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IDENTIFYING THE RELATIONSHIP BETWEEN HEMOLOGICAL DISORDERS USING FISH MODEL

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ABSTRACT

It has become more crucial to use fluorescence in situ hybridization (FISH) as a diagnostic and monitoring technique for the detection of disease-related chromosomal aberrations. Interphase FISH (iFISH) analysis was performed on 92 individuals. For the detection of common cytogenetic rearrangements linked with haematological malignancies, we have employed five distinct FISH probes. Patients with BCR/ABL gene rearrangements were tested in a total of 83 cases. 37.3 percent of patients (31/83) had iFISH patterns of BCR/ABL gene rearrangements that varied from 10 percent to 98 percent. While three individuals with AML had t (15; 17) (12%), and inv (16; 16) (8.3%), t (8; 21) was absent in the study. There were 6.5 percent of all instances in this investigation where secondary chromosomal abnormalities were found to be non-random. Patients with CML who have BCR/ABL gene rearrangements are likely to benefit from using this information to monitor their treatment. In addition, atypical patterns may have clinical consequences. The function of AML1/ETO, PML/RARA, CBFB and p53, as well as the particular chromosomal locations and interacting genes, must be studied in bigger groups of patients.

KEYWORDS: - Fluorescence in Situ Hybridization, BCR/ABL, Gene Rearrangements

INTRODUCTION

When examining the relationship between chromosomal aberrations and hematologic malignancies, fluorescent in situ hybridization (FISH) serves as a significant complement to traditional cytogenetics and molecular research As DNA probes and procedures used in FISH are not normally authorized by the Food and Drug Administration, their use as reagents specific to analytes necessitates stringent pre- and postanalytical conditions. Our goal is to educate labs on how to conduct credible metaphase and interphase FISH testing by outlining the many technical factors that go into the process. In-depth instruction for technologists on particular probe types and how to evaluate data consistently, covering both normal and abnormal outcomes, is provided. The precise FISH nomenclature for results reporting, as well as the application of FISH in conjunction with other laboratory tests in the continuing monitoring of illness, are all covered in detail. FISH testing programmes may be effectively implemented or assessed using this article's extensive guidelines in combination with existing rules, allowing for best patient care. In clinical laboratory research, a fluorochrome-labeled DNA probe is hybridised to an in situ chromosomal target using the FISH technique. It is possible to use FISH on a wide range of

specimens. Using metaphase preparations from cultivated cells for cytogenetic investigation is regarded as the "gold standard" since chromosomal shape and signal location are clearly visible. FISH, on the other hand, offers the benefit of being able to analyse non-dividing interphase cells. It is possible to identify particular chromosomal rearrangements or numerical aberrations associated with haematological malignancies using interphase nucleus evaluation from uncultured samples. Additionally, bone marrow cell suspensions, paraffin-embedded tissue slices, or disaggregated cells from bone marrow, or blood smear, and touch-preparations of cells from lymph nodes or solid tumours may be employed for interphase analysis, as well.

LITERATURE REVIEW

Tariq Ahmad Bhat (2017) - For finding particular DNA sequences, diagnosing genetic illnesses, mapping the genome, and identifying new oncogenes or genetic abnormalities that contribute to many forms of cancer, fluorescence in situ hybridization (FISH) is the most reliable technology. FISH is a technique that uses fluorescent reporter molecules to monitor the annealing of DNA or RNA probes to a particular target sequence in the sample's DNA under fluorescence microscopy. Multicolor whole chromosome probe approaches, such as multiplex FISH or spectral karyotyping, or an array-based method employing comparative genomic hybridization, have recently been improved to allow simultaneous screening of the whole genome. Fluorescence in situ hybridization (FISH) has transformed cytogenetics, making it a reliable diagnostic and discovery tool in the battle against genetic disorders.

Zubair Ahmed Ratan (2017) - In the realm of cytology, the macromolecule identification method known as fluorescence in situ hybridization (FISH) is being hailed as a groundbreaking innovation. When it was first created, it was used to map genes on chromosomes. As a result, biomedical researchers have taken advantage of FISH's precision and adaptability. DNA analysis and chromosomal research may be separated by this aesthetically attractive method. Using a hybridizing DNA probe, FISH is able to mark cells either directly or indirectly, depending on the method. Fluorescent nucleotides are employed for direct labelling, whereas reporter molecules, which are recognized by fluorescent antibodies or other affinity molecules, are utilised for indirect labelling. For example, FISH may be used to identify gene fusions, aberrant cell chromosome numbers, or the loss of one or more chromosomes or regions of chromosomes. Gene mapping and the discovery of new oncogenes are only two examples of how this technology is used in research. An examination of FISH as a medical concept, as well as its practical applications and benefits, is provided in this article.

