

# **FLUORESCENCE IN SITU HYBRIDIZATION ANALYSIS OF ADULT AML ACCORDING TO RECENT UPDATES OF CLINICAL CYTOGENETICS GUIDELINES AND QUALITY ASSURANCE**

*Thesis*

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### *List of Abbreviations*

<b><i>Abb.</i></b>	<b><i>Full Term</i></b>
<b>ABL</b> .....	Abelson murine leukemia (viral oncogene homolog)
<b><math>\alpha</math>-KG</b> .....	Alpha ketoglutarate
<b>ALL</b> .....	Acute lymphoblastic leukemia
<b>AML</b> .....	Acute myeloid leukemia
<b>ANAE</b> .....	Alpha naphthyl acetate esterase
<b>Anti- RC84</b> .....	Anti-human erythroleukemia cell line antigen
<b>APL</b> .....	Acute promyelocytic leukemia
<b>AS203</b> .....	Arsenic
<b>ATRA</b> .....	Alltrans retinoic acid
<b>BAALC</b> .....	Brain and acute leukemia cytoplasmic
<b>BCR</b> .....	B – Cell receptor
<b>BM</b> .....	Bone marrow
<b>BMT</b> .....	Bone marrow transplantation
<b>BrdU</b> .....	Bromodeoxyuridine
<b>CAE</b> .....	Chloroacetate esterase
<b>CALGB</b> .....	Cancer and leukemia group B
<b>CAN</b> .....	Candida gene
<b>CBC</b> .....	Complete blood count
<b>CBF</b> .....	Core – binding factor
<b>CD</b> .....	Cluster of differentiation
<b>cDNA</b> .....	Complementary deoxyribonucleic acid
<b>CEBPA</b> .....	CCAAT/ enhancer binding protein A
<b>CEP</b> .....	Centromeric Probe
<b>CGH</b> .....	Comparative genomic hybridization

<b><i>Abb.</i></b>	<b><i>Full Term</i></b>
<b>CLL</b> .....	Chronic lymphocytic leukemia
<b>CML</b> .....	Chronic myeloid leukemia
<b>CN-AML</b> .....	Cytogenitically normal AML
<b>CNS</b> .....	Central nervous system
<b>CR</b> .....	Complete remission
<b>CSF-1</b> .....	Colony stimulating factor
<b>DA</b> .....	Distamycin A
<b>DAPI</b> .....	Diamino-phenyl indole
<b>DEK</b> .....	Dog embryo kidney
<b>del</b> .....	deletion
<b>D-FISH</b> .....	Double fusion Fluorescence insitu hybridization
<b>D2HG</b> .....	D2- hydroxyglutarate
<b>DIC</b> .....	Disseminated intravascular coagulopathy
<b>DW</b> .....	Distilled Water
<b>ECOG</b> .....	Eastern cooperative oncology group
<b>EDTA</b> .....	Ethylene diaminetetraacetic acid
<b>EFS</b> .....	Event free survival
<b>ELN</b> .....	European leukemia net
<b>EM</b> .....	Electron microscope
<b>EVI1</b> .....	Ectopic viral integration site 1
<b>FAB</b> .....	French-American-British
<b>FBS</b> .....	Fetal bovin serum
<b>FCM</b> .....	Flow cytometry
<b>FITC</b> .....	Fluorescein isothiocyanate
<b>FLT3</b> .....	FMS-like tyrosine kinase 3
<b>G-banding</b> .....	Giemsa banding
<b>G-CSF</b> .....	Granulocyte colony stimulating factor

