

AMERICAN COLLEGE OF MEDICAL GENETICS

Prenatal Interphase Fluorescence In Situ Hybridization (FISH) Policy Statement

Introduction

The past decade has witnessed significant advances in molecular biology that have already had a profound impact on research in human genetics. Such developments have been applied to the clinical practice of medical genetics with a speed perhaps unmatched in any other field of biomedical science. Fluorescence in situ hybridization (FISH), already commonly used as an additional staining method for metaphase analysis, is now being applied to interphase chromosome analysis. During the past 5 years, FISH has begun to demonstrate its considerable diagnostic potential. The development of molecular probes by using DNA sequences of differing sizes, complexity, and specificity, coupled with technological enhancements (direct labeling, multicolor probes, computerized signal amplification, and image analysis) make FISH a powerful investigative tool.

The rapid detection of numerical chromosomal anomalies is made possible through centromere-specific repetitive-DNA probes and chromosome-specific unique-sequence probes. The successful application of FISH to interphase cells, once the specificity and sensitivity of appropriate probes are determined, will be a welcome addition to the diagnostic armamentarium of clinical cytogenetics, providing rapid results and possibly overcoming much of the labor-intensive activity of tissue culture and karyotyping.

Although FISH has great potential in a variety of cytogenetic studies, particular attention has been focused on its use in prenatal diagnosis of chromosomal anomalies, because of the speed with which results are attainable. However, as with all emerging technologies, the transition from the developmental phase to application as a standard diagnostic procedure must be accompanied by assurance of reliability, reproducibility, and accuracy, as well as by guidelines for appropriate use.

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Clinical Considerations

For clinical applications, FISH must still be considered an investigational procedure. Clinical indications for FISH as an additional analytical technique are similar to those for standard cytogenetic analysis. In addition, FISH can be used to assess maternal contamination in chorionic villus sampling (CVS); to screen CVS or amniotic fluid cells to determine which cultures should be expedited for full cytogenetic analysis; and to provide rapid preliminary results in pregnancies of advanced gestational age that are complicated by fetal anomalies, where waiting for standard cytogenetic results would limit clinical management options. However, the nature of interphase FISH analysis leads to unique clinical considerations. The frequency of fetal numerical chromosomal aneuploidies increases in pregnancies of advanced maternal age, in the presence of specific ultrasonographically detected abnormalities, and in high-risk pregnancies identified by maternal serum-screening programs. Hence, FISH may be most applicable in these situations. Recent data suggest a significantly higher proportion of structural abnormalities detected at prenatal diagnosis than were detected in the 1970s, because of increased levels of chromosome resolution today. Since FISH analysis cannot distinguish numerical from structural imbalances, positive screening results may suggest additional, more detailed diagnostic testing of individual chromosomes.

At this time, FISH is not a standard procedure in cytogenetic laboratories, and therefore it should be used as an adjunct test only, with standard chromosomal analysis serving as the primary diagnostic and confirmatory evaluation. Appropriate informed physician and patient consent should be obtained, and patient-management decisions should not be based on results obtained by FISH alone.

Technical Considerations

Although FISH has demonstrated potential, its current limitations preclude its introduction as a “stand-alone” diagnostic tool for interphase cyto-

netic analysis in prenatal diagnosis. These limitations include the following:

1. *The paucity of specific probes.*—The inability of any specific probe to detect most structural chromosomal rearrangements restricts the application of FISH to numerical abnormalities. Furthermore, mechanisms for increasing the availability and distribution of probes to the genetics community must be developed.

2. *Signal interpretation.*—Attention must be paid to both technical (cross-hybridization, nonspecific hybridization, simultaneous use of multiple probes, and stringency conditions) and biological problems (mosaicism and differentiating between G_1 and G_2 cells). Moreover, when FISH is appropriately applied to structural abnormalities, (e.g., microdeletion syndromes), the smallest region of overlap, defining the appropriate probes, must be delineated. Additional research is necessary to resolve these difficulties.

3. *Uniformity, availability, and application of probes.*—Different probes, each demanding individual optimization of conditions, are currently used for the identification of the same chromosome. Reagents and protocols must be standardized. The most useful probes, which will facilitate the widest introduction of the technology, must be identified. Widespread testing of the procedures must be conducted, and a common basis for interpretation of results must be established.

4. *Test performance.*—Since positive signals are detected in only a proportion of the potential targets by any FISH study, “in-house” norms for background rates and detection frequencies must be developed. The frequency of uninformative cases (as high as 10%–15%) may contain a disproportionate number of abnormalities, potentially reducing the overall detection rate of the test.

Test Validation Considerations

The potential diagnostic success of FISH is suggested by a relatively few published and many unpublished series from individual laboratories. These findings represent a limited body of data, obtained under diverse experimental settings and with widely divergent goals and results. Well-designed prospective studies are needed to address the statistical/epidemiologic problems of sensitivity and specificity of the test, protection from false positives and negatives, establishment of “acceptable” error rates and sample size (number of cells to be screened), etc. Establishment of the clinical validity, safety, and efficacy of the test must occur in a manner

consistent with regulatory guidelines and standards established by recognized professional authorities and governmental agencies.

At this time, the foremost unresolved issues in the utilization of FISH for interphase analysis in prenatal cytogenetics are the following:

- a. Expanding the experience with the technology to a large number of prenatal diagnostic centers via independent FISH trials. This would increase the chance of identifying those rare events that could lead to diagnostic dilemmas.
- b. Defining appropriate prenatal applications of unique-sequence probes, chromosome-specific repetitive probes, and whole-chromosome painting probes.
- c. The lack of uniform laboratory interpretative criteria for diagnosis and further investigation of equivocal results.
- d. The establishment of an integrated external (national) quality-assurance scheme for FISH.
- e. Improved availability of and access to appropriate probes.
- f. Evaluation of future advances in FISH technology—such as expanding probe sets and multicolor probe analysis—for their appropriate and rapid introduction into the clinical laboratory.

Recommendations

1. The investigational nature of FISH testing should be described clearly to all patients and health-care providers.
2. Proper informed consent should be obtained following explanations of the purpose, accuracy, potential risks, and limitations of FISH testing.
3. Until accepted as a standard laboratory technique, FISH should be used in prenatal interphase cytogenetics only in conjunction with standard cytogenetic analysis.
4. Irreversible therapeutic action should not be initiated on the basis of FISH analysis alone.
5. Well-designed, multicenter prospective trials should be undertaken to assess the reproducibility, sensitivity, specificity, and positive and negative predictive values, as well as the general applicability of FISH analysis in prenatal diagnosis.
6. Appropriate quality assurance/quality control for reagents, as well as techniques in the development of standardized protocols, must be established for FISH analysis.