Meenakshi A. (2015) - The reciprocal translocation of chromosomes 9 and 22 leads in the creation of the chimeric fusion gene BCR-ABL, which causes the Philadelphia chromosome (Ph) to be detected in over 95% of Chronic Myeloid Leukaemia (CML) patients. When it comes to CML diagnosis and treatment, this is a critical breakthrough. BCR-ABL fusion signals in interphase and metaphase spreads of bone marrow samples may be detected using the molecular cytogenetics method of fluorescence in situ hybridization (FISH). There have been a few studies that show peripheral blood white cells may act as a substitute for bone marrow. Patients with Chronic Myeloid

Leukaemia were given peripheral blood samples to examine the accuracy and speed with which FISH could detect Ph positive cells. Patients' cultured peripheral blood samples with the BCR/ABL Translocation, Dual fusion probe were subjected to FISH. GTG banding method was used for chromosomal analysis. The existence of reciprocal translocation t (9; 12) was verified by FISH and karyotyping (q34.1; q11.2). FISH is a fast, sensitive, and quantitative approach that may be utilised for the assessment of CML in peripheral blood, as shown by our data. Minimal residual disease and disease recurrence with a limited fraction of aberrant cells may be detected using FISH. Ph-positive cells are more likely to be found in high concentrations when the WBC count is abnormally high, according to our observations.

Linping Hu (2014) - Studies of genetic aberrations in human illnesses that have taken place during the last two decades have shown that many malignancies are linked to recurring genomic abnormalities. Microarrays and next-generation sequencing, two cutting-edge high-throughput genetic diagnostics, have been created and implemented into ordinary clinical practise throughout the years. Fluorescence in situ hybridization (FISH), a low-throughput cytogenetic test, is not showing indications of waning; on the contrary, it has become an essential aspect of the fast-emerging area of personalized medicine. De novo discovery and routine FISH detection of chromosomal rearrangements, amplifications or deletions related with the aetiology of different cancers have been discussed in this article, as well as the most recent breakthroughs in FISH application. We also looked at the most recent changes in FISH technique.

Susan Mahler Zneimer (2014) - There are several different types of fluorescence in situ hybridization (FISH) techniques, but one of the most often used is FISH. Centromere probes, locus-specific probes, fusion probes, and break apart probes are the most popular kinds of FISH probes utilised in the research of neoplastic illnesses. A variety of molecular techniques are now being utilised to detect alterations in DNA for the diagnosis of cancer, including flow cytometry, PCR, conventional cytogenetics, and FISH. The company or laboratory that created the FISH probe may use a different DNA composition than the one used to make the probe. Interphase FISH investigations using different probe designs and the use of several probes in a single test are the focus of this chapter. FISH analysis using breakpoints is outlined below in order to further explain the probes that were used. Hematological cancers are often treated with bone marrow transplantation.

RESEARCH AND METHODOLOGY

The iFISH analysis was performed on 92 individuals with recognized haematological disorders, including 50 (54.34 percent) CML, 25 (27.2 percent) AML, 7 (7.6 percent) ALL, 4 (4.35 percent) CLL, and 6 (6.52 percent) MDS patients diagnosed at the Departments of Hematology. Only 59 men and 33 women were present. From the age of twenty to the age of eighty-one, they had a mean age (SD) of 50, 45 15, 19 years (Tables 1 and 2).

Slide Preparation and FISH

All patients had a 2-ml venous blood sample drawn to check for chromosomal abnormalities such as t (9;22), t (8;21), t (15;17), and/or inv (16) and/or p53 gene

