

Interphase Chromosome Profiling in the Workup of Products of Conception and Hematologic Malignancies. Time to do away with Classical Karyotype?

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Interphase Chromosome Profiling

INTRODUCTION

Karyotype plays an important role in establishing the diagnosis of malignancies and in determining the genetic basis of first trimester losses. However, current karyotypic methods are limited to the availability and the analyses of adequate mitotically active cells. These limitations can potentially give false negative results if present in a minor clone or if the relevant cells are not mitotically active in sufficient numbers in culture. In addition, culture failure is a frequent occurrence in many tissue types. Therefore, the increasing role of genetics in diagnosis and patient management necessitates the development of sensitive and failure-proof high resolution cytogenetic methods. To meet this demand, we recently developed and validated a novel cytogenetic technology 'Interphase Chromosome Profiling (ICP)' to assess the molecular karyotype of any tissue using interphase cells (Cytogenet Genome Res 2014;142:226, Abstract # 22). The idea behind this approach is to detect all numerical, most balanced and unbalanced structural aberrations including all Robertsonian translocations.

OBJECTIVES

The original validation study (referenced above) consisted of 20 bone marrows and 42 Products of conception (POC) tissues which showed a near-perfect concordance with the karyotype results. In the current study, we further tested the main attribute of this technology, i.e. to make it 'failure-proof and more sensitive' compared to the conventional cytogenetics.

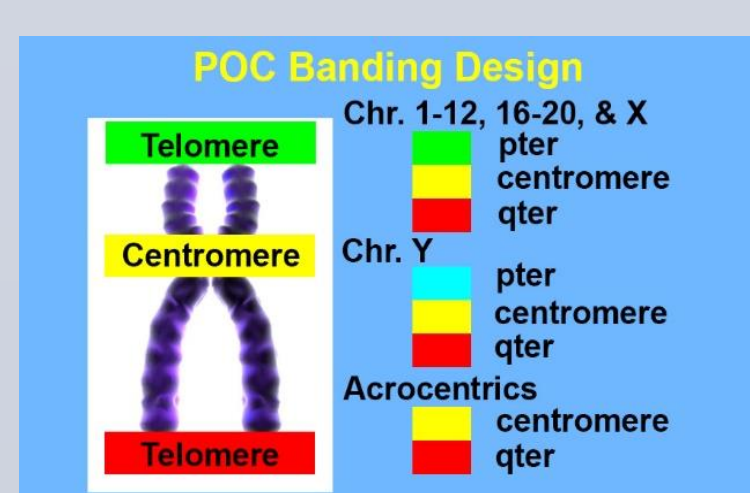
MATERIALS AND METHODS

The study consisted of 38 samples with karyotype results and 30 cases with failed cytogenetics. For both POC (4 samples) and oncology (64 samples), while each autosome was analyzed separately, the sex chromosomes were analyzed together for POC samples and separately for oncology samples. Individual chromosome hybridizations were done on four slides with six areas of hybridization on each slide, following standard FISH protocols. Appropriate filter sets were used to detect fluorochromes DEAC, Fluorescein-12, Cyanine555, Cyanine647, and CF594. A minimum of 20 interphase cells were analyzed for each chromosome. Since the entire chromosome was profiled as opposed to standard targeted FISH, the usual guidelines of metaphase analysis were followed with minor adjustments in defining the abnormal clone - four cells for both structural and numerical abnormalities.

For the assessment of Robertsonian translocations, similar to standard FISH studies, a normal cut-off had to be established. The cut off was 20% for any two pericentromeric probes to co-localize by random chance or by satellite association. Any juxtaposition of two signals greater than 20% of cells is considered abnormal. This was necessary since only one probe was used on each chromosome.

The Interphase Chromosome Profiling design is based on the concept of placing the FISH probes in an equidistant manner along the whole length of the chromosome as depicted in ICP Illustrations. The total number of bands in any chromosome arm was largely dependent on the overall length of that arm. Each chromosome arm consisted of a minimum of one and a maximum of six bands. Telomeres and centromeres were given pure color band while the interstitial bands were either pure or hybrid color (see illustrations). This configuration provides approximately a 600 band resolution equivalent karyotype, and each band on any given chromosome is molecularly distinct from its adjacent band or any other band on that chromosome. Therefore, any deviation of the expected number and/or position of the bands signifies an abnormality. Based on the specific characteristics of the signal patterns, it is classified either numerical or structural and further classified into particular category of abnormality.