<b><i>Abb.</i></b>	<b><i>Full Term</i></b>
<b>GM-CSF</b> .....	Granulocyte-monocyte colony stimulating factor
<b>GVHD</b> .....	Graft – versus – host disease
<b>Hb</b> .....	Haemoglobine
<b>HCT-CI</b> .....	Hematopoietic cell transplantation specific co-morbidity index
<b>HER2</b> .....	Human epidermal growth factor receptor 2
<b>HLA-DR</b> .....	Human leukocytic antigen
<b>HMF</b> .....	Hypermetaphase FISH
<b>HSCT</b> .....	Hematopoietic stem cell transplantation
<b>IDH1</b> .....	Isocitrate dehydrogenase 1
<b>IFN-<math>\alpha</math></b> .....	Interferon alpha
<b>IL</b> .....	Interleukin
<b>Inv</b> .....	Inversion
<b>IPT</b> .....	Immunophenotyping
<b>IR</b> .....	Incomplete remission
<b>ISCN</b> .....	International system for human cytogenetic numenclature
<b>ITD</b> .....	Internal tandem duplication
<b>LDH</b> .....	Lactate dehydrogenase
<b>LSI</b> .....	Locus specific identifier
<b>LSP</b> .....	Locus specific probe
<b>MDS</b> .....	Myelodysplastic syndrome
<b>m-FISH</b> .....	Multicolour- FISH
<b>MLL</b> .....	Mixed lineage leukemia
<b>MN1</b> .....	Meningioma 1 gene
<b>MPN</b> .....	Myeloprolifirative neoplasm
<b>MPO</b> .....	Myeloperoxidase

<b><i>Abb.</i></b>	<b><i>Full Term</i></b>
<b>MRD</b> .....	Minimal residual disease
<b>m-RNA</b> .....	Messenger RNA
<b>NaF</b> .....	Sodium fluoride
<b>NCCN</b> .....	National comprehensive cancer network
<b>NEC</b> .....	Non-erythroid compartment
<b>NPM1</b> .....	Nucleophosmin 1
<b>NS</b> .....	Non - significant
<b>NSE</b> .....	Non- specific esterase
<b>OS</b> .....	Overall survival
<b>PAS</b> .....	Periodic acid schiff
<b>PB</b> .....	Peripheral blood
<b>PBS</b> .....	Phosphate buffer saline
<b>PCR</b> .....	Polymerase chain reaction
<b>PCS</b> .....	Phycoerythrin – cyanine 5
<b>PE</b> .....	Phycoerythrin
<b>Ph</b> .....	Philadelphia chromosome
<b>PI3-Kinase</b> .....	Phosphatidyl inositol 3-kinase
<b>PML</b> .....	Promyelocytic leukemia
<b>RARA</b> .....	Retinoic acid receptor alpha
<b>Rb</b> .....	Retinoblastoma gene
<b>RE</b> .....	Restriction enzyme
<b>RFS</b> .....	Relapse free survival
<b>RPMI</b> .....	Rosewell park memorial institute
<b>RT-PCR</b> .....	Reverse transcriptase polymerase chain reaction
<b>S</b> .....	Significant
<b>SBB</b> .....	Sudan black B
<b>SBH</b> .....	Southern Blot Hybridization

<b><i>Abb.</i></b>	<b><i>Full Term</i></b>
<b>SCE</b> .....	Sister chromatide exchange
<b>SCFR (c-Kit)</b> .....	Stem cell factor receptor
<b>SCT</b> .....	Stem cell transplantation
<b>SF-FISH</b> .....	Single fusion – Fluorescence insitu hybridization
<b>SSC</b> .....	Standard saline citrate
<b>SWOG</b> .....	Southwest oncology group
<b>t</b> .....	Translocation
<b>TdT</b> .....	Terminal deoxynucleotidyl transferase
<b>TEM</b> .....	Transmission electron microscopy
<b>TKD</b> .....	Tyrosine kinase domain
<b>TKI</b> .....	Tyrosine kinase inhibitor
<b>TLC</b> .....	Total leukocytic count
<b>TNF</b> .....	Tissue necrosing factor
<b>UD</b> .....	Undetermined
<b>VEGF</b> .....	Vascular endothelial growth factor
<b>WCP</b> .....	Whole chromosome painting probe
<b>WHO</b> .....	World health organization
<b>WT1</b> .....	Wilms tumor 1
<b>-</b> .....	Monosomy
<b>+</b> .....	Trisomy