deletion. No incubation was employed in the harvesting or slide preparation processes. Fluorescence in situ hybridization was done on slides that had been incubated at room temperature overnight. We used the LSI BCR/ABL-ES Dual Color Translocation Probe (Vysis), LSI PML/RARA Dual Color Dual Fusion Translocation Probe (Vysis), LSI AML1/ETO Dual Color Dual Fusion Translocation Probe (Vysis), LSI CBFB Dual Color Break Apart Rearrangement Probe (Vysis), and LSI p53, 17p13.1, Spectrum Orange Probe (Vysis) for these experiments. To begin, slides were prepared for 5 minutes at room temperature with 2XSSC before being submerged for 30 minutes at 37 degrees Celsius in a solution containing HCl (1N), water, and pepsin A (2:200:2 v/v/v). Slides were promptly rinsed with water when the time period had expired. After that, they were treated with paraformaldehyde for 2 minutes, PBS for 2 minutes, PBS/MgCl2. 6H2O for 10 minutes, and PBS/MgCl2. 6H2O with paraformaldehyde for 10 minutes before being dehydrated with 70, 85, and 100 percent ethanol for 3 minutes each. After that, the slides were allowed to air dry. A coverslip was applied to each slide, and 10 l of each of the probe mixes was immediately applied to the slides using rubber cement. It was necessary to denaturate the slides for five minutes at 95°C before hybridizing them for an overnight period at 37°C in the ThermoBrite Denaturation/Hybridization System. 0.4XSSC/0.3% Tween 20 for 2 minutes at 73 C and 2XSSC/0.1% Tween 20 for 1 minute at room temperature were used to wash slides after the post-hybridization procedure. The slides were then left to dry in a pitch-black chamber. The next procedure was vortexing the DAPI tube and counterstaining the slides with 10 l of the dye, followed by 30 minutes at –20 C. Fluorescent microscopy was used to examine slides using red, green, and DAPI filters towards the end. A BX51 Olympus fluorescent microscope coupled with Cytovision Probe Software was used to examine interphase cells (Applied Imaging, Santa Clara, CA). A minimum of 100 interphase cells were examined for the signal patterns for each instance and probe.

DATA ANALYSIS

Researchers examined many indices [t (9; 22), t (8; 21), t(15; 17), inv (16; 16) and p53] in 92 haematological patients, including 50 (54.34%) of those with CML, 25 (27.17%) of those with AML, 4 (4.35%) of those with CLL, and 6 (6.52%) of those with MDS (Table 1).

Patients with BCR/ABL gene rearrangements were tested in a total of 83 cases. IFISH patterns for BCR/ABL gene rearrangements varied from 10% to 98% in the vast majority of patients studied, including most CML cases (25/48, 52.1%), AML (4/22, 18.2%), ALL (1/7, 14.3%), and MDS (1/6, 16.7%). (Tables 2 and 3). More than half of CML patients had a translocation of the Ph chromosome, whereas less than half had the chromosome in their DNA. Ph-positive individuals had the expected FISH signal pattern in around 96.2 percent of cases. There were four distinct iFISH patterns for BCR/ABL gene rearrangements. One fusion-der (22), one greennonrearranged 22 (29/83), and two red-der (9) with the nonrearranged chromosome 9 signals (1F2R1G) comprised the normal iFISH pattern (Pattern A). 1F1R1G and 2FG among the ph-positive patients had BCR/ABL fused gene rearrangements on chromosome 9 or the depletion of the rearranged chromosome 9; coexistence of der(9q) and der(22q) deletions (2/25, 7.7 percent) (Figure 1).

iFISH patterns (Pattern A) were seen in 27 individuals, including 23 patients with CML, two patients with AML, one with ALL, and one with MDS (Table 3, Figure 1). Pattern B (one fusion, one red and one green signals) was detected in three individuals (3.6 percent), one of whom had CML and the other two had AML, as shown in Figure 1 and listed in Table 4. Only 2% of CML patients showed pattern C (one fusion, one red and two green signals) (Table 4, Figure 1). In our patients, the t(15;17) was found in three AML patients [3/41 (7.31 percent) examined patients for PML/RARA]; C28, C47, and C67, with the rates of 11%; 79%; and 86%, respectively (Tables 2 and 3). This treatment was tested on 19 people. Only 12/100 interphase cells from 12 different AML patients (C11) revealed this inversion (Tables 2 and 3, Figures 2(c) and (d)). Seven individuals with CML, AML, and CLL were examined for p53 gene deletion. C54, the only CML patient tested positive, had a 10% chance of remission. The p53 gene was not deleted in any of the other cases (Tables 2 and 3).

Table 1. The distribution of hematological cancers in the present study.