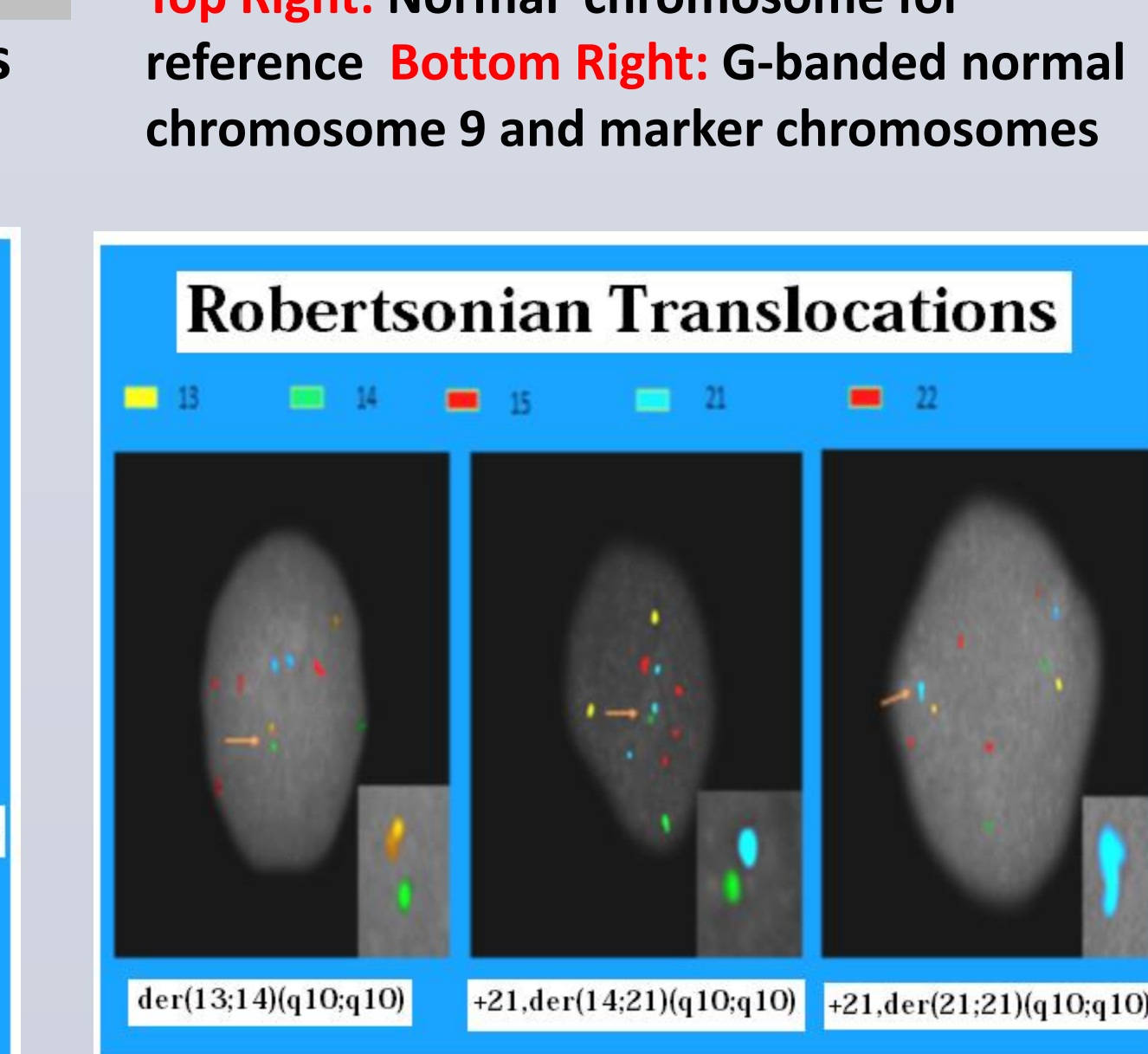
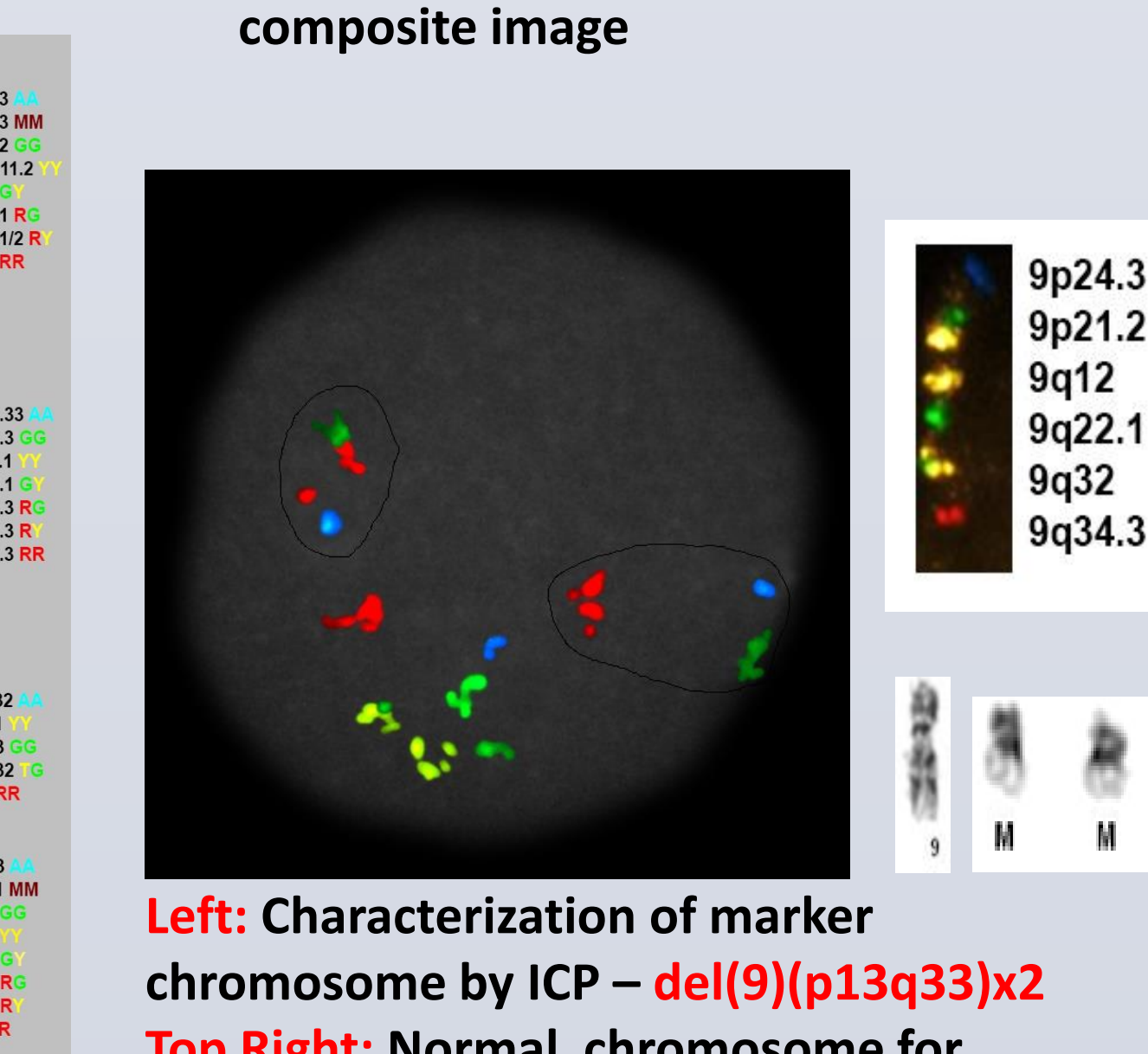
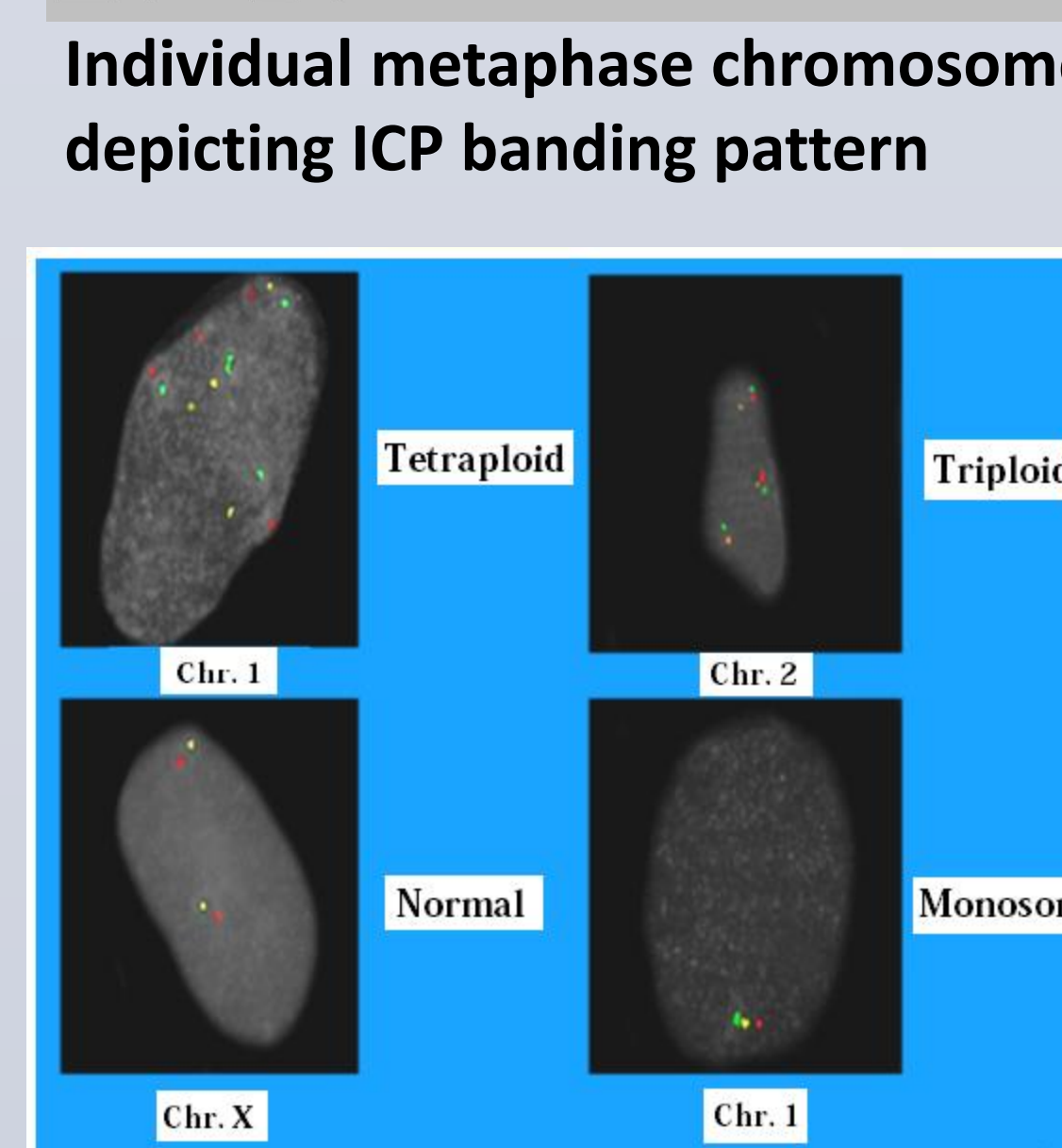