## List of Tables

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
<b>Table (1):</b>	Incidence of Hematopoietic Malignancies In Arab Countries Including leukemia.....	6
<b>Table (2):</b>	Genetic disorders implicated in AML pathogenesis.....	8
<b>Table (3):</b>	Environmental factors that contribute to AML.....	8
<b>Table (4):</b>	Examples of some genes involved in AML pathogenesis. ....	11
<b>Table (5):</b>	FAB classification of acute myeloid leukemia. ....	17
<b>Table (6):</b>	WHO classification system of AML in 2008. ....	18
<b>Table (7):</b>	Cytochemical stains in AML .....	27
<b>Table (8):</b>	Immunophenotypic patterns in AML subtypes. ....	29
<b>Table (9):</b>	Risk group definition based on cytogenetic evaluation at diagnosis .....	31
<b>Table (10):</b>	Prognostic value of molecular aberrations in AML: .....	36
<b>Table (11):</b>	Risk group definition by combining cytogenetics& molecular markers evaluated at diagnosis.....	37
<b>Table (12):</b>	Selected New Agents under Study for Treatment of Adults with AML.....	40
<b>Table (13):</b>	Indication for allogeneic stem cell transplantation in acute myeloid leukemia.....	42
<b>Table (14):</b>	Examples of some chromosomal abnormalities in AML. ....	44
<b>Table (15):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of t (8; 21) .....	46
<b>Table (16):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of t (15; 17) .....	48
<b>Table (17):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of inv 16.....	50
<b>Table (18):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of t (9; 11) (p22; q23).....	53
<b>Table (19):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of t (6; 9) DEK/CAN. ....	57

## List of Tables (Cont...)

<i>Table No.</i>	<i>Title</i>	<i>Page No.</i>
<b>Table (20):</b>	Epidemiological, morphological and immuno-phenotypic characteristic of inv (3) (q21q26.2).....	58
<b>Table (21):</b>	Clinical correlation of common genetic aberrations in AML .....	68
<b>Table (22):</b>	The main types of chromosome banding methods.....	72
<b>Table (23):</b>	Clinical & laboratory findings, IPT and outcome of newly diagnosed AML patients.....	122
<b>Table (24):</b>	Clinical and lab data of the 41 newly diagnosed AML patients .....	124
<b>Table (25) :</b>	Immunophenotyping pattern of newly diagnosed AML .....	125
<b>Table (26):</b>	FISH pattern of newly diagnosed AML patients .....	127
<b>Table (27):</b>	Results of AML patients' outcome in relation to different prognostic factors.....	128



## List of Figures

<i><b>Fig. No.</b></i>	<i><b>Title</b></i>	<i><b>Page No.</b></i>
<b>Fig. (1):</b>	Model of leukemogenesis with two cooperating classes of mutations .....	9
<b>Fig. (2):</b>	The interplay of different molecular alterations in the development of AML .....	13
<b>Fig. (3):</b>	Schematic presentation of some intracellular signaling pathways with relevance for the pathogenesis of AML .....	16
<b>Fig. (4):</b>	Algorithmic Approach To The Diagnosis of Acute Myeloid Leukemia. ....	19
<b>Fig. (5):</b>	Auer Rods seen in AML, Specific to M2 type.....	25
<b>Fig. (6):</b>	A high power view of a bone marrow smear of M0/M1 subtype of AML .....	25
<b>Fig. (7):</b>	A high power view of a bone marrow smear of M1 Subtype of AML .....	25
<b>Fig. (8):</b>	A high power view of a bone marrow smear of M2 Subtype of AML .....	25
<b>Fig. (9):</b>	A high power view of a bone marrow smear of M3 subtype Of AML .....	26
<b>Fig. (10):</b>	A high power view of a bone marrow smear shows M3v Subtype of AML .....	26
<b>Fig. (11):</b>	A high power view of a bone marrow smear shows M4 Subtype of AML .....	26
<b>Fig. (12):</b>	A high power view of a bone marrow smear shows M5a subtype of AML .....	26
<b>Fig. (13):</b>	A high power view of a bone marrow smear shows M5b subtype of AML .....	26
<b>Fig. (14):</b>	A high power view of a bone marrow smear shows M6 subtype of AML .....	26
<b>Fig. (15):</b>	A high power view of a bone marrow smear shows M7 Subtype of AML .....	27
<b>Fig. (16):</b>	MPO positive.....	28
<b>Fig. (17):</b>	Flow chart for the therapy of newly diagnosed acute myeloid leukemia.....	38

