Acute Lymphocytic Leukemia Interphase Chromosome Profiling (ALL-ICP): A Simple and Comprehensive Method to Detect Chromosome Abnormalities in ALL Samples

R. Babu[1], D.L. Van Dyke[2], J. Tepperberg[3], E. Fuentes[1], S. Fuentes[1], S. Kopuri[1], C. Williamson[2], M. Liu[4], S. Papa[1], S. Schwartz[3], P. Papenhausen[3], P. Koduru[5]

[1]Department of Research and Development, InteGen LLC, Orlando, FL 32819; [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55902; [3]Department of Cytogenetics, Lab Corp of America, Research Triangle Park, NC 27709; [4]Department of Clinical Cytogenetics, Genetics Associates Inc., Nashville, TN 37203 [5]Department of Pathology, UT Southwestern Medical Center, Dallas, TX 75390

InteGen LLC
Interphase Chromosome Profiling

INTRODUCTION

B- and T-cell acute lymphocytic leukemia (ALL) affects both children and adults. Cytogenetic and recent molecular genetic findings at diagnosis constitute important, independent prognostic factors in all age groups. Due to the typically poor morphology of the chromosome preparations, conventional cytogenetic analysis can miss one or more recurrent abnormalities and most ALL FISH panels only target select abnormalities. Therefore, a simple, fast, and comprehensive improvement to karyotype analysis would greatly enhance the diagnostic capabilities of clinical service as well as research laboratories for ALL.

MATERIALS & METHODS

We recently developed and validated a novel molecular technology, Interphase Chromosome Profiling (ICP) (Babu et al., 2017). We tested a variation (ALL- ICP) of our original method for interrogating interphase cells and metaphase chromosomes in ALL samples. The design consisted of a multiplex approach with analysis of six chromosomes per hybridization site. Telomeres and pericentromeric regions on each chromosome are targeted and the resulting fluorescent signals are spectrally distinct and easily recognizable from each other using two dual filter sets from Chroma. An additional probe set targeting common deletions and duplication/amplification was included. Overnight hybridization is done on one slide in four areas and whenever possible 20 metaphase spreads are analyzed for each chromosome. When 20 metaphases are unavailable, interphase nuclei were used to complete the 20-cell analysis. Modified culture conditions included increased time and exposure to dilute concentrations of Colcemid and/or exposure to a chemical known to induce premature chromosome condensation during the last three hours of culture. Forty-eight ALL samples were studied blindly by the laboratory that developed the technology. Three institutions provided cell pellets with known cytogenetic and FISH results.

