**Letter to the Editor**

**Application and Usefulness of FISH (Fluorescent *In Situ* Hybridization)**

**Method in Cytogenetic Researches**

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**Abstract:** Fluorescent *in situ* hybridization (FISH) is a sensitive molecular cytogenetic method which points to a specific numerical or structural chromosomal aberration. FISH method is a mix of procedure between conventional cytogenetics and molecular genetics. The advantages of this method are the ability to detect genetic aberration in cells in interphase, application on both, fresh and previously fixed samples and short duration of analysis. The sensitivity of this method is great as a possibility of analysis on a large number of cells. The method has a wide range of applications in cytogenetic laboratories for routine analysis in prenatal diagnostics, pediatrics, hematology, oncology and another fields of medicine. FISH method is very important and useful method for quick diagnosis, early and on time prognosis of the disease and initiation of adequate therapy.

**Key words:** FISH method, Cytogenetic study, application, advantages, sensitivity

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*In situ* hybridization (ISH) is a method that allows the detection of specific target sequences within the cell while preserving cellular and tissue morphology. ISH is based on the principle of complementarity, it means that denatured the probe will hybridize exactly to the complementary target nucleic acid sequence (most often it is DNA). Combining the denatured probe and target allows the annealing of complementary DNA sequences. The term *in situ* means "in the usual place" hybridization took place within the cell where the target DNA or RNA is normally located. This method has improved significantly in the last ten years and due to day can be used various types of probes. The probe is a short segment of nucleic acid that is marked by someone fluorescent dye (e.g. in FISH) and which is complementary to the target sequence which wants to detect. The test needs to be marked so that it can be accurately determined localization and that the amount of the target sequence could be determined. With accordance that in normal cell (except for genes located on the X and Y chromosomes in men) are present two copies of each gene, by in situ hybridization, are confirm two yield signals for each probe which is used. Contrary of cytogenetic analysis, ISH methods do not require cell culture or mitotic cell culture and can be applied directly on the cells in interphase. Also, ISH analysis can be applied to a wide samples of cell culture, fresh or frozen samples, cytological samples, tissues fixed in formalin and on tissue microarrays (1-3).

The *in-situ* hybridization method was employed for the first time in 1969 by Gall and Pardue, and was modified in 1981 by Langer, who used a non-radioactive *in situ* hybridization procedure. The technique of FISH has been developed as a complementary method for rapid gene mapping within the Human Genome Project (4). In FISH method the probe is visualized with fluorochrome, while in CISH for visualization of the probes is used chromogens.

 According to the method of binding of the fluorescent marker, FISH method can be direct and indirect. In the direct analysis the probe is bound to fluorochrome, while in the indirect analysis the nucleotides are the probes bind to biotin or digoxigenin, and then visualized with antibodies to biotin or digoxigenin bound to fluorochrome.

The all procedure for FISH analysis during approximately two days and the procedure has several important steps: tissue pretreatment, hybridization, and evaluation itself. Pretreatment is the first step when the tissue is actually prepared for hybridization and the gene of the chromosome makes available for probe, which used in analysis. Sodium thiocyanate (NaSCN) is used in tissue pretreatment, with intention to break the DNK protein complex. This step is followed by protein digestion which must be optimally adjusted for individual tissue. The next crucial step is hybridization, it is important to monitor the temperature on which it depends the very success of binding the probe to the target site. If the temperature is too low then try it will not bind to the target site on the chromosome and will be seen under a fluorescent microscope only weak signal or none at all. Lastly, professionals and well-trained staff are needed to evaluate preparation and adequately analyze its validity and assess whether key steps have been performed under optimal conditions. There are several types of FISH methods that vary by the number of analyzed genetic sequences, binding site of the probes and the number of probes used in the analysis: whole chromosome painting probes, locus-specified probes i spectral karyotyping (SKY, multicolor FISH). With accordance to this, with FISH method can be detected numerical and structural rearrangements of chromosomes utilizing different chromatid or chromosome-specific probes (5, 6).

The method has a wide range of applications in cytogenetic laboratories for routine analysis in prenatal (preimplantation) diagnostics, detection of microdeletions, microduplications, marker chromosomes and other cryptic and complex chromosomal rearrangements in different fields of medicine (7-11).

FISH method has been widely used for research purposes such as:

* gene mapping, analysis of nuclear organization during the lifetime of a cell
* to follow the dynamics of DNA reparation
* discovering unsolved cases of mental retardation and multiple anomalies
* most common microdeletion syndromes (Prader-Willi syndrome, DiGeorge syndrome ets.)
* diagnosis of certain hematological diseases: acute myeloblastic leukemia, acute lymphoblast leukemia, chronical granulocytes leukemia, myelodysplastic syndrome, chronical lymphocytes leukemia, multiple myeloma, lymphoma, follicular lymphoma, mantle cell lymphoma, Burkitt lymphoma etc.
* Pediatrics, oncology and another fields of medicine
* phylogenetic studies

In a fact that some of the microdeletion syndromes give a wide variety and overlapping of the phenotypic signs, clinical diagnosis can be difficult and application of FISH probes is a prerequisite for confirmation (7).

**Scope for Further Research**

 The advantages of FISH method are the ability to detect genetic aberration in cells in interphase, application on both, fresh and previously fixed material and short duration of analysis. These advantages are very important for quick diagnosis, early and on time prognosis of the disease and initiation of adequate therapy. The sensitivity of this method is great as a possibility of analysis on a large number of cells. False positives results can be result of poor processing of the specimen and inadequate microscopic analysis (fluorochrome artifact as a signal probe bind with the chromosome). False-negative results may occur if there is an aneuploidy, if the cell nucleus is damaged or overlaps of the cells or overlapping part of the genome of one cell.

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**Conflict of interest:**

The authors declared no conflict of interest

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