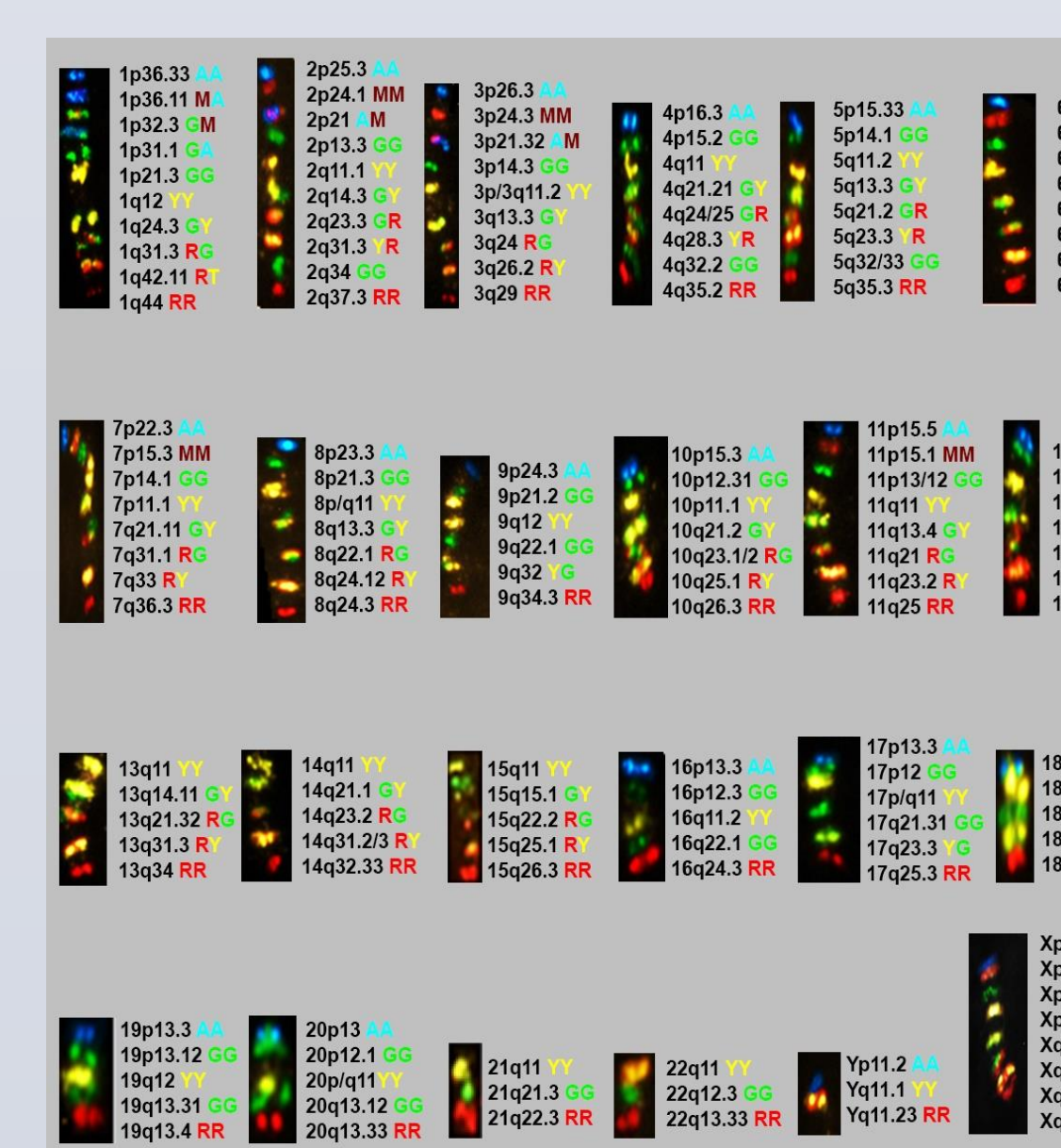
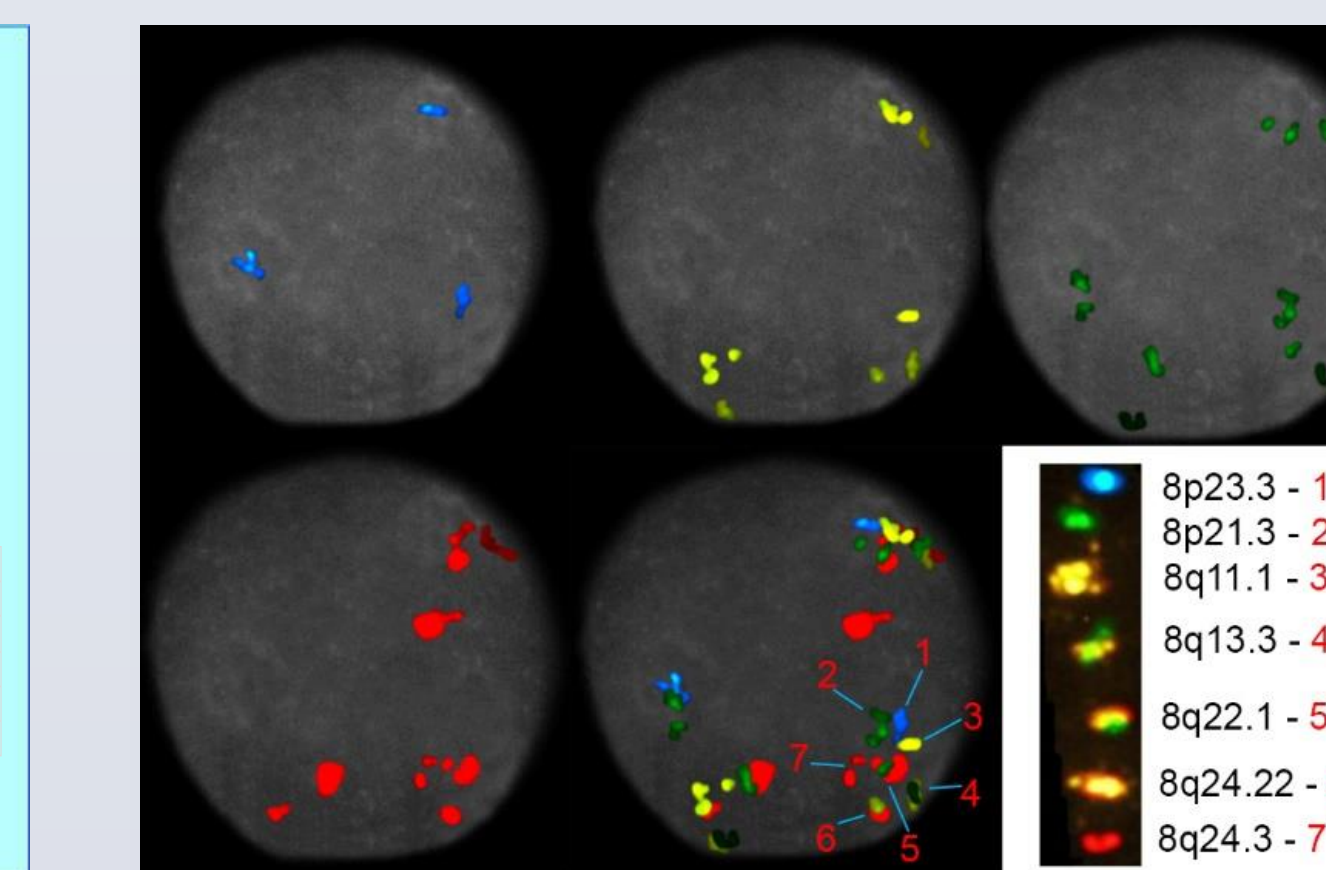
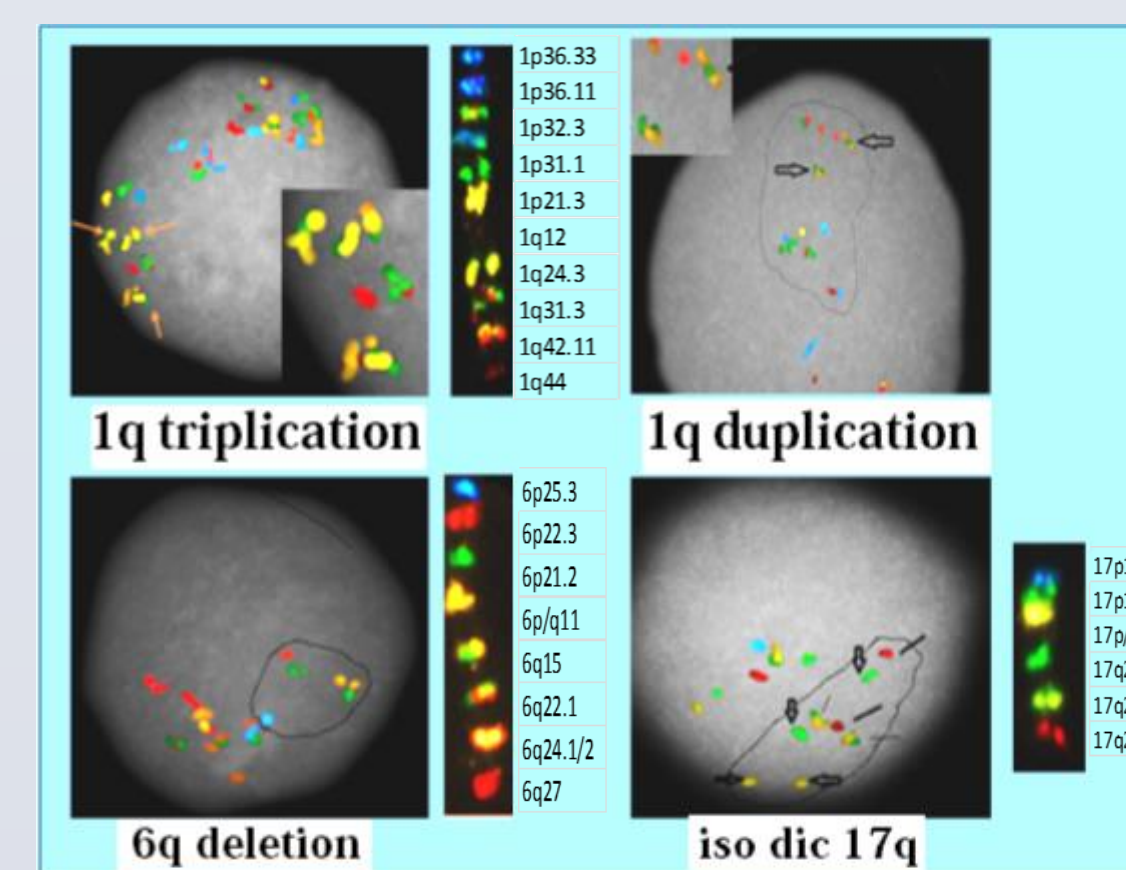