RESULTS

Sample Number	Cytogenetic Findings	ICP Results	Additional changes found and/or characterized by ALL -ICP
1	Hyperdiploid, +der	Concordant +	Characterized the derivative
2	Add(5q), inv(7)	Inv missed	Add origin 15q
3	Tetraploid, add(3p), 6(p) 1-3 mar	Concordant +	Deletion 14q, markers origin 13q and 10q
4	Complex abnormalities	Concordant +	14q deletion and dim 14q
5	Complex, add and interstitial deletions	Missed interstitial deletions	Add origin 19q
6	Complex, add(5q), add(17q), interstitial deletions	Concordant +, Missed interstitial deletions	Add origin 5q and 8q resulting in duplications
7	Complex with markers	Concordant +	One mar is der(11)
8	-X and 6q deletion – 1 cell	Concordant +	t(12;21) and 14q deletion
9	Hyperdiploid, i(7q)	Concordant	None
10	Hyperdiploid, add(19p)	Concordant +	Add origin 12q
11	Hyperdiploid	Concordant +	+9 is der(9)t(8;9); +15 is +18
12	dic(9;20),+21	Concordant	None
13	t(1;19)(q23;p13.3)	Concordant +	14q deletion
14	t(1;19)(q23;p13.3)	Concordant	None
15	t(9;22)(q34;q11.2)	Concordant +	Homozygous 14q deletion
16	t(9;22)(q34;q11.2)	Concordant +	14q deletion
17	+i(21)(q10)x2	Concordant +	t(5;10), 16q and 22q deletions
18	Hyperdiploid	Concordant +	+14 is der(14)t(14;21), deletions (subtelomere) 18p and 15q
19	del(13)(q14q34)	Concordant +	t(12;21), 14q deletion
20	Hypodiploid, t(9;22), dup(3q)	Missed duplication	None
21	Hyperdiploid with dup(1q)	Missed duplication	del(3p) and 13 identified as der(3); deletion 16p and -20
22	t(6;22)(q23;q12)	Concordant +	t(12;21) and multiple rearrangements involving chromosomes 6, 9, 8, 10
23	Complex with variant t(9;22)	Concordant +	12p deletion
24	add(21)(q22)	Concordant +	21q deletion, *RUNX1* duplication
25	+X,+21c	Concordant +	21q deletion, 14q deletion, der(15)
26	del (12)(p11.2p13)	Concordant	None
27	Variant t(9;22)	Concordant +	Iso dic(17q)
28	del(6)(q13q27)	Concordant	None
29	Complex with add(10q)	Concordant +	Clarified add(10q) as iso dic(10q)
30	Deletions 9p and 6q	Concordant +	-19
31	add (6q)	Concordant +	Add origin 5q; der(19)t(1;19), 14q deletion
32	Hyperdiploid	Concordant +	10p and 21q deletions
33	9p (*CDKN2A*) deletion	Concordant	None
34	12p deletion; add chromosome	Concordant+	t(12;21), add material from chromosomes X and 11
35	Complex; FISH positive for *ETV6-RUNX1*	Concordant+	t(12;21), 14q32 homozygous deletion, 8p deletion, 6q deletion, *RUNX1* amplification
36	t(7;17)	Concordant+	*CDKN2A* dim (partial deletion), t(1;7)
37	t(2;14), +10	Concordant+	11q deletion; homozygous 14q deletion
38	t(9;22)	Concordant+	Homozygous *CDKN2A* deletion; 12p deletion, 14q deletion, 4p deletion
39	Complex karyotype; 'add' chromosomes	Concordant+	Add material from chromosomes 13q, 3p, 11/9q
40	Hyperdiploid; t(9;22); cyto 1 cell	Concordant+	ICP 20 cells abnormal
41	Hyperdiploid plus mars; FISH 19p deletion	Concordant+	mars are copies of extra chromosomes; *TCF3* deletion (19p13.3) missed by ICP – not targeted
42	6q deletion in two cells; 9p homozygous deletion	Concordant+	6q deletion in 20 cells
43	45,XY,dic(20;21)(q13.3;q22)	Concordant+	dic t(20;21) has tandem duplication of *RUNX1* as well as telomere; 20q deletion confirmed
44	9q deletion in 80% of the cells	Concordant+	9p deletion; balanced t(5;11)
45	Marker chromosome, -12; *ETV6-RUNX1* by FISH	Concordant+	Marker is possible variant translocation t(12;19;21)
46	46,XX,t(2;7)(p21;p22)	Concordant+	14q deletion; possible t(2;12)
47	Hyperdiploid	Concordant+	Possible 12p deletion
48	Hyperdiploid	Concordant	None