Hematological disorder	**n (%)**
Chronic myeloid leukemia (CML)	50 (54.34)
Acute myeloid leukemia (AML)	25 (27.17)
Acute lymphoblastic leukemia (ALL)	7 (7.6)
Chronic lymphoblastic leukemia (CLL)	4 (4.35)
Myelodysplastic syndrome (MDS)	6 (6.52)
Total	**92**

Table 2. The demographic information and iFISH results of the study population.

Case No	**Age**	**Sex**	**Disease**	***BCR/ ABL* [t (9; 22)] %**	***AML1/ ETO* [t (8; 21)] %**	***PML/ RARA* [t (15; 17)] %**	***CBFB* [inv (16; 16)] %**	***P53* [del (p13.1)] %**	**The other chromosomal aberrations (%)**
C1	42	M	CML	**10**	–	–	–	–	–
C2	64	M	CML	**21**	–	–	–	–	–
C3	49	F	CML	**77**	–	–	–	–	–
C4	38	F	CML	**12**	–	–	–	–	–
C5	57	M	CML	**50**	–	–	–	–	–
C6	70	F	AML	–	–	0	–	–	–

C7	58	F	CML	**80**	–	–	–	–	–
C8	64	M	CML	0	–	–	–	–	–
C9	62	M	CML	**84**	–	–	–	–	–
C10	44	F	CML	0	–	–	–	–	–
C11	70	M	AML	3	7	4	**12**	–	–
C12	49	M	CML	**86**	5	9	5	–	–
C13	58	F	CML	7	5	5	8	–	–
C14	77	M	MDS	**11**	2	3	5	–	–
C15	70	M	AML	0	3	3	8	–	–
C16	68	F	ALL	6	3	6	3	–	–
C17	45	M	CML	**88**	–	–	–	–	–
C18	29	M	CML	**88**	–	–	–	–	–
C19	46	F	CML	**37**	–	–	–	–	–
C20	30	M	CML	**26**	–	–	–	–	–
C21	37	M	CML	**89**	2	5	1	–	–
C22	44	F	ALL	5	2	7	5	–	–
C23	70	M	CML	0	–	–	–	–	–
C24	41	M	CML	0	–	–	–	–	–
C25	37	F	AML	8	2	4	1	–	–
C26	35	F	AML	–	2	5	2	–	–
C27	43	M	AML	**12**	8	5	3	–	–
C28	44	F	AML	**55**	2	**11**	3	–	–
C29	52	M	AML	**10**	2	4	2	–	–
C30	59	M	ALL	**21**	–	–	–	–	–
C31	81	F	CML	6	1	6	0	–	–
C32	38	F	CML	**97**	–	–	–	–	–
C33	39	F	MDS	9	0	3	–	–	–
C34	36	M	ALL	6	4	4	–	–	–
C35	74	F	AML	5	3	8	–	–	**Monosomy 9 (70%)**
C36	21	M	AML	**11**	–	9	–	–	–
C37	75	M	AML	6	5	9	–	–	–
C38	54	M	MDS	5	1	6	–	–	**Trisomy 8 (16%)**
C39	70	F	CML	6	–	–	–	–	–
C40	20	F	CML	0	2	0	–	–	–
C41	60	M	CML	**86**	0	0	–	–	–
C42	77	M	CML	0	–	–	–	–	–
C43	59	M	MDS	7	–	–	–	–	–
C44	51	M	CML	**88**	–	–	–	–	–
C45	60	F	CML	**93**	–	–	–	–	–
C46	46	M	CLL	–	–	–	–	–	–
C47	55	M	AML	2	1	**79**	–	–	–
C48	70	M	MDS	0	0	1	–	–	–
							–	–	**Monosomy 17 (25%)**