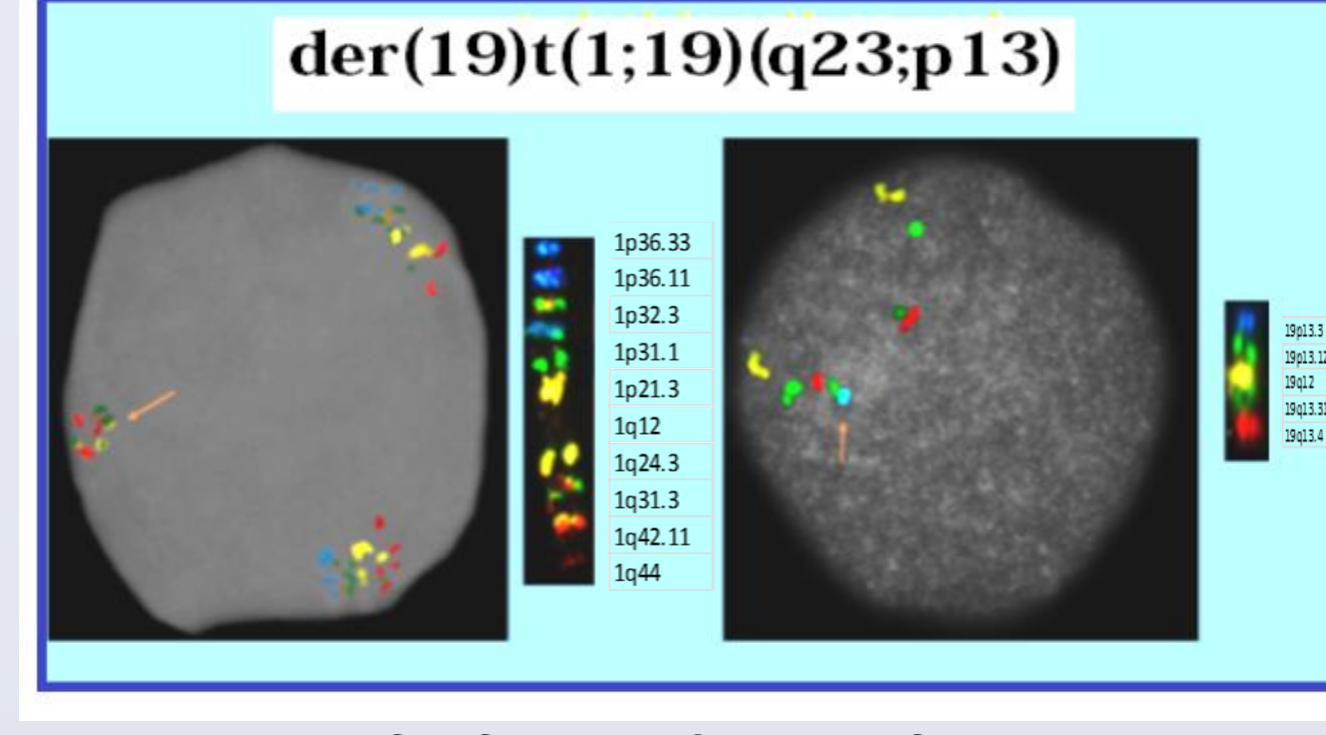
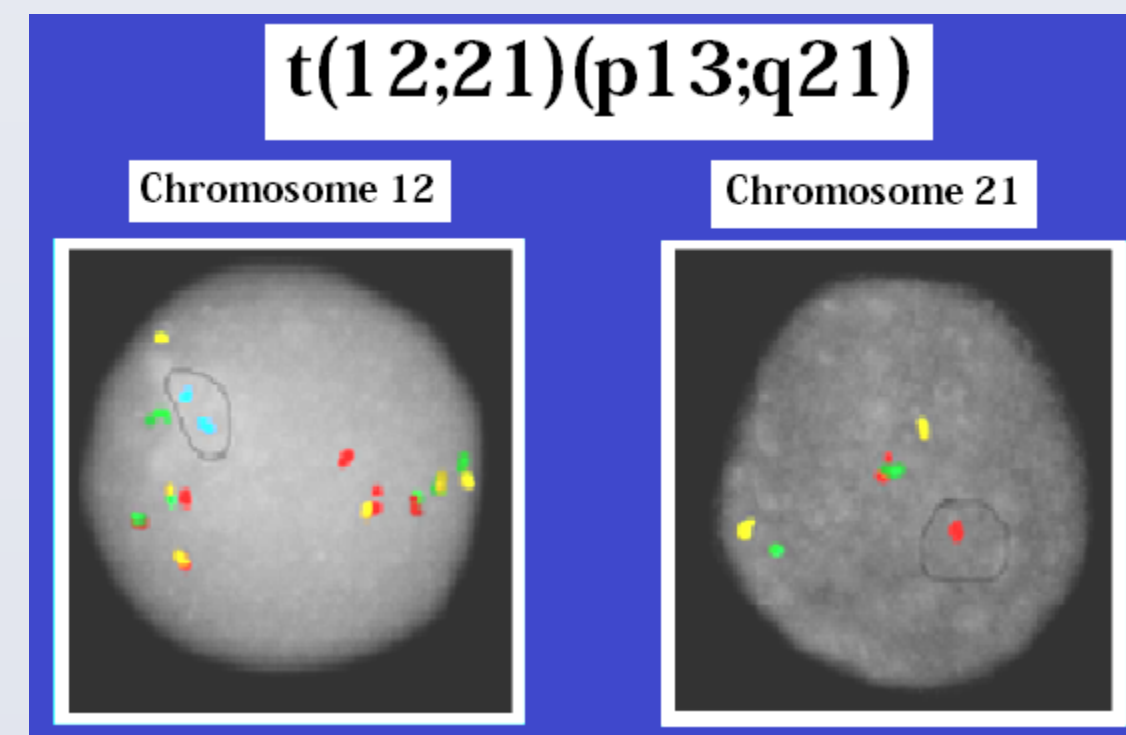
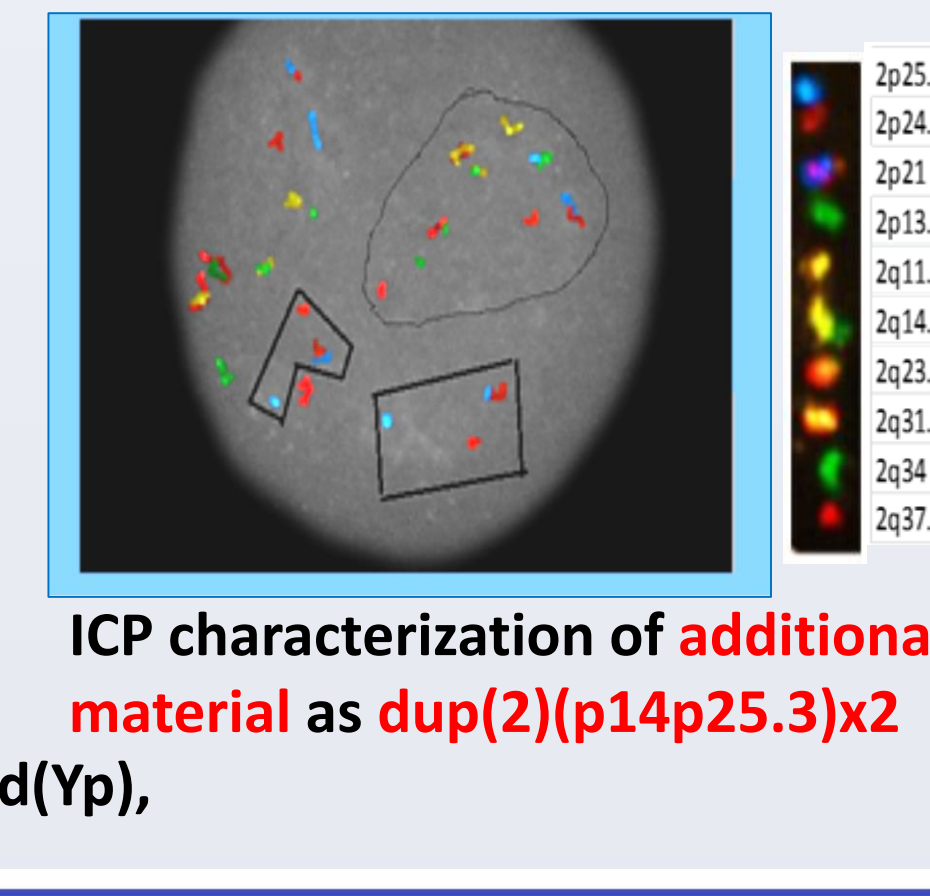
