not been

cleared by the FDA for clinical use.

7. Hard Copy Prints. Most systems are capable of

producing near photographic quality, printer

generated hard copy of the metaphase images and

karyotypes.

8. Generating Reports. Many instruments have

capabilities of generating (preparation &

printing) a final summary for the referring

clinician and of handling the billing process and

other bookkeeping.

9. Computerized Patient Data Storage, Retrieval and

Archival Systems. A data base may include

metaphase images and karyotypes, patient

identifying information, and final reports.

10. Training Feature (in development). This feature

permits the operator to teach the system to

recognize the chromosomes preparations particular

to a given laboratory. It allows the operator to

"train" the instrument to recognize different

staining preparations. Although this feature is

currently being used for research purposes, it has

not been cleared by the FDA for clinical use.

11. Telecommunication Features allows for site-to-site

image transmission.

12. Networking features provide networking between

workstations and local areas.

III. Specific Performance Characteristics

Support specific parameters of importance to the operation

of the instrument by data generated with the device.

Demonstrate that the device is substantially equivalent to a

legally marketed predicate device. Conduct performance

studies to demonstrate that the device is safe and effective

by comparing the device's performance to the manual

reference method of chromosome analysis. See section II.B.,

"Device Description" for details of performance

characteristics required for specific feature.

Address all aspects of performance characteristics as stated

in section III.C. in the Performance Characteristics section

of the Labeling. Provide the following specific information

on reproducibility/precision and accuracy for instruments

with features that warrant such studies. Include a detailed

study protocol, generated data and statistical analysis of

the data in any submission to the include a summary of the

performance data in the Performance Characteristics section

of the Labeling

A. Analytical/Laboratory/in vitro Studies

1. Reproducibility Studies

Study a sufficient number of control specimens and

test specimens with the types of chromosome

abnormalities or characteristics for which claims

are made (e.g., normal, aneuploidies, structural

rearrangements, fragile sites, sister chromatin

exchange, etc.) to demonstrate:

a. Within Sample Reproducibility

Does the device give the same results on

repeated trials (analysis) of a given

procedure? e.g., does it locate the same

cells on a given slide, rank cells in the

same manner, give the same chromosome count

on a given cell, generate the same karyotype

for a given cell, etc.

b. Between Instrument Reproducibility

Do different devices give the same results

for a given procedure? e.g. do they locate

the same cells on a given slide, rank the

cells in the same manner, give the same

chromosome count on a given cell, generate

the same karyotype for a given cell, etc.

2. Comparison Studies

Comparison studies provide data on the ability of

the instrument to determine accurately specific

results as compared to the manual method for

chromosome analysis. (If a method other than the

manual method is used justify the choice of the

method and include pertinent references.)

Perform the test on a sufficient number of

specimens with and without chromosome aberrations

and calculate the following parameters:

a. Relative Diagnostic Sensitivity: the

probability that the instrument will

correctly identify an abnormality determined

to be abnormal by the reference method.

b. Relative Diagnostic Specificity: the

probability that the instrument will

correctly identify as normal a specimen

determined to be normal by the reference

method.

3. Specifications

Describe the relative quality: Does the

instrument achieve the same (or better band

resolution compared to the reference method

according to the ISCM (1985) guidelines10?

B. Software Documentation

All computer software should comply with the FDA's

Policy for Regulation of Computer Products. For

general information contact the FDA Division of Small

Manufacturers (phone, 800-638-2041). For specific

information contact the Division of Product

Surveillance (phone, 301-427-8156).

C. Special Considerations for Specific Features Described

in II.B.

All devices that have any decision making features

should also have a feature which allows the operator to

interact, edit and override the work generated by the

device.

Several features of imaging analysis computer systems

need special consideration which are addressed below.

For each of the following features, provide

reproducibility and comparison studies, specifications,

and software documentation unless otherwise specified.

Incorporate the following considerations as appropriate

in the Labeling (e.g., Intended Use, Methods,

Limitations, etc.).

1. Metaphase Finders

a. State which staining methods may be used with

the device. State what types of preparations

may be analyzed (e.g., air dried, grown on

coverslips, primary colonies, etc.)

b. Provide data to demonstrate that the device

does not introduce bias in selection of

metaphases with respect to chromosome number

(e.g., aneuploidy, polyploidy),

endoreduplication, poor chromosome morphology

as in some malignant cells, structural

chromosome abnormality (e.g., translocations,

dicentrics, fragments, etc.), or chromosome

staining factors.

c. Provide data to demonstrate the performance

of the metaphase ranking feature. State in

the Labeling whether metaphase finders will

or will not detect certain cellular

abnormalities such as micronuclei, nucleolar

organizing region (NOR) alterations and other

abnormalities of the cytoplasm or nucleus

which would be noted by an astute observer.

d. Since these instruments are not always

accurate for finding and ranking metaphases,

place the following or (similar) statement in

the Limitations Section of the Labeling.

"The cytogenetic technologist/

cytogeneticist should always review

slides independently of the metaphase

scanner/finders."

e. State whether or not it is appropriate to use

the metaphase finder for studies such as drug

sensitivity, tumor hard tissue, etc. State

any limitations in the Limitations section of

the Labeling.

2. Chromosome Counters

a. State which staining methods may be used with

the device.

b. State limitations imposed by how well the

chromosomes are spread (e.g., overlapping

chromosomes, too much spread, broken cells,

too many metaphases in one location, etc.).

c. Provide data to demonstrate that bias is not

introduced into chromosome counts by

aneuploidy, polyploidy, endoreduplication,

radial formations, chromosome pulverization,

poor chromosome morphology as in some

malignant cells, and structural chromosome

abnormality (dicentrics, fragments, etc.).

