Tumor specific fluorescence activated cell sorting (FACS) improves sensitivity of fluorescence in situ hybridization (FISH) assays: clinical implications for patients with hematologic malignancies

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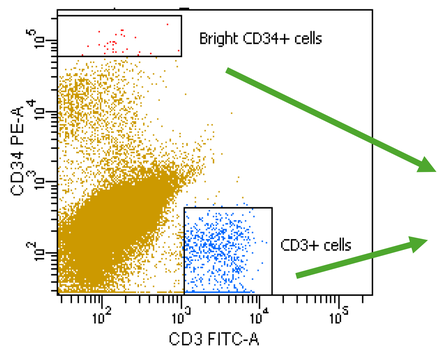
Introduction: FISH is a standard assay for the diagnostic work-up and disease monitoring of patients with hematologic malignancies, albeit with sensitivity limitations compared to other assays like PCR and flow cytometry. To enhance sensitivity, we routinely perform FISH on cell populations sorted by FACS.

Case studies:

(1) An 11-year-old female with AML and monosomy 7, status post transplant. The flow cytometric findings showed no evidence of aberrant myeloid antigen expression or abnormal myeloblasts, however, rare myeloid progenitor cells were observed below the limit of enumeration.



Cell Fraction	Probe	Loci	Results	Pattern	ISCN
CD34++ progenitor cells	del(7q)	7q22/7q31	**ABNORMAL**	1R1G (23.5%)	nuc ish(D7S796,D7S2543)x1[12/51]
CD3+ T cells			Normal	2R2G	nuc ish(D7S796,D7S2543)x2[200]

Confirmation of MRD

(2) A 23-year-old male with Philadelphia-chromosome positive leukemia. The differential diagnosis includes B-ALL and CML in blast crisis.

Cell Fraction	CML Panel	Loci	Results	Pattern (%)	ISCN
CD33+ myeloid cells	BCR/ABL1 + ASS1	t(9;22)(q34;q11.2)	**ABNORMAL**	1R1G2F (89%)	nuc ish(ASS1x2,ABL1x3,BCRx3) (ABL1 con BCRx2)[178/200]
CD19+ B cells			**SUSPICIOUS**	1R1G2F (5.5%)	nuc ish(ASS1x2,ABL1x3,BCRx3) (ABL1 con BCRx2)[11/200]
CD3+ T cells			Normal	2R2G	nuc ish(ASS1,ABL1,BCR)x2[200]

FISH results indicate CML in blast crisis

(3) A 14-year-old male with *RUNX1*::*RUNX1T1* positive AML, post therapy. Concurrent flow cytometric findings showed no evidence of aberrant myeloid antigen expression or abnormal myeloblasts while RT-PCR studies are positive for *RUNX1*::*RUNX1T1* fusion transcripts.

Cell Fraction	Probe	Loci	Results	ISCN
CD34+,CD117+ Myeloid Progenitor cells	RUNX1T1/RUNX1	t(8;21)(q21.3;q22)	Normal	nuc ish(RUNX1T1,RUNX1)x2[200]
CD117++, CD34- Mast cells			**ABNORMAL**	nuc ish(RUNX1T1,RUNX1)x3 (RUNX1T1 con RUNX1x2)[96/100]
CD3+ T cells			Normal	nuc ish(RUNX1T1,RUNX1)x2[200]

Clonal mast cells, no MRD

Conclusion: FISH on FACS sorted cell populations can improve the standard-of-care diagnostic testing for patients with hematologic malignancies by providing cell lineage specific genetic information not obtainable via regular direct FISH.