REFERENCES

1 Mechoso B, Vaglio A, Quadrelli A et al. (2007) A de novo complex rearrangement involving chromosomes 2, 3, 5, 9 and 11 detected prenatally and studied postnatal byconventional cytogenetic and molecular cytogenetic analysis

2 Lin CC, Li YC, Liu PP et al. (2007) Identification and characterization of a new type ofasymmetrical di-centric chromosome derived from a single maternal chromosome.

3. Shurtleff SA, Buijs A, Behm FG et al. (1995) TEL/AML fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL.

4 Barber JC, Maloney VK, Huang S et al. (2008) 8p23.1 duplication syndrome; a novel genomic condition with unexpected complexity revealed by array CGH.

5. Poetsch M, Dittberner T, Woenckhaus C et al. (2000) Use of inter phase cytogenetic indemonstrating specific chromosomal aberrations in solid tumors – new insights in thepathogenesis of malignant melanoma and head and neck squamous cell carcinoma.

6. Wordsworth S, Papanicolas I, Buchanan J et al. (2008) Molecular testing for somaticcancer mutations: a survey of current and future testing in UK laboratories.

7. Bastian BC, Wesselmann U, Pinkel D et al. (1999) Molecular cytogenetic analysis ofSpitz nevi shows clear differences to melanoma J Invest Dermatol.

8 Busam KJ, Fang Y, Jhanwar S et al. (2012) Diagnosis of blue nevus-like metastatic uvealmelanoma confirmed by Fluorescence In -Situ Hybridization (FISH)

9. Gerami P, Wass A, Mafee M et al. (2009b) Fluorescence In -Situ Hybridization fordistinguishing nevoid melanomas from mitotically active nevi.

10. Liehr T (2009) Fluorescence In- Situ Hybridization (FISH)—Application Guide. Springer: Berlin, Germany

11. Marty M, Prochazkova M, Laharanne E et al. (2008) Primary cutaneous T-celllymphomas do not show specific NAV3 gene deletion or translocation.

12. O'Connor C (2008) (FISH).

Dr Lal PathLabs

FISH Lens

FLUORESCENCE IN-SITU HYBRIDIZATION (FISH)

INTRODUCTION

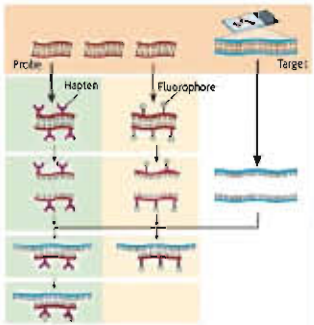
The introduction of Fluorescence In-Situ Hybridization (FISH) almost 30 years ago marked the beginning of a new era for the study of chromosome structure and function. Conceptually, Fluorescence In-Situ Hybridization (FISH) is a Cytogenetic technique, used to detect the presence or absence and location of specific gene sequences. It can visualize specific cytogenetic abnormalities (copy number aberrations), such as Chromosomal deletion, Amplification and Translocation. FISH has been used in prenatal diagnosis and has served both as a diagnostic and as a prognostic marker for various Leukemias and Sarcomas.

With respect to biomarker detection, a series of innovative high-throughput molecular tests, such as array-based Comparative Genome Hybridization (aCGH), Single Nucleotide Polymorphism (SNP) arrays and Next Generation Sequencing have recently been developed and incorporated into routine clinical practice. In fact, FISH has become increasingly important in clinical diagnosis due to its simplicity and reliability in evaluating key biomarkers in various tumors. In this newsletter, we aim to review the advantages of FISH for disease biomarker detection and personalized medicine applications along with its limitations.

HOW IS FISH PERFORMED?

FISH involves the binding or annealing of fluorescence-labeled, target-specific nucleic acid probes to their complementary DNA or RNA sequences and the subsequent visualization of these probes within cells in the tissue of interest. The tissue of interest can either be formalin-fixed, paraffin-embedded sections or fresh-frozen tissue. First, the DNA or RNA sequences from the tissue of interest are allowed to denature to become single stranded. Next, a FISH probe is selected and applied. The selection of an appropriate FISH probe is a critical step for enhancing its value as a diagnostic test because FISH only detects those chromosomal abnormalities that are specifically targeted by the probes used. Once the probe is selected, the fluorescence labeling of the probe can be done either directly or indirectly.

Figure 1 Principles of FISH technique



WHAT FISH DOES

- Fish is used to visualize specification cytogenetic abnormalities.
- It can serve as a supplementry diagnostic tool in pigmented lesions. However, it should not be used as a stand-alone test.
- FISH cannot replace traditional histopathologic analysis.
- FISH must correlate clinical, pathologic and molecular information.