## List of Figures (Cont...)

<i><b>Fig. No.</b></i>	<i><b>Title</b></i>	<i><b>Page No.</b></i>
<b>Fig. (18):</b>	Morphological and chromosomal features in t (8; 21).....	45
<b>Fig. (19):</b>	The t (15; 17) (q22; q21) PML/RAR- $\alpha$ ).....	47
<b>Fig. (20):</b>	Normal structure of chromosome 16 and the pericentric inversion of chromosome 16 .....	51
<b>Fig. (21):</b>	MLL gene and partner genes.....	54
<b>Fig. (22):</b>	t (6; 9) (p21; q34).....	57
<b>Fig. (23):</b>	5q-deletion.....	59
<b>Fig. (24):</b>	A normal G-banded bone marrow karyotype .....	72
<b>Fig. (25):</b>	Principle of FISH technique.....	76
<b>Fig. (26):</b>	Signal pattern positive for BCR/ABL fusion gene by LSI BCR/ABL single fusion, dual color probe.....	82
<b>Fig. (27):</b>	Signal pattern positive for BCR/ABL fusion gene by LSI BCR/ABL double fusion, dual color probe .....	83
<b>Fig. (28):</b>	Examples of FISH probe designs commonly used in hemato- lymphoid disorders and their resulting hybridization patterns.....	84
<b>Fig. (29):</b>	Whole chromosome paint .....	85
<b>Fig. (30):</b>	Multicolor FISH.....	86
<b>Fig. (31):</b>	Comparative genomic hybridization.....	89
<b>Fig. (32):</b>	Schematic drawing of the PCR cycle.....	94
<b>Fig. (33):</b>	cDNA microarray .....	101
<b>Fig. (34):</b>	Nucleic acid hybridization .....	107
<b>Fig. (35):</b>	A pie Chart results of numerical aberrations in all patients .....	129
<b>Fig. (36):</b>	A pie Chart results of structural aberrations in all patients.....	129
<b>Fig. (37):</b>	A Bar Chart of TLC in relation to patient outcome in newly diagnosed patients .....	130
<b>Fig. (38):</b>	A Bar Chart of Numerical aberrations in relation to patient outcome in newly diagnosed patients.....	130
<b>Fig. (39):</b>	A Bar Chart of Structural aberrations in relation to patient outcome in newly diagnosed patients.....	131

# **PROFESSIONAL GUIDELINES FOR CLINICAL CYTOGENETICS OF ACUTE MYELOID LEUKAEMIA**

## **I-INTRODUCTION**

Professional guidelines for Cytogenetics laboratories incorporate the standards imposed by regulatory bodies (Clinical Pathology Accreditation (CPA)) and by statute (Clinical Governance) (**Association for Clinical Cytogenetics, 2012**).

## **II-BACKGROUND**

Cytogenetic analysis is integral to the diagnosis and/or classification of both AML and MDS, and is recommended in clinical guidelines (**Milligan et al., 2006 and Dohner et al., 2010**). Detection of disease-specific abnormalities can allow the correct diagnosis to be reached, provide information on prognosis and allow a risk classification to be made. It can also be useful in assessing disease progression or response to treatment. This information can be used by the clinician to counsel the patient and to select the appropriate mode of treatment.

### **A) AML Clonal karyotype:**

Abnormalities are found in leukaemic cells in approximately 55% of adult AML cases and 78% of childhood AML (**Milligan et al., 2006 and von Neuhoff et al., 2010**), detection of these abnormalities can aid the diagnosis by demonstration of clonality. Many abnormalities are not specific

to AML and general markers of myeloid neoplasia are common. Within the World Health Organisation Classification of Tumours of Haematopoietic & Lymphoid Tissues 2008 (Swerdlow et al., 2008), a number of diagnoses within AML are now defined by specific cytogenetic abnormalities.

## **B) Cultures:**

It is recommended that a method of cell counting is used, so that final culture densities can be optimised to approximately  $1 \times 10^6$  /ml. When there is sufficient sample, at least two culture types should be initiated to increase the chance of obtaining analysable abnormal metaphase preparations and detecting the abnormal clone. Standard one and/or two day cultures are appropriate for all myeloid disorders. It has been reported that growth factor supplements, such as granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor and interleukin-3, may improve the quality of cytogenetic preparations and facilitate chromosome analysis in myeloid malignancies (Earle et al., 2007). Occasionally, AML may present as a solid, extra-medullary granulocytic sarcoma either with or without concurrent bone marrow infiltration. Cytogenetically,  $t(8;21)(q22;q22)$  or  $inv(16)(p13q22)$  are frequent findings. Tissue samples should be disaggregated to produce a cell suspension; this may then be cultured, processed and analysed in the same way as bone marrow.