C49	61	F	AML	3	0	5			**Trisomy 8 and monosomy 21 (37%)**
C50	76	M	CML	8	–	–	–	–	–
C51	56	M	CLL	–	–	–	–	0	–
C52	70	F	CML	1	–	–	–	–	–
C53	70	F	AML	2	–	–	–	0	–
C54	60	M	CML	–	–	–	–	**10**	–
C55	63	F	CML	**96**	–	–	–	–	–
C56	56	M	CML	**6**	–	–	–	–	–
C57	56	M	CML	**5**	–	–	–	–	–
C58	43	M	CML	**3**	–	–	–	–	–
C59	59	M	CML	**86**	–	–	–	–	–
C60	69	M	CML	**7**	–	–	–	–	–
C61	51	M	CML	–	–	–	–	0	–
C62	23	F	ALL	**7**	–	–	–	–	–
C63	40	M	AML	**6**	–	2	–	–	–
C64	35	F	MDS	**6**	0	4	–	–	–
C65	53	M	AML	**0**	0	8	0	–	–
C66	69	F	AML	–	1	4	2	–	Trisomy 21 (85%)
C67	35	F	AML	**4**	-	86	0	–	Trisomy 8 (3%)
C68	33	M	AML	**3**	4	5	–	–	–
C69	54	M	AML	**2**	-	6	0	–	–
C70	54	M	AML	**0**	0	3	–	–	–
C71	47	M	AML	**9**	0	4	–	–	Trisomy 8 (98%)
C72	64	M	AML	**4**	0	4	–	–	–
C73	42	M	AML	**0**	–	0	0	–	–
C74	30	F	CML	**85**	–	–		–	–
C75	54	M	CML	**1**	0	3	–	–	–
C76	38	F	ALL	**4**	0	0	–	–	Tetrasomies 8 and 21 (55%)
C77	48	M	AML	**5**	4	0	–	–	Trisomy 8 (89%)
C78	23	M	CML	**4**	–	–	–	–	–
C79	46	F	CML	**0**	–	–	–	–	–
C80	60	M	CLL	–	–	–	–	0	–
C81	49	M	CML	**45**	–	–	–	–	–
C82	58	M	CML	**93**	–	–	–	–	–
C83	54	F	CML	**6**	–	–	–	–	–

C84	25	F	CML	**5**	–	–	–	–	–
C85	47	M	CML	**8**	–	–	–	–	–
C86	46	M	CLL	–	–	–	–	4	–
C87	28	M	CML	**20**	–	–	–	–	–
C88	41	M	CML	**5**	–	–	–	–	–
C89	26	M	CML	**90**	–	–	–	–	–
C90	25	M	CML	**98**	–	–	–	–	–
C91	22	F	ALL	**3**	0	4	–	–	–
C92	44	M	CML	**0**	–	–	–	–	–

Table 3. The distribution of the patients according to the results of BCR/ABL, AML/ETO, PML/RARA, CBFB and *P*53.

	% (Positive results/total number of patients)				
Hematologic disorder	**BCR/ABL [t(9; 22)]**	**AML/ETO [t(8; 21)]**	**PML/RARA [t(15; 17)]**	**CBFB [inv(16; 16)]**	**p53 [del(p13.1)]**
CML	52.1(25/48)	0 (0/7)	0 (0/7)	0 (0/4)	50 (1/2)
AML	18.2(4/22)	0 (0/18)	12.5 (3/24)	8.3 (1/12)	0 (0/1)
ALL	14.3(1/7)	0 (0/5)	0 (0/5)	0 (0/2)	–
MDS	16.7(1/6)	0 (0/5)	0 (0/5)	0 (0/1)	–
CLL	–	–	–	–	0 (0/4)
Total	83	35	41	19	7

Table 4. Distribution of typical and atypical iFISH patterns with the ES probe in BCR/ABL+ leukemias studied at diagnosis.

iFISH pattern with *BCR/ABL* ES probe	**Chromosomal localization of signals**			**Number of Ph positive cases (%)**			
	F	R	G	CML (*n* = 25)	AML (*n* = 4)	ALL (*n* = 1)	MDS (*n* = 1)
A: 1F 2R 1G	1F(Ph)	2R (9)	1G (22)	**23 (92%)**	**2 (50%)**	1 (100%)	1 (100%)
B: 1F 1R 1G	1F(Ph)	1R (9)	1G (22)	1 (4%)	**2 (50%)**	–	–
C: 1F 1R 2G	1F(Ph)	1R (9)	2G (22,22)	1 (4%)	–	–	–

F:fusion, R: red, G: green, A: Representative schemes of nuclei carrying typical *BCR/ABL*; B and C: atypical *BCR/ABL* fused gene rearrangements on chromosome 9 or 9q deletion of the rearranged chromosome 9; coexistence of der(9q) and der(22q) deletions.

8/92 of our patients, on the other hand, had numerical chromosomal deficits or increases. In C35, a 74-year-old AML patient, 70 out of 100 cells were found to have monosomy 9. Trisomy 8 cells were found in 16 percent of MDS cells, 3 percent of AML cells, 98% of AML cells, and 89% of AML cells from the C38, C67, C71, and C77 cell lines. Additionally, the research discovered trisomy 21 in C66 (AML) (85%), tetrasomies 8 and 21 in C76 (ALL) (55%), and trisomy 8 in combination with monosomy 21 (37%) and monosomy 17 (25%) in C49 (AML) (Table 2, Figure 3).