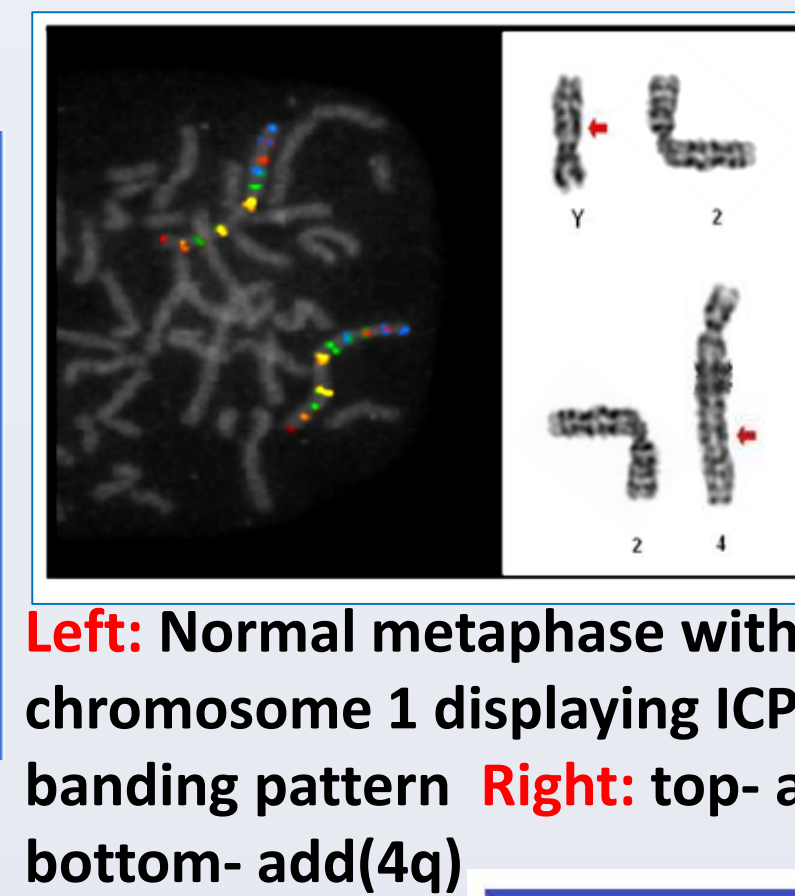
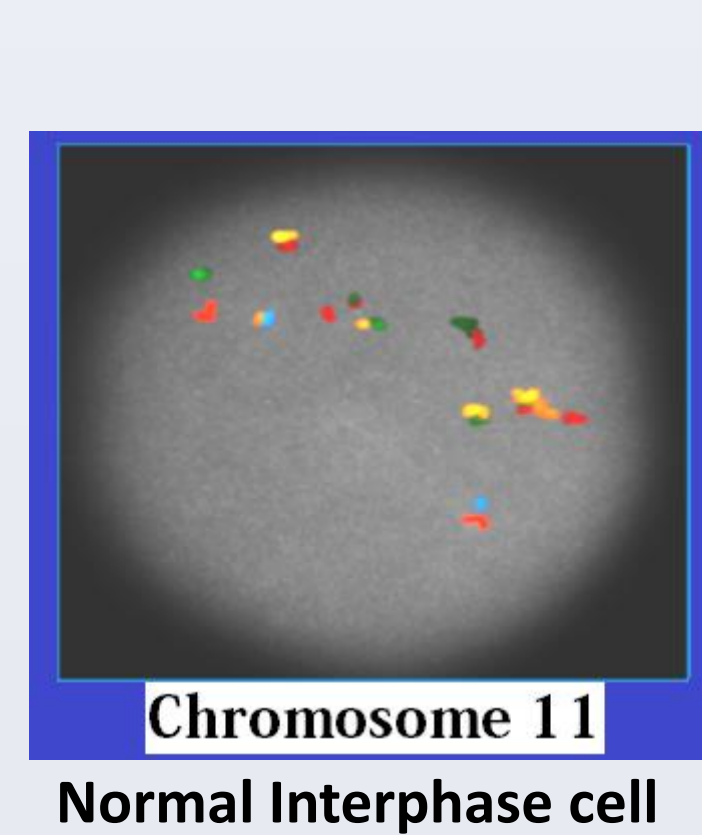
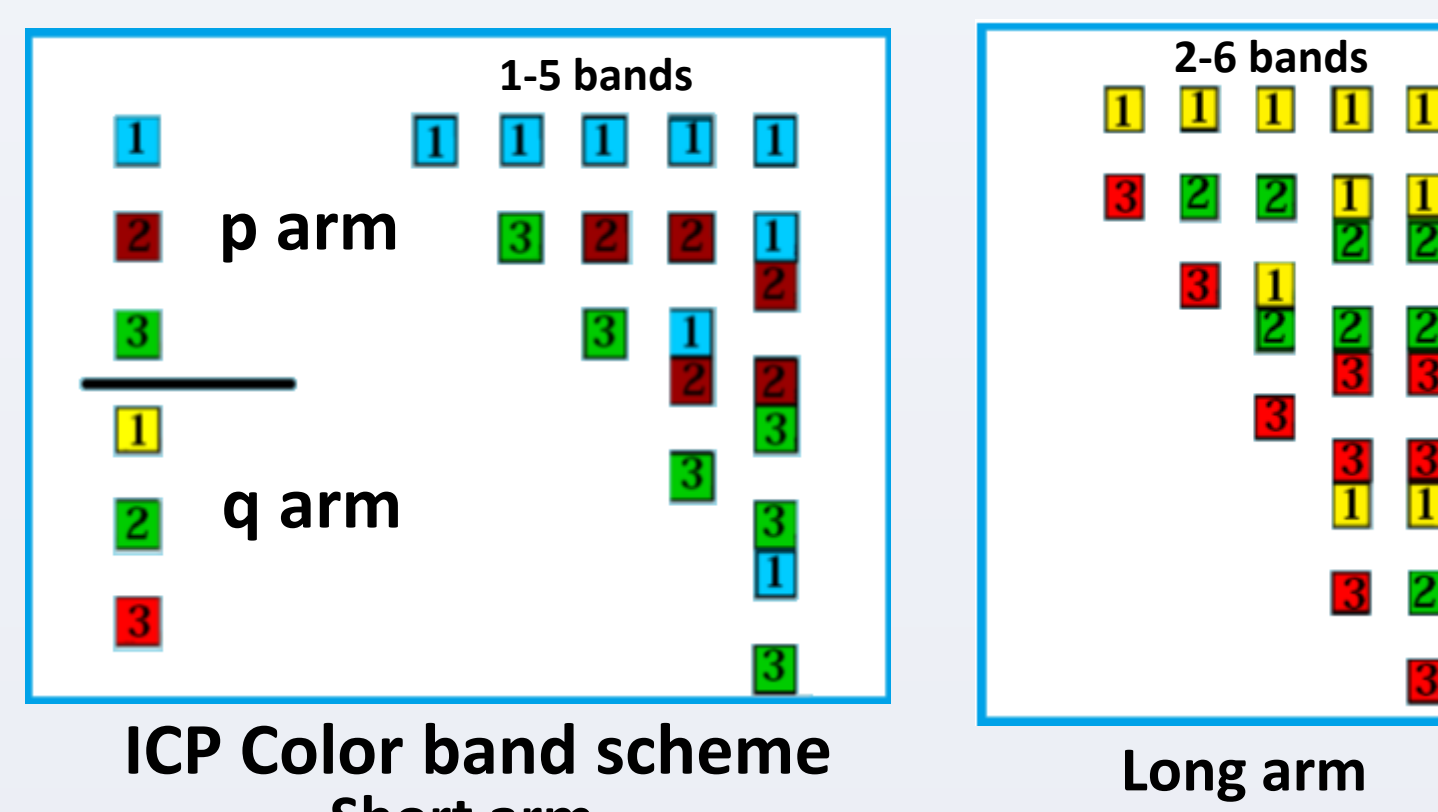
To assess the abnormalities commonly encountered in POC samples, the chromosome profiling design was simplified by targeting only telomeres and centromeres (see illustrations). A typical hybridization slide is shown below.