## **C) Analysis:**

### **1- G-banded analysis for AML and MDS at diagnosis:**

For all diagnostic cases, if no clonal abnormality is detected by G-banding, a minimum of 20 metaphases must be

examined, with at least 10 of these being fully analysed. If fewer than 20 suitable metaphases are available, the result must be reported with a 'partial analysis' caveat. If 10 cells cannot be fully analysed in a case with normal cytogenetics then this must be considered a failed analysis. Where an abnormal clone is detected, examination of 10 metaphases is recommended, with at least five of these being fully analysed. Abnormal results may, if necessary, be reported on fewer metaphases, provided that the ISCN criteria for a clone are fulfilled (i.e. two or more cells with same extra chromosomes or structural rearrangements, or three or more with the same chromosome loss). FISH may be useful to corroborate the presence of abnormalities tentatively identified in such restricted analyses. In the case of finding a single cell with a potentially significant abnormality, such as loss of chromosome 7, it is recommended that a minimum of 10 additional cells are scored in order to exclude this, or that FISH analysis is considered.

## **2-FISH/RT-PCR analyses at diagnosis:**

**a)Acute promyelocyticleukaemia(APL):** If APL is suspected at presentation, rapid analysis of RARA rearrangement status may be necessary and this may be best achieved by FISH or RT- PCR. It is important to distinguish between t(15;17)(q24;q21) PML-RARA and t(11;17)(q23;q21) ZBTB16(PLZF)-RARA in APL since these have differing responses to ATRA (**Sanz et al., 2009**). In the situation where metaphase chromosomes are unavailable and FISH indicates RARA rearrangement but not PML-RARA fusion, then analysis should be undertaken to rule out ZBTB16 (PLZF) involvement. Since there are currently no commercial FISH probes for

ZBTB16(PLZF)-RARA, this may require referral to another laboratory.

It is important to note that variant rearrangements can occur which are both chromosomally cryptic and also normal by FISH analysis using both PML-RARA dual fusion and RARA break apart probes. RT-PCR analysis must be performed in such cases; this may require referral to another laboratory. NB/ the recently redefined location for PML is 15q24; this should be used in the translocation ISCN.

**b)Inv(16)(p13q22):** This subtle rearrangement may be overlooked in poor quality preparations. In cases where G-banding shows apparently normal chromosomes 16, FISH or RT-PCR analyses must be carried out if (i) the haematologist reports bone marrow morphology consistent with inv (16), or (ii) secondary abnormalities associated with inv(16), such as deletion of 9q or trisomy 22, are identified in the karyotype. Even if an inv (16) is detected by G-banding, it is recommended that FISH or RT-PCR be used to confirm the interpretation. bi-phenotypicleukaemia / leukaemia in infants.

### **3-Unsuccessful Karyotype – FISH panels :**

When chromosome analysis is unsuccessful due to lack of metaphases or poor quality preparations, interphase FISH and/or RT-PCR should be considered to investigate abnormalities of established prognostic importance. The need for such studies will vary from case to case, taking into account the potential impact on clinical management approaches.

## **D) Interpretation and Reporting :**

Abnormal results must be linked where possible to WHO classification subtypes (**Swerdlow et al., 2008**). If a patient is known to be entered onto a particular trial, then the appropriate risk stratification should be given according to the trial classification. If there is a discrepancy with more recent data, then this should be highlighted.

### **1- Karyotype complexity :**

Historically, the criteria used to classify karyotype complexity have not been clearly or consistently defined. The number of abnormalities used to define complexity differs in AML and MDS, and so reports must refer to a particular prognostic scheme; this can be problematic since a diagnosis is often not confirmed at the time of requesting cytogenetics, it is recommended to use the classification of complexity described in Grimwade et al. 2010 (**Grimwade et al., 2010**) which stipulates  $\geq 4$  abnormalities. The classification of Chun et al. 2009 (**Chun et al., 2009**) is recommended for scoring the number of cytogenetic abnormalities in MDS.

### **2-Prognosis :**

Many cytogenetic abnormalities in AML carry a prognostic association. Information regarding prognosis may be derived from WHO (**Swerdlow et al., 2008**), or where a specific prognosis is based on multiple publications or inclusion on national or international trials. In such cases, prognostic associations must be reported.