LIMITATION

- Probe design requires knowledge of specific chromosomal abnormalities to be studied.
- Cut-off signals may differ among laboratories.
- Processing errors, imperfect hybridisation, non-specific binding, photobleaching, interobserver variability and false-positive and negative results are possible.

DIAGNOSTIC APPLICATIONS OF FISH

- Prenatal diagnosis
- Cancer diagnosis
- Molecular cytogenetic of birth defects and mental retardation
- The identification of specific chromosome abnormalities
- The characterization of marker chromosomes
- Interphase FISH for specific abnormalities in cases of failed cytogenetic samples
- Monitoring the success of Bone Marrow Transplantation

ADVANTAGES OF FISH

- Dual colored probes for fast, sensitive, and specific detection
- Works on metaphase spread, paraffin embedded and frozen tissue
- Identify gene amplification, loss and translocation
- High signal to noise ratio
- Low cross reactivity
- Faster result than conventional cytogenetic analysis

TYPES OF SAMPLES USED

- Fixed cell suspension
- Formalin fixed paraffin embedded tissue
- Buccal smear and cerebrospinal fluids

TYPES OF PROBES

Centromere probes

- Alpha and Satellite III probes
- Generated from repetitive locus at the end of specific chromosome sequences found in centromeres
- Centromere regions are stained brighter

Telomere

- Specific for telomeres
- Specific to the 300 kb

Whole chromosome

- Collection of probes that bind to the whole length of chromosome
- Multiple probe labels are used probes called localization probes
- Hybridize along the length of the chromosome probes

Locus

- Deletion
- Translocation probes
- Gene detection and gene amplification

INTERESTING CASE PRESENTATIONS

Case Presentation 1

- Cytogenetic evaluation of a 10 months-old girl with Dysmorphic features, Developmental delay and Mental retardation. She was the fifth child born to Non-consanguineous parents

- The clinical examination revealed Marked growth retardation, Microcephaly, Prominent glabella, Short philtrum, Micrognathia, High forehead, Preauricular tags with low set ears, Narrow external auditory canals, Strabismus, Hypertelorism, Iris coloboma, Wide nasal bridge,Downturned corners of the mouth with a fish-type appearance and Hyperconvex fingernails

Result

- Microdeletion of 4p detected by FISH using a probe for the Wolf-Hirschhorn Syndrome (WHS), Critical region (red) and Chromosome 4 centromere (green)

Normal appearing chromosome 4

- Deletion between 2-4 Mb in 25-30% of patients with WHS
- Must have suspicion of WHS to run this probe

Case Presentation 2

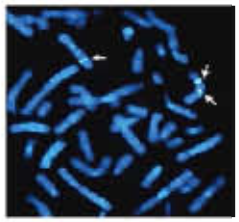
- 3 year old boy presented with Facial dysmorphism and mild intellectual disabilities for Karyotyping
- Facial dysmorphism includes broad forehead, an upturned nose, a long philtrum, full lips, small chin
- Karyotyping shows normal chromosome pattern in 400-450 band level. As per clinical feature, we advised FISH for William syndrome

Result

Karyotype Report

Normal appearing chromosome 7

FISH Report



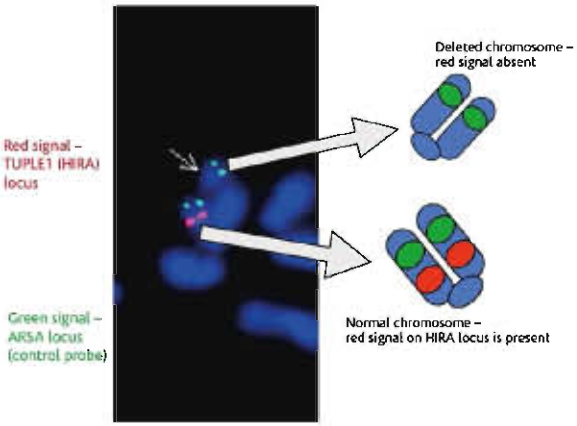
ELN gene absent

Case Presentation 3

- Inborn with cardiac defect (e.g tetralogy of fallot), Thymic hypoplasia (or aplasia)
- Physical appearance: Hypertelorism, Micromandibula, Low set dysplastic ear, Antimongoloid slant of eyelids
- Suggested for FISH

Result

Microdeletion confirmed (loss of one red signal)