ICP ILLUSTRATIONS

Chr.	Chr.	Chr.			
1	p5/q5	9	p2/q4	17	p2/q4
2	p4/q6	10	p2/q5	18	p1/q4
3	p4/q5	11	p3/q5	19	p2/q3
4	p2/q6	12	p2/q5	20	p2/q3
5	p2/q6	13	p0/q5	21	p0/q3
6	p3/q5	14	p0/q5	22	p0/q3
7	p2/q5	15	p0/q5	X	p3/q5
8	p2/q5	16	p2/q3	Y	p1/q2

Number of bands in each arm of respective chromosomes



RESULTS

Comparison of Cytogenetics and ICP Results

Method	Number Failed	Normal	Abnormal	Abnormal and Revised	TOTAL
Cytogenetics	30	12	26	NA	68
ICP	0	26 (1*, 3**)	31 (7***)	11	68

Details of Discordant Normal, Abnormal and Abnormal/Revised and Redefined cases (ABN/RV)

Discordant Category	Cytogenetics Result	ICP Result
Normal		
*1 (POC sample)	Female	Male/Female
**1	Inversion 3q	Normal
**2	Inversion 11q	Normal
**3	Low level clone (2 cells)	Normal
Abnormal ***		
1	Normal	trisomy 12
2	Normal	+3,+7,+5,del(16q),+19
3	Normal	+12,+15,t(14;18),del(17q),del(19q)
4	Normal	t(11;14),del(18q)
5	Normal	t(11;15;17)
6	Normal	Monosomy 13
7 (POC sample)	Normal	Trisomy 22
ABN/RV	A = Additional changes	R = Revised and Redefined
1	Trisomy 12	A: del(19q)
2	t(14;18)(q32;q21)	A: -Y
3	hyperdiploid	A: t(8;22)(q24;q11)
4	complex	A: del(19p), del(20p)
5	complex	A: hyperdiploid
6	add(6q)	A: del(17q) R: dup(15)(q22q26.3)
7	monosomy 9, marker x2	R: del(9)(p13q33)x2
8	complex; monosomy 5, monosomy 20, marker x2	R: del(5q), del(20p)
9	add(Yp), add(4q)	R: dup(2)(p14p25.3)x2
10	marker	R: dup(1)(p32.3q24.3)
11	t(2;7)(p21;q22)	R: t(2;7)(p11.2;q21)

DISCUSSION AND CONCLUSIONS

Discussion: Obtaining the diagnostic chromosome abnormality at the initial workup of patients with hematologic disorders is extremely crucial for disease classification and management. In seven of the cases in this study, only normal karyotypes were obtained, but one or more clonal abnormalities were detected in these by ICP, including one with a variant t(15;17) characteristic of Acute Promyelocytic leukemia. Incorrect breakpoint assignment of structural abnormalities has obvious clinical implications. In one case with limited cells for karyotype and poor morphology, the initial breakpoint assignment of the t(2;7)(p21;q22) failed to recognize the potential involvement of CDK6 gene at 7q21, which was identified by ICP. In several cases, ICP was able to completely characterize the marker chromosomes and the "additional" material and identified other 'new' abnormalities. Moreover, in one case ICP allowed us to establish the mechanism of marker formation with a likely neo-centromere. This was inferred because ICP detected the marker in many cells proving its stability despite the absence of the traditional centromere signals. Finally, ICP also detected abnormalities otherwise missed by standard FISH panels. In the POC samples, ICP identified male cells and chromosomal abnormalities missed by conventional cytogenetics.

The one discordant case was an oncology sample where, due to a very low level clonal size, ICP missed the clonal abnormality where the abnormality was only observed in two cells by cytogenetics. Both inversion 11q and inversion 3q were missed by ICP. This is an inherent limitation of the current design of ICP and therefore alternate methods including standard FISH should be utilized to rule out inversions.

Conclusion: Since ICP is failure-proof and can detect both numerical and structural aberrations including Robertsonian translocations, we propose that it should be the first choice of method in the investigation of POC samples. For the workup of hematologic malignancies with failed cytogenetics and with "normal" results in plasma cell myeloma cases, ICP should be considered a REFLEX test since standard FISH panels do not detect all clinically relevant abnormalities.

REFERENCES AND CONTACT

- Trask B, Pinkel D (1990): Fluorescence in situ hybridization with DNA probes. Methods Cell Biol 33:388-400.
- Shearer BM, Thorland EC, Carlson AW et al., (2011): Reflex fluorescent in situ hybridization testing for unsuccessful product of conception cultures: a retrospective analysis of 5555 samples attempted by conventional cytogenetics and fluorescent in situ hybridization. Genet Med 13(6):545-52.
- Pe'rez-Simo'n JA, Garcia-Sanz R, Tabernero MD et al., (1998): Prognostic Value of Numerical Chromosome Aberrations in Multiple Myeloma: A FISH Analysis of 15 Different Chromosomes. Blood 91(9): 3366-3371.

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