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**SUMMARY: SAFETY AND EFFECTIVENESS INFORMATION FOR CEP 8 SpectrumOrange DNA Probe Kit**

The CEP 8 SpectrumOrange DNA Probe is a SpectrumOrange fluorescent labeled DNA probe specific for the centromeric region of chromosome 8. This assay is designed to provide a method for the detection and quantification of chromosome 8 in both interphase nuclei and metaphase spreads by fluorescence *in situ* hybridization (FISH).

Standard cytogenetic analysis detects cytogenetic abnormalities such as trisomy 8 by karyotyping metaphase spreads after staining the chromosomes with a dye in cultured tissue cells.

Safety and effectiveness issues relevant to FISH CEP 8 assay may include cross-reactivity, poor sensitivity, poor specificity, or poor reproducibility. The following summarize the specific performance characteristics of CEP 8 assay:

## Analytical Sensitivity and Specificity

### Hybridization Efficiency

In a pilot study, the average percentage of cells with no hybridization signal was 0.22% (s.d.=0.20%) on 35 bone marrow (BM) specimens. In a pivotal study, the average percentage of cells with no hybridization signal was 0.11% (s.d.=0.21%) on 60 BM specimens. Thus, <2% cells with no signal is a realistic standard of acceptance, especially for FISH metaphase analysis.

### Analytical Sensitivity

The analytical sensitivity of the CEP 8 probe was tested in the reproducibility study described below. In that study, the 0% specimen was estimated with a mean of 0.95% (s.d.=0.51%) tri-signaled nuclei and the 5% specimen, 5.37% (s.d.=0.98%). There was little overlap between the 0% and 5% specimens; the upper 95% confidence limit for the 0% specimen was 1.95% and the lower 95% confidence limit for the 5% specimen was 3.45%. Thus, the limit of detection for CEP 8 in interphase cells is estimated to be 4.0%.

### Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Vysis QC protocols. A total of 62 metaphase spreads were examined sequentially by G-banding to identify chromosome 8, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 62 cells examined; hybridization was limited to the centromere region of chromosome 8.

## Reproducibility

In a pilot study, the reproducibility of CEP 8 interphase analysis for the %tri-signaled cells cells was assessed for inter-site, inter-day, and inter-observer reproducibility, using one lot of the CEP 8 DNA probe. One normal bone marrow specimen was evaluated for the %tri-signaled cells according to the instructions for signal enumeration in the package insert. Statistically significant site-to-site and observer-to-observer variations were observed, reflecting the subjectivity of the visual enumeration process. The results of classification of slides as positive or negative for trisomy 8 (using a cutoff of 2.2%) was 96% correct (1 of 24 had 2.4% tri-signaled nuclei) for this normal specimen. For the overall variation, the N, mean, SD, and percent CV of the observed %tri-signaled nuclei was 24, 0.99%, 0.57%, and 58%, respectively.

In a pivotal study, the CEP 8 interphase analysis for the %tri-signaled cells was again assessed for inter-site, inter-lot, inter-day and inter-observer reproducibility. One low level (approximately 7%) trisomy 8 bone marrow specimen was evaluated for the %tri-signaled cells according to the instructions for signal enumeration in the package insert. Statistically significant site-to-site and observer-to-observer variations were observed, reflecting the subjectivity of the visual enumeration process. The results of classification of slides as positive or negative for trisomy 8 (using a cutoff of 2.2%) was 100% correct for this low level (7%) trisomy 8 bone marrow specimen. For the overall variation, the N, mean, SD, and percent CV of the observed percentage of tri-signaled nuclei was 24, 7.70%, 1.45%, and 19%, respectively.

## Methods Comparison: Clinical Specimens

A multi-center, blinded, controlled, comparative study was conducted to further define the performance characteristics of the CEP 8 probe. The objective of the study was to determine the sensitivity and specificity of the CEP 8 assay relative to standard cytogenetic analysis, the standard of care. Four laboratories provided 368 archived bone marrow specimens for assay at three investigation sites. Site 1 provided 101 specimens; Site 2 provided 57 specimens; Site 3 provided 130 specimens; Site 4 provided 80 specimens. Specimens from Site 4 were analyzed at Site 3. By standard cytogenetic analysis, 151 of these specimens were classified as positive for trisomy 8; 201 negative for trisomy 8; and 16 ambiguous for trisomy 8 (1 trisomy 8 per 30 metaphases analyzed). Fifteen of the 16 ambiguous cases were selected "purposefully" after study completion. These specimens were derived from patients with one of the following diagnoses.

1. Acute myeloid leukemia (**AML**): 102 specimens
2. Myeloproliferative disorder (**MPD**), including polycythemia vera: 44 specimens
3. Myelodysplastic syndrome (**MDS**): 80 specimens
4. Chronic myelogenous leukemia (**CML**): 72 specimens
5. Hematological disorder, not otherwise specified (**HDNOS**): 70 specimens (including hyperproliferative states such as leukemoid reaction, lymphoproliferative disorders or chronic lymphocytic leukemia, without trisomy 8).