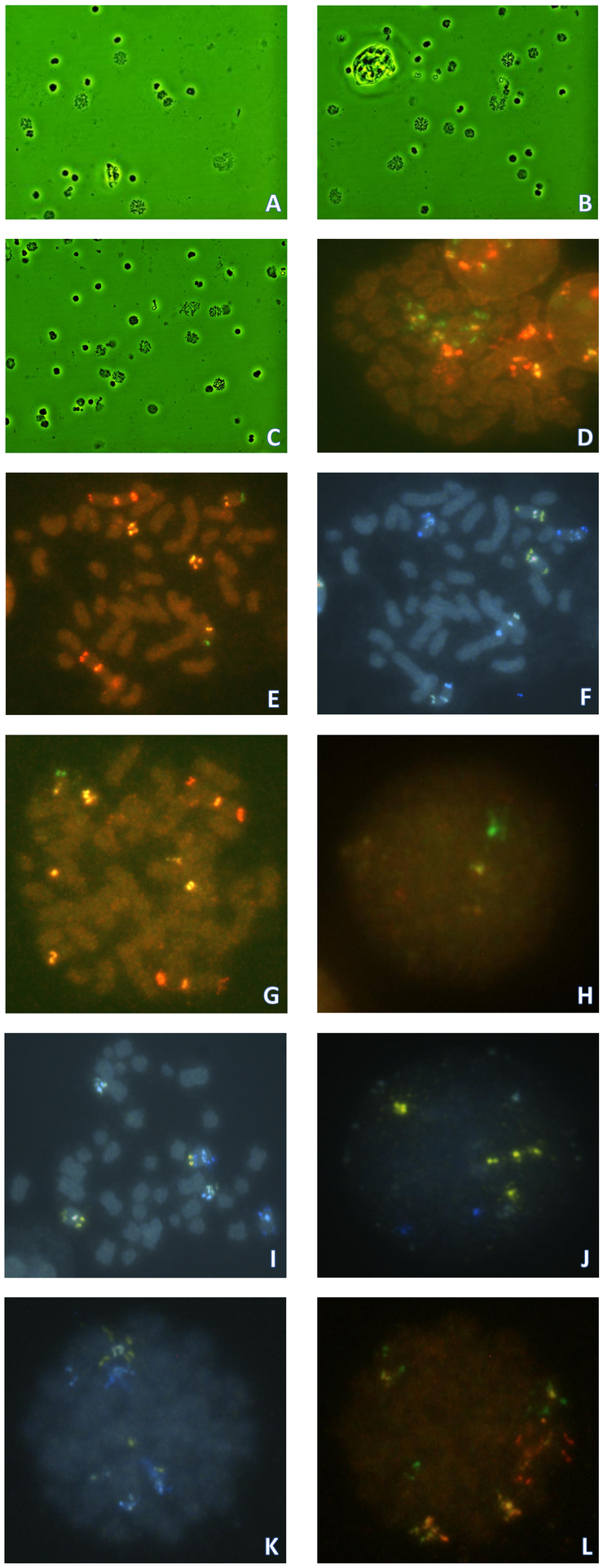
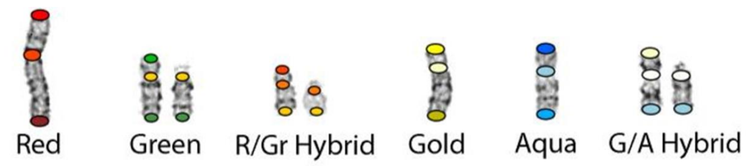


FIGURE LEGEND

A – C: Representative images showing a 3-fold increase in mitotic index; D: Trisomy 4 and 10; E: Normal chromosomes 2, 14 and 22 Mix 1; F: Normal chromosomes 8, 9 and 11 Mix 1; G: 14q32 (*IGH*) deletion; H: Homozygous 9p deletion and 6q deletion; I: der(6)(5;6)(q;q); J: *RUNX1* Amplification; K – L: t(12;21)(p13;q22)

DESIGN



CHROMOSOME COLOR SCHEME

Color Pattern	Mix 1	Mix 2	Mix 3	Mix 4	Mix 5
Red	2	1	4	7	9p21.3
Green	14	12	10	16	6q21
R/G Hybrid	22	17	Y	13	13q14.2
Gold	8	X	5	3	21q22.12
Aqua	9	19	6	18	Xp22.33
Y/A Hybrid	11	21	20	15	

CONCLUSION

- All numerical abnormalities identified by karyotype or FISH are detected by ALL-ICP.
- ALL-ICP refined the breakpoints and clarified marker and derivative chromosomes.
- Novel 14q32 (IgH) deletions and several previously unknown sub-telomere deletions were identified.
- There appears to be an association between t(9;22) and 14q deletions – nearly 50% with this deletion had the translocation.
- ALL common deletions and amplification of RUNX1 were detected.
- Non-recurring interstitial deletions and duplications not targeted by ALL-ICP were not detected.
- All known and new balanced translocations were detected.
- ALL-ICP detected additional abnormalities in 85% of the cases.
- A three-fold increase in mitotic index was observed in modified cultures.

ALL-ICP is a simple method that saves valuable time and detects all clinically relevant recurrent B- and T-cell chromosome abnormalities. Additionally, it characterizes the marker chromosomes and 'add' material. The improved diagnostic capabilities, supported by the study results, make ALL-ICP ideal for the genetic diagnostic workup of Acute Lymphocytic Leukemia on standard as well as modified cytogenetic harvests.

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