If any incorrect chromosome counts result,

this should be declared in the limitations

section of the package insert.

d. Include the following or similar statement in

the Limitations Section of the Labeling:

"In general there will be one or more

errors in determining the correct

chromosome number for the population of

cells studied. It is the responsibility

of the operator to determine the correct

modal chromosome number."

3. Interactive Karyotyping Systems (no decision

making ability)

Reproducibility and comparison studies are not

required since this feature has no decision making

ability.

4. Automated Karyotyping

Reproducibility and comparison studies are

required only for the decision making features of

the device.

Include the following or similar statement in the

Limitations section of the Labeling:

"In general, there will be one or more errors

in the computer-generated karyotype

Therefore, it must always be examined and

edited as a final interactive manual

operation by a qualified cytogeneticist or

cytogenetic technologist."

5. Enhancement, Alteration and Manipulation Features

Altering chromosome morphology in any way (aside

from improving culturing and staining techniques)

is not an accepted standard of professional

practice in cytogenetic analysis.

a. Comparison data are not required for

enhancement, alteration or manipulation

features.

b. Any device with features that in any way

alter chromosome morphology should also have

a built-in feature that automatically and

permanently marks/designates these

alterations in the karyotype.

c. Digitized straightening of chromosomes will

often artificially induce extra bands and

thus make it impossible to determine,

unequivocally, whether the straightened

chromosome is, indeed, normal. Designate any

straightened chromosomes as stat.

d. Assure that the device does not have the

capacity to induce artifacts.

e. Features that "lift" cellular background

should not be automatic and should have a

built-in feature that requires operator

activation. Such a feature should not be

used unless the operator has already examined

the cell to assure that the material to be

lifted is true artifact and not chromatin

material such as double minutes.

f. Include a statement about the potential for

misuse of each feature under the Limitations

section of the Labeling and elsewhere in the

operators manual whenever use of these

features are described. Use the following or

a similar statement about features listed in

this section:

"It is the responsibility of the

cytogenetics technician and/or the

cytogeneticist to utilize all features

in compliance with standard laboratory

practice and regulatory guidelines."

6. Photomicroscopy and Dark Room Process

a. Reproducibility and comparison data are not

required.

b. Compare the resolution of the digitized

images to standard microscopic resolution in

terms of the resolution standards (400, 500

and 850 bands) of the ISCN, 1985 guidelines10.

c. For devices that have no decision making

ability, include the following or similar

statement in the Intended Use section of the

Labeling:

"The device does not locate metaphase

spreads; it does not rank the given

cells according to quality; it does not

automatically classify chromosomes; it

does require and relies completely on

the operator to manipulate the digitized

microscope images."

7. Hard Copy Prints

a. Reproducibility and comparison data are not

required.

b. Describe the quality and resolution of the

hard copy print in the Principle of the

Procedure section of the Labeling. State

whether the quality/resolution of the

computer generated print is equivalent to (as

good as) standard photomicroscopy (levels of

gray) and whether the device meets the

minimum resolution standard set by the ISCN,

1985 guidelines.

c. Describe the composition, quality and

durability of the photo image paper and

whether contrast deteriorates with age.

i. A precaution/warning statement in the

Precautions section of the Labeling will

be required for paper containing mercury

or other toxic substances.

ii. State how long the prints will remain of

archival quality. The durability of

these hard copy prints is important

since some proficiency/licensing

agencies require long term storage of

metaphase images (up to 25 years for New

York State).

8. Computerized Patient Data Storage, Retrieval and

Archival System.

a. Reproducibility and comparison data are not

required.

b. This feature should comply with section

III.B. of this document. The following

issues are of special concern and should be

addressed in the Principles of Procedure

section of the labeling:

i. adequate security control which may

require several levels of "password"

security to assure protection and

confidentially of patient information;

ii. adequate safe guards to protect against

accidental or virus generated deletion

of the data

iii. an explanation of where and how the data

are stored; and

iv. a recommendation of multiple identifying

codes (e.g., for patient identifying

data, metaphases, karyotypes, final

report, etc.) to assure correct and

usable storage and retrieval of

information from the data base and

archival system.

IV. Labeling Considerations

The Labeling (Operator's Manual or Package Insert) should

include all information listed in the in vitro diagnostic

Labeling regulations 21 CFR ~ 809.10(b)(6) plus additional

pertinent headings found under 21 CFR ~ 809.10(b):

A. Intended Use Statement

Describe concisely the functions/features of the

device. State clearly that a qualified cytogenetic

technologist and/or cytogeneticist must edit and/or

confirm all computer-generated data/results and make

the final judgment/decision.

For devices that have no decision making ability,

include the following or similar statement in this

section:

"The device does not locate metaphase spreads; it

does not rank the given cells according to

quality; it does not automatically classify

chromosomes; it does require and relies completely

on the operator to manipulate the digitized

microscope images."

B. Limitations of the Device

Include the following:

1. a statement that decision making capabilities of

the instrument do not relieve the

cytogeneticist/cytogenetics technologist of the

responsibility to review and edit all work

generated by the device and that the final

decision must be made by a qualified

cytogeneticist;

2. appropriate statements of precaution outlined for

each feature in the Performance Characteristics

section (III-C). In some cases, limitation

statements for more than one features may be

combined; and

3. these general statements for all devices that have

features with decision making ability:

"In general, there will be one or more errors

in the computer-generated data/karyotypes.

Therefore, it must always be examined and

edited as a final interactive manual

operation by qualified cytogenetic

personnel."

"The final clinical diagnosis must be made by

qualified medical personnel."

C. Performance Characteristics

Provide a summary of all reproducibility and comparison

studies (sensitivity and specificity) when performed as

requested by Section III.

Also address other performance characteristics that relate

to specific features (as described in section III-C).

V. Bibliography

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