Interphase Fluorescence In situ Hybridization Analysis: A Study Using Centromeric Probes 7, 8, and 12*†

LIAN ZHAO, M.D., CLSp(CG),
ZEBUNNISA KHAN, M.S., CLSp(CG),
KIMBERLY J. HAYES, B.S., CLSp(CG),
and ARMAND B. GLASSMAN, M.D.

Division and Department of Laboratory Medicine,
Section of Cytogenetics,
The University of Texas M.D. Anderson Cancer Center,
Houston, TX 77030

ABSTRACT

Interphase fluorescence in situ hybridization (I-FISH) is a useful technique for detecting chromosomal numerical abnormalities in tumors and is gaining acceptance as a tool in cytogenetics and clinical diagnoses. Performance and quality control information about commercial products are necessary in order to implement an individual FISH probe as a routine clinical laboratory test. Interphase FISH analysis was performed with three commercially available alpha-satellite chromosome-specific DNA centromeric probes (D7Z1/D7Z2; D8Z2; and D12Z3) on bone marrow material prepared for conventional cytogenetic analysis. The results were interpreted following enumeration of the signals in 500 interphase nuclei each by two different observers. A mean of 93.92 percent (±1.3 percent, 1 SD) was found for chromosome 7; a mean of 93.91 percent (±1.5 percent, 1 SD) was found for chromosome 8, and a mean of 92.85 percent (±1.4 percent, 1 SD) was found for chromosome 12. The results of the study demonstrated that I-FISH using chromosome centromeric probe(s) is a reliable, reproducible, and accurate technique. This technique can be integrated into routine clinical practice with proper quality control protocols.

Introduction

Fluorescence in situ hybridization (FISH) is used increasingly in clinical practice.1 Improved sensitivity, speed of detection, and other inherent advantages of this technique have enabled the cytogeneticist to detect specific deoxyribonucleic acid (DNA) sequences and to visualize their distribution in interphase nuclei. The technique has been successful in our research laboratory.2,3 To apply this technique in the clinical laboratory, information about product performance needed to be generated.

The purpose of this study was to establish reference ranges for FISH. Three chromosome-specific centromeric probes (alpha-satellite DNA 7, 8, 12) were chosen. These three chromosomes are commonly involved in...
human leukemias and lymphomas. Our data demonstrated that: (1) interphase FISH, using chromosome centromeric probe(s), is a reliable, reproducible, and accurate technique, and (2) this technique can be integrated into clinical cytogenetics practice.

Material and Methods

Control Sample Preparations

Bone marrow cells from 10 untreated leukemia subjects with conventional cytogenetically normal chromosomes 7, 8, 12 were used. Chromosome preparations were made according to standard cytogenetic procedures. Briefly, cells were cultured overnight in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 20 percent fetal bovine serum, and then harvested using colcemid by routine cytogenetic methods.

DNA Probes

Three centromeric alpha satellite probes (D7Z1/D7Z2, D8Z2, and D12Z3) were used to determine the number of chromosome centromeres in both interphase nuclei and metaphase chromosomes. The probes were digoxigenin labeled and commercially available.

In situ Hybridization

The hybridization protocol, as recommended by Oncor, Inc. with modification, was applied. Slides were treated with 2x standard saline citrate [SSC] for 30 min at 37°C, dehydrated in a 70 percent/85 percent/100 percent ethanol series for 2 min each, denatured (70 percent formamide, 2x SSC [pH 7.0] at 70°C for 2 min, dehydrated again at room temperature, and air-dried. The probes were mixed with Hybrisol VI and denatured at 72°C for 5 min, immediately cooled on ice, and then placed onto slides. The slides were then covered with a 22 x 22 mm coverslip, sealed with rubber cement, and incubated overnight at 37°C in a humidified chamber.

Probe Detection and Microscopy

After incubation, the slides were treated in 0.25x SSC for 5 min at 70°C. The signal of the cells was detected by anti-digoxigenin conjugated with fluorescein isothiocyanate (FITC). Slides were then washed in phosphate buffer with detergent [PBD] three times, for 2 min each. Finally, the slides were counterstained with propidium iodide/antifade solution. The slides were examined under a Zeiss fluorescent microscope. A dual wave length filter (Zeiss) was used to view both FITC and propidium iodide simultaneously.

| TABLE I |
| Criteria For Analyzing by Fluorescence In Situ Hybridization Results |
| 0 Signal | No signal can be seen in a cell. Do Not include cells in S-phase, which has a diffuse FITC background. |
| 1 Signals | After focusing, just one clear signal can be seen in a cell. |
| 2 Signals | Two clear signals can be seen in a cell. |
| 3 Signals | Three clear separated signals are seen in a cell. Do Not confuse with sister chromatid split. |
| 4 Signals or > 4 | Four or more separate, clear signals in a cell. |
| Not analyzable (N/A) | There are signals in a cell, but the signal is hardly distinguishable. |

FITC = fluorescein isothiocyanate.
Evaluation of FISH Results

The FISH results were evaluated by two trained observers (L.Z. & Z.K.) using the criteria in table I. Five categories of interphase cells with signals are shown in figure 1. All slides were coded so that the observers had no knowledge of the probes that had been used on each slide. In each sample, a total of 500 nuclei were evaluated by each observer in dif-

![Figure 1](image-url)
different areas of the slides. In addition, certain numbers of metaphase were identified with FISH signals on each slide.

