Development and Validation of a simple, comprehensive, single FISH assay for multiple myeloma

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INTRODUCTION

Multiple myeloma is a common adult plasma cell neoplasm. Classical cytogenetic techniques are less valuable in the diagnostic and prognostic workup of bone marrow samples of multiple myeloma. It has become a standard of practice to enrich plasma cells with cd138 and depending on the enrichment method used, the number of cells recovered varies. Chromosome abnormalities play an important role in establishing the diagnosis and providing prognosis for treatment. Current methods involve use of several FISH probes in multiple independent hybridizations. This practice is labor intensive and expensive. In some instances, there may not be enough cells to carry out all the necessary hybridizations. Therefore, a simplified yet comprehensive approach in assessing the abnormalities from multiple myeloma samples, in a single hybridization will be extremely useful and cost-effective.

MATERIALS & METHODS

We have designed a multiplex assay that targets 10 different chromosomal locations. The design allows the detection of all chromosome changes commonly observed in multiple myeloma such as IGH rearrangements, ploidy changes, deletions and duplications of specific chromosomal regions. Based on our previous observations of a duplication on the long arm of one X chromosome in males (Babu and Koduru 2015), we included a probe to detect this duplication. The assay design with the spectral characteristics of the resulting fluorescence signals, target locations is listed below.

Seven cases with known cytogenetic and FISH results were tested with the probes developed for the single multiplex assay. A step-wise analysis of 20 interphase cells at a time was undertaken to assess the need for the commonly accepted guideline of 200 cells.

For simultaneous detection of all 10 color signals, two dual color filter sets from chroma were used: a red/green and gold/aqua. Raw images were acquired using a color CCD camera. No background subtraction or image manipulation through software packages was employed.

RESULTS

TABLE 1 – Results of 60 Cell Analysis

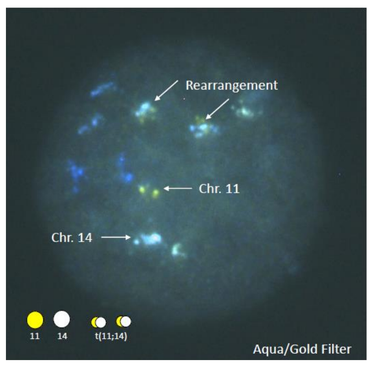
Case ID	Abnormality	20 Cells	40 Cells	60 Cells
IGS-060415-8	dup(1q)	50%	53%	52%
	+3	50%	50%	50%
	+5	50%	50%	48%
	+7	50%	50%	48%
	+9	50%	50%	48%
	+11	50%	53%	52%
	-13	25%	33%	33%
	+15	40%	40%	42%
	-16	50%	53%	52%
IGS-112117-2	dup(1q)	55%	60%	63%
	t(4;14)	85%	80%	78%
	+9	15%	18%	18%
	-11	50%	63%	58%
	+15	75%	75%	75%
	del(17p)	60%	63%	63%
IGS-060415-10	+5	25%	23%	23%
	+7	25%	23%	23%
	+9	25%	23%	23%
	+11	25%	23%	23%
	del(17p)	5%	5%	7%
	+19	25%	23%	23%
	dup(Xq)	20%	20%	22%
IGS-112117-1	+1, +1	35%	35%	35%
	+3	45%	48%	47%
	+4, +4	40%	40%	40%
	+5	45%	48%	47%
	+6	40%	40%	40%
	+7, +7	45%	48%	47%
	+9, +9	45%	48%	47%
	+11, +11	40%	40%	40%
	+13, +13	40%	40%	40%
	t(14;16)	40%	40%	40%
	+15	40%	45%	45%
	+16, +16	50%	45%	42%
	+19	45%	48%	47%
	+20	30%	30%	32%
	+X, +X	40%	40%	38%
IGS-050813-7	dup(1q)	30%	25%	25%
	+3	35%	35%	33%
	+5	40%	40%	38%
	+7	40%	38%	37%
	+9, +9	45%	40%	38%
	t(11;14)	20%	20%	22%
	+15	40%	38%	37%
	+19	35%	35%	33%