At one trial site, approximately 50% of the archived bone marrow specimens failed to produce informative FISH results. Further examination of a subset of these specimens revealed a lack of specimen integrity and it was determined that specimens at this site were stored at 4°C rather than at the recommended temperature of -20°C. The conclusion was that some specimens and/or slide preparations were inadequate.

FISH Interphase Analysis versus Standard Cytogenetics

From the same multi-center comparative study described above, the analysis of interphase nuclei by FISH compared to standard cytogenetics was performed. Based on the cutoff point of 2.2% tri-signaled nuclei that was validated by the same pivotal clinical study, the relative sensitivity was 96.03% (145/151) [95% C.I. 92.55 - 99.51%] and the relative specificity was 98.01% (197/201) [95% C.I. 96.08 - 99.94%] for the CEP 8 interphase analysis.

Among the 10 discrepant cases, the standard cytogenetic analysis results ranged from 0/20 to 8/22 metaphase cells with trisomy 8; the CEP 8 interphase results of % tri-signaled nuclei ranged from 0.6% to 6.0%. Among the 16 ambiguous cases, the range of % tri-signaled nuclei by CEP 8 interphase analysis was from 0.2% to 2.2%.

From the 368 clinical specimens, the correlation coefficient of trisomy 8 between standard cytogenetic metaphase analysis and CEP 8 interphase analysis was 0.91. The regression coefficient of CEP 8 assay on cytogenetic analysis was 0.71. The following equation describes the plot of CEP 8 assay results on cytogenetic analysis results:

$$y = 1.2944 + 0.7112x$$

where: x = percent metaphase spreads with trisomy 8 by standard cytogenetics
y = percent tri-signaled interphase nuclei by CEP 8 analysis

FISH Metaphase Analysis versus Standard Cytogenetics

A total of 348 cases were included in the comparison of CEP 8 metaphase analysis to standard cytogenetics. Twenty of the 368 cases included in the CEP 8 interphase analysis were excluded due to insufficient number of metaphase spreads for analysis with the CEP 8 assay (a specimen must have ≥ 20 metaphase spreads).

By the rules of standard cytogenetic analysis, a case is declared positive for trisomy 8 if two or more metaphase spreads are trisomic for chromosome 8. Using this cutoff of ≥ 2 tri-signaled metaphases, the relative sensitivity was 89.19% (132/148) [95% C.I. 84.19 - 94.19%] and the relative specificity was 91.30% (168/184) [95% C.I. 87.22 - 95.37%] for the CEP 8 metaphase analysis. Among the 16 ambiguous cases by standard cytogenetic analysis, 15 were negative and 1 was ambiguous for trisomy 8 by CEP 8 metaphase analysis.

From the 348 clinical specimens, the correlation coefficient of trisomy 8 between cytogenetic metaphase analysis and CEP 8 metaphase analysis was 0.91. The regression coefficient of CEP 8 assay on cytogenetic analysis was 0.83. The following equation describes the plot of CEP 8 assay results on cytogenetic analysis results:

$$y = 1.6979 + 0.8325x$$

where: x = percent metaphase spreads with trisomy 8 by standard cytogenetics
y = percent tri-signaled metaphase spreads by CEP 8 analysis

FISH Interphase Analysis versus FISH Metaphase Analysis

In addition to the methods comparison between FISH and standard cytogenetics described above, a comparison between FISH interphase and FISH metaphase was made. There was a 90.8% [(133+183)/348] concordance between FISH interphase and FISH metaphase.

From the 348 clinical specimens, the correlation coefficient of trisomy 8 between CEP 8 interphase analysis and CEP 8 metaphase analyses was 0.95. The regression coefficient of CEP 8 metaphase analysis on interphase analysis was 0.81. The following equation describes the plot of CEP 8 assay metaphase results on CEP 8 interphase analysis results:

$$y = 1.0942 + 0.8119x$$

where: x = percent tri-signaled interphase cells by CEP 8 analysis
y = percent tri-signaled metaphase spreads by CEP 8 analysis

## Conclusions

Consistent performance of CEP 8 is guaranteed by the Vysis Quality Control Procedures. When the CEP 8 SpectrumOrange DNA Probe is used as instructed in the package insert, the above statements describe its performance.

DEPARTMENT OF HEALTH & HUMAN SERVICES

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Vysis
c/o Dr. Russel K. Enns
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Re: k953591
Trade/Device Name: CEP 8 SpectrumOrange DNA Probe Kit
Regulation Number: 21 CFR §866.4700
Regulation Name: Automated fluorescence in situ hybridization (FISH) enumeration systems.
Regulatory Class: Class II
Product Code: OYU, KIR
Dated: October 21, 1996
Received: October 22, 1996

Dear Dr. Enns:

This letter corrects our substantially equivalent letter of November 29, 1996.

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related

adverse events) (21 CFR 803); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Maria M. Chan, Ph.D.
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