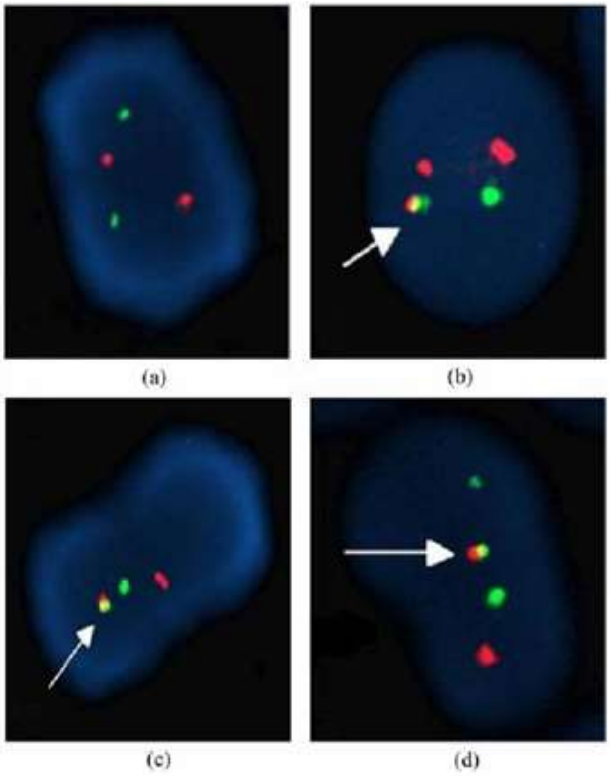

Figure 1. Different interphase FISH (iFISH) patterns found with the LSI *BCR/ABL* ES Dual Color Translocation probe (a)Normal nuclei, (b) 1F 1G 2R pattern (pattern A), (c) 1F 1G 1R pattern (pattern B), (d) 1F 2G 1R pattern (pattern C).

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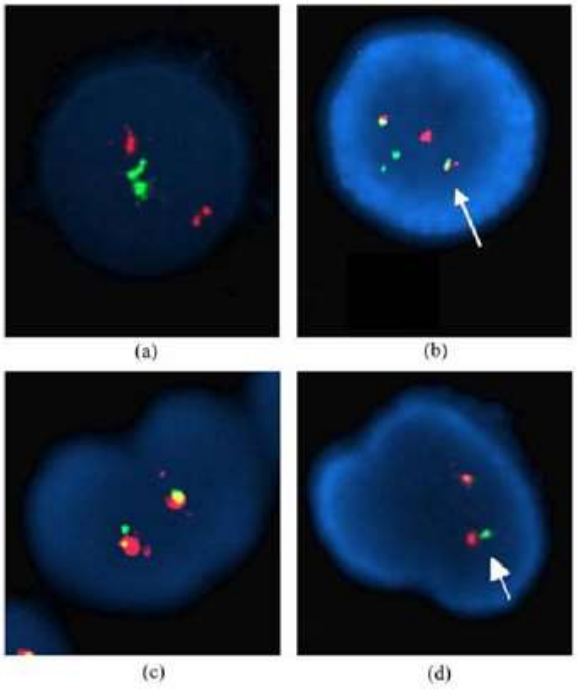

Figure 2. Interphase nuclei showing normal pattern and t (15;17) found with LSI *PML/RARA* Dual Color Dual Fusion Translocation Probe (a-b), normal pattern and inv (16) found with LSI *CBFB* Dual Color Break Apart Rearrangement Probe (c-d).

CONCLUSION

Now, FISH is a vital technique in the identification and monitoring of acquired chromosomal abnormalities associated with many haematological and other neoplastic diseases. This demands a fairly methodical approach to the validation of the FISH probes and technical processes, as well as the training of the persons who will be doing the testing. It also necessitates a method that is both thorough and intelligible for reporting out the findings. The variety of FISH probes and unique probe sets will surely improve as the number of key loci implicated in neoplastic chromosomal rearrangements or numeric aberrations increases. As a diagnostic tool, FISH has become an essential tool for both defining the disease process's early chromosomal abnormalities, as well as a reliable way of tracking the response to treatment and the remission of illness.

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