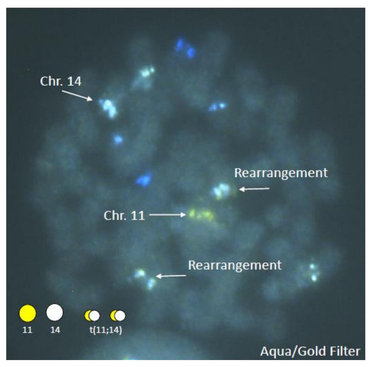
TABLE 2 – Results of 200 Cell Analysis

Case ID	Abnormality	20 Cells	40 Cells	60 Cells	80 Cells	100 Cells	120 Cells	140 Cells	160 Cells	180 Cells	200 Cells
IGS-012717-5	dup(1q)	45%	48%	48%	50%	49%	48%	49%	49%	49%	50%
	+3	45%	43%	42%	44%	46%	45%	46%	47%	47%	47%
	+5	60%	63%	62%	59%	59%	58%	58%	58%	58%	58%
	+7	60%	58%	57%	55%	56%	55%	56%	56%	57%	57%
	+9, +9	60%	63%	62%	61%	59%	58%	58%	58%	57%	57%
	+11, +11	65%	63%	62%	60%	58%	56%	56%	56%	56%	56%
	+15	60%	60%	60%	58%	57%	56%	56%	56%	57%	58%
	+19	45%	50%	48%	49%	50%	49%	49%	49%	49%	50%
	+20	15%	20%	23%	25%	24%	24%	24%	24%	24%	25%
	dup(Xq)	60%	58%	58%	58%	56%	56%	56%	55%	54%	55%
IGS-060415-9	+3	10%	10%	10%	9%	9%	9%	9%	9%	9%	9%
	+5	15%	13%	13%	13%	13%	13%	11%	11%	11%	11%
	+7	5%	8%	8%	9%	9%	8%	8%	8%	7%	8%
	+9	15%	13%	12%	10%	10%	10%	11%	11%	10%	10%
	+11	5%	8%	8%	9%	8%	8%	9%	8%	8%	8%
	+15	10%	10%	10%	9%	9%	8%	9%	9%	8%	9%
	+19	10%	10%	10%	10%	9%	9%	9%	9%	9%	9%
	+21	15%	13%	13%	13%	13%	13%	11%	12%	12%	11%
	+X	5%	5%	7%	8%	8%	8%	8%	7%	7%	7%

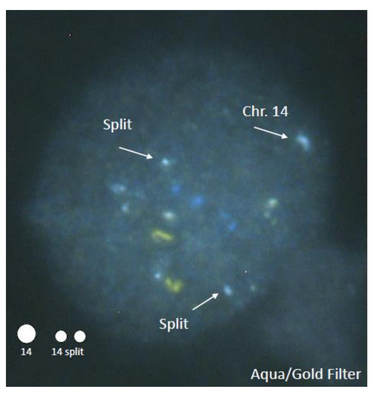
IMAGES

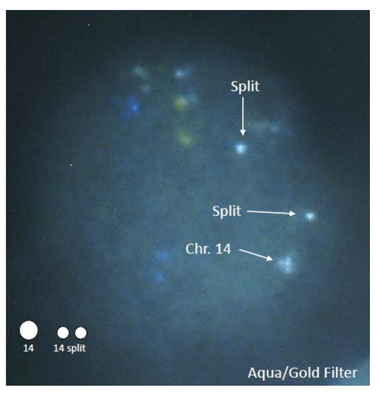
t(11;14)(q32;q13)



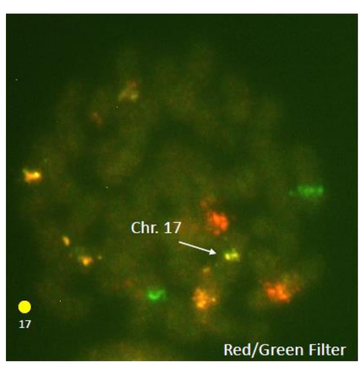


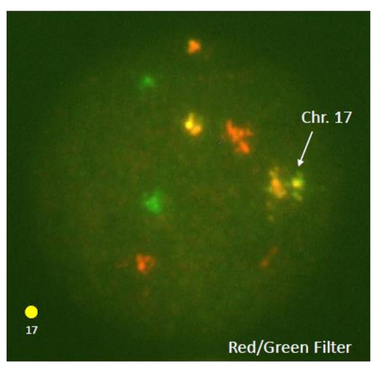
t(14;?)(q32;?)