Results

Ten non-leukemic diploid specimens based on conventional cytogenetics were randomly selected for each group. Three probes had been used on each sample. The same hybridization condition was used on a total of 30 slides. The results were interpreted following enumeration of the signals in 500 interphase nuclei. From each group of 10 samples, a mean of 93.92 percent (±1.3 percent, 1 SD) was found for chromosome 7; a mean of 93.91 percent (±1.5 percent, 1 SD) was determined for chromosome 8, and a mean of 92.85 percent (±1.4 percent, 1 SD) was observed for chromosome 12 (figure 2). On each slide, the signal on metaphases was counted to confirm the probe locus. Two signals on all intact metaphases were found (table II). The probability distribution from each observer is summarized in table III.

Discussion

In this study, commercially available digoxigenin-labeled centromeric DNA probes specific for chromosome 7, 8, and 12 were used for routine cytogenetic evaluation. The probes* provided a rapid and reliable method for detecting and quantifying chromosomes 7, 8, and 12 in interphase nuclei. The results were interpreted following enumeration of the signals in 500 interphase nuclei in different areas of the slides by two trained observers. Six categories for the control groups were established: no signal (0); 1 signal; 2 signals; 3 signals; 4 or more signals, and N/A cells (not analyzable). A cut-off value for each category along with the two expected signals was determined. Similar, or even considerably higher, percentages of false monosomic or trisomic nuclei, have been seen by other investigators for a variety of alpha-satellite probes.5,6,7

Loss of target DNA, poor penetration of probe, incomplete or inefficient hybridization,

* Oncor, Gaithersburg, MD.
<table>
<thead>
<tr>
<th>Slide Number</th>
<th>Chromosome 7</th>
<th>Comment</th>
<th>Chromosome 8</th>
<th>Comment</th>
<th>Chromosome 12</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>19M:2S 1M:1S</td>
<td>The metaphase with one signal does not contain 46 chromosomes</td>
<td>18M:2S 1M:1S 3M:1S</td>
<td>The one and three signal, metaphases are poor quality</td>
<td>20M:2S</td>
<td></td>
</tr>
<tr>
<td>Control 2</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 3</td>
<td>10M:2S</td>
<td>12M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 4</td>
<td>20M:2S</td>
<td>10M:2S</td>
<td>8M:2S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 5</td>
<td>18M:2S</td>
<td>19M:2S</td>
<td>1M:3S 19M:2S</td>
<td>The morphology of the metaphase with the three signals is poor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 6</td>
<td>1M:4S 19M:2S</td>
<td>The metaphase with four signals is tetraploid</td>
<td>19M:2S 1M:4S</td>
<td>The metaphase with four signals is tetraploid</td>
<td>18M:2S</td>
<td></td>
</tr>
<tr>
<td>Control 7</td>
<td>20M:2S</td>
<td>17M:2S</td>
<td>1M:4S</td>
<td>The metaphase with four signals is tetraploid</td>
<td>No metaphases</td>
<td>found</td>
</tr>
<tr>
<td>Control 8</td>
<td>5M:2S</td>
<td>3M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 9</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td>20M:2S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 10</td>
<td>20M:2S</td>
<td>4M:2S</td>
<td>8M:2S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M = metaphase.
S = signal.


or signal overlap are all possible causes leading to a weak or absent signal. Some cells were difficult to score because of signal problems. These cells were classified as not analyzable (N/A) cells. The percentage of these N/A cells may have been a reflection of the counting skill of the laboratory person, the binding efficiency of the probe, or otherwise technically related. The efficiency of each probe is indicated in part by the number of cells having no signal. The N/A cells are not included in the total percentage for each category. Once the laboratory normal control range is established, the percent N/A cells can be used to compare with each specimen. Only the cells that contain clear signals should be counted. Fluorescence in situ hybridization, using alpha-satellite DNA centromeric probes 7, 8, and 12, is highly reproducible and capable of providing reliable quantitative information in the clinical cytogenetic laboratory.

Acknowledgment

This work was supported in part by the Olla S. Stribling chair for Cancer Research. The authors thank Leslie Wildrick for her editorial assistance. The secretarial assistance of Isabel Hernandez, Pinkie D. Oji, and Bernadine Williams is greatly appreciated.

References