Microdeletion 22q11.2 is associated with

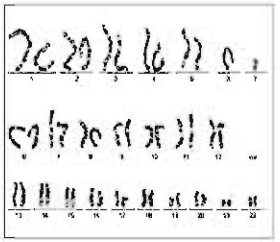
DiGeorge syndrome

Case Presentation 4

- 2-years old boy with mental retardation
- Inborn cardiac defect – Supravalvular aortic stenosis
- Phenotypic features and inborn defects are typical for Williams-Beuren syndrome
- This syndrome is caused by microdeletion of the long arm of the chromosome 7 (sub-band 7q11.23)
- In 95% of patients, this microdeletion could be examined by the FISH method
- Before the molecular cytogenetic analysis, basic cytogenetic examination is recommended

Results

Karyotype

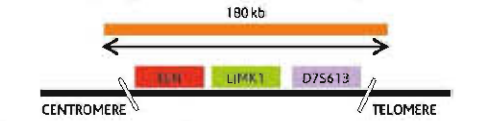


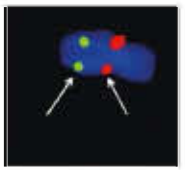
Normal finding:
46,XY

↓

Microdeletion should be confirmed by the FISH analysis

Molecular cytogenetic analysis of 7q11.23 microdeletion

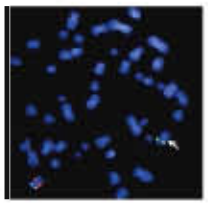




- **LOCUS SPECIFIC PROBE FOR THE CRITICAL REGION ELN/LIMK/D7S613**
  - (labeled with the spectrum orange, red signal)
- **CONTROL PROBE D7S522**
  - (labeled with the spectrum green, green signal)

Conclusion of the Molecular cytogenetic examination

- Microdeletion of 7q11.23 chromosome confirmed
- Diagnosis: Williams-Beuren syndrome



CONCLUSIONS

The advent of FISH in Cytogenetics has proved invaluable for both diagnostics and research. The power offers ability to identify specific genetic aberrations, which has propelled FISH-based techniques to the forefront of screening procedures for Prenatal, Pediatric and adult cases in a wide variety of cell types, including Paraffin-embedded tissue, making FISH analysis data a useful tool in the decision of therapy to combat cancer. This is supported by a recently conducted survey by Wordsworth et al.

The ultimate goal of FISH utilization would be an array based screen using the complete oncogenic repertoire to diagnose any prenatal or postnatal aberration(s). Any mutation could be rectified accordingly using gene therapy as a method of cancer prevention. However, the prevalence of mutations in human cancers are highly variable, each with a unique assortment of abnormalities that contribute towards tumor genesis at different developmental stages and extents. Improved aetiology through techniques, such as FISH maybe crucial in the fight against cancer with the knowledge acquired effectively directed towards the research and development of better treatment strategies to benefit the sufferers of diseases based on these genetic aberrations.

TEST RANGE ON FISH

TEST CODE	TEST NAME	ADVANTAGES
XX011	FISH FOR HER-2 NEU ONCOGENE AMPLIFICATION	Analysis of 50 interphase nuclei from invasive carcinoma cells
XX031	ALK1 REARRANGEMENT BY FISH	t (2;5) is associated with almost 60% of anaplastic large-cell lymphomas The EML4-ALK fusion gene is responsible for approximately 3-5% of non-small-cell
Z787	FISH for Non-Small Cell Lung Carcinoma(NSCLC) with ALK1 & ROS	FISH is better-suited than molecular testing to detect the spectrum of variants of ALK and ROS1 rearrangements. The ALK gene has more than 20 known rearrangement partner genes, with 15 variants of the most common EML4-ALK fusion. ROS1 has seven partners described so far in NSCLC while molecular assays must be designed to individual and unique fusions, FISH detection encompasses all described and as-yet undescribed rearrangements.
XX036	Aneuploidy detection, Products of Conception (POC)-FISH using chromosomes 13,18,21,X and Y	Rapid detection of common chromosomal aneuploidies or triploidy
XX037	FISH for trisomy 21 or Down syndrome	Rapid detection of Down's Syndrome
XX038	FISH-Williams syndrome / 7q11.23 deletion	The elastin gene, ELN, has been mapped to 7q11.23 (Williams syndrome chromosome region, and is reportedly hemizygous in up to 96% of patients with WS. The deletion of an elastin gene locus cannot be detected by conventional high-resolution chromosome analysis in the vast majority of cases due to the small size of this deletion.
Z789	Lung Cancer Mutation Panel *ALK1 & *EGFR	ALK rearrangement testing by FISH along with EGFR molecular testing are recommended for recurrent or metastatic cases with histological subtypes of Aden Carcinoma, Large cell Carcinoma or NSCLC NOS (not otherwise specified), and Squamous cell carcinoma in non-smokers or when biopsy specimens are small. FISH technique is the gold standard in detecting ALK-1 gene rearrangements.