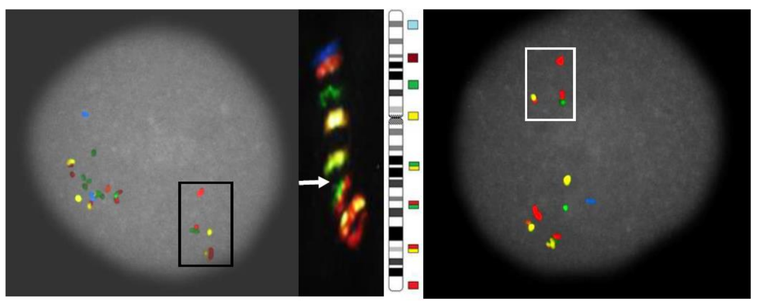


p53 deletion

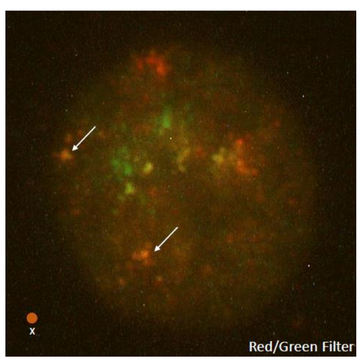


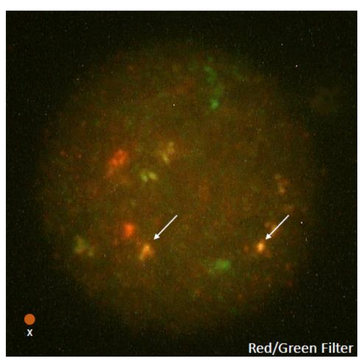


Xq21-qter duplication (male)



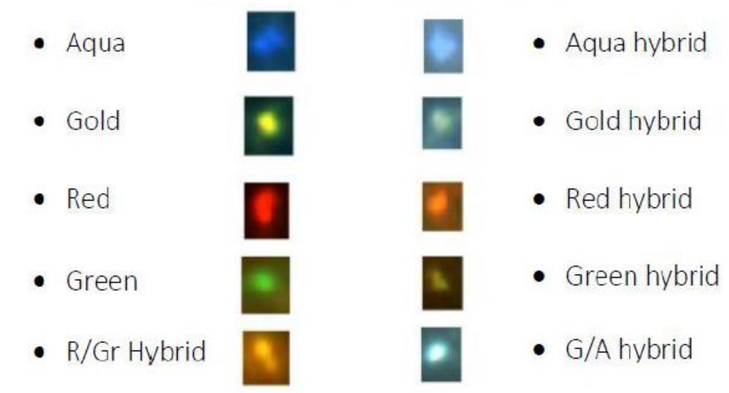
Xq duplication (male)





DESIGN

Signal Characteristics



Chromosome Color Scheme

	Aqua	Gold	Red	Green
Chr.	4p16.3	11q13.2	1q21.3	13q14.2

	R/Gr Hyb.	Aqua Hyb.	Gold Hyb.	Red Hyb.	Green Hyb.	G/A Hyb.
Chr.	15	9	16q23.2	Xq28	17p13.1	14q32.33

DISCUSSION

The multiplex, single assay detected all of the clinically relevant abnormalities commonly encountered in multiple myeloma. These included a balanced translocation t(11;14)(q13;q32); a translocation of IGH with unknown partner – t(14;?)(q32;?); a p53 deletion; a 1q21 duplication; hyperdiploidy; and a novel duplication of Xq28. When the IGH partner is unknown in the initial test, a reflex algorithm can be used to detect the other translocations i.e., t(4;14), t(14;16), t(6;14) and t(14;20) all in one test using the multiplex approach similar to the initial testing. If additional chromosomes commonly observed in hyperdiploid cases are needed, up to five chromosomes can be included in the second hybridization along with the translocations listed above.

We wondered if a smaller number of cells would be adequate to detect even low level clonal abnormalities in multiple myeloma, using our single multiplex assay. In the step-wise analysis of 20 cells at a time, we were able to show that all clonal abnormalities, even at low levels, could be detected in 20, 40, or 60 cells. The results were similar to the 200 cell analysis in every case. Based on these results, we recommend a 60 cell analysis that would save significant technologists' time thus providing cost savings. This should have no impact on reimbursement because the payment is the same for 60 or 200 cells.

By cutting down the number of hybridizations from 6-7 to a single one, substantial savings of technologist time can be realized.

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