Shorter Hybridisation Times Using Cytocell Aquarius® Probes

S Chatters, F Partheniou, A Hobbs, G Fonseka, A Castro-Justo, A Gocza-Blasko, K Mak-Hannah, R Frodsham, M Lawrie

Cytocell Ltd., 3-4, Technopark, Newmarket Road, Cambridge CB5 8PB UK

Introduction

Fluorescence *in situ* hybridisation (FISH) analysis is the 'gold-standard' method for the detection of balanced and unbalanced chromosomal rearrangements plus gains and deletions in neoplastic specimens. Standard FISH protocols incorporate an overnight hybridisation step; however, shorter hybridisations are sometimes desired as a result of laboratory operational requirements. The Cytocell Aquarius FISH probe range from Oxford Gene Technology (OGT) delivers bright, clear and precise signals when hybridised overnight. The aim of this project was to determine whether the hybridisation times for standard Cytocell 'off-the-shelf' CE-marked IVD probes could be reduced without detrimental effect on probe performance.

Materials and Methods

Ten Cytocell Haematology CE-marked IVD probe kits were selected for analysis, as shown in Table 1, below.

Cat. No.	Probe Name
LPH007	BCR/ABL Translocation, Dual Fusion
LPH013	MLL Breakapart
LPH014	IGH Breakapart
LPH017	P53 Deletion
LPH020	Del(20q) Deletion
LPH024	Del(5q) Deletion
LPH025	Del(7q) Deletion
LPH038	BCR/ABL Plus Translocation, Dual Fusion
LPH039	CKS1B/CDKN2C (P18) Amplification/Deletion
LPH052	P53/ATM Probe Combination

Table 1: Probe selection

Following the standard Cytocell protocol, (Table 2), four replicates of each probe set were hybridised to Carnoy's-fixed peripheral blood lymphocyte and bone marrow samples and each replicate hybridised for five differing times: one hour, two hours, three hours, four hours and overnight (sixteen hours), making a total of 160 separate hybridisations.

1	Spot slide with 10µl of cell sample and dehydrate.
2	Apply 10µl of Aquarius probe onto dehydrated cell sample.
3	Place coverslip onto slide and seal.
4	Denature on a hotplate at 75°C (+/–1°C) for 2 minutes.
5	Hybridise the slide in a humid, lightproof container at 37°C (+/–1°C).
6	Wash the slide in 0.4xSSC (pH 7.0) at 72ºC (+/– 1ºC) for 2 minutes.
7	Wash the slide in 2xSSC+0.05% Tween-20 (pH 7.0) at RT for 30 seconds.
8	Apply DAPI counterstain provided and view under a fluorescent microscope.

Table 2: Outline of Standard Cytocell FISH Procedure

Each of the hybridisation replicates were analysed and assessed for individual probe component intensity and sensitivity by two independent analysts, scoring a target number of one hundred interphase nuclei each, according to Cytocell standard QC procedure, and validated against individual probe intensity measured using MetaSystems® Isis and Metafer image analysis software.

Results and Discussion

The independent scoring and intensity data were normalised against the data obtained for standard overnight hybridisation. This enabled relative sensitivity and intensity relative values to be calculated.

Absolute intensity, by pixel, and eye intensity values were combined to give a signal intensity index for each probe set. These indices were analysed by fluorophore colour and sample type, using a standard overnight hybridisation as baseline to give a measure of any reduction in signal intensity. Sensitivity data were normalised in the same manner, giving an indication of the probe sensitivity, alongside standard deviation values. These results are shown in Tables 3 and 4, and Figures 2 and 3.

At a hybridisation of four hours, the intensity and sensitivity indices for all sets of data are above 0.8 – the cut-off for Cytocell QC analysis. This indicates the presence of bright easily-scored signals with little dropout for all fluorophore types in all cell types analysed. Furthermore, many of the indices for hybridisation times below four hours are above or approaching the 0.8 value, which still indicates presence of strong bright signals, confirming the robustness of Cytocell probes when used with shorter hybridisation times. It is of note that even with a reduction of signal intensity below that of the Cytocell QC cut-off, signals remained bright and scoreable.

Hyb Time	All Probe Sets	Green Signals Only	Red Signals Only	Aqua Signals Only	PB Lymphs Only	Bone Marrow Only
ON	1.00	1.00	1.00	1.00	1.00	1.00
4	0.88	0.92	0.85	0.80	0.85	0.90
3	0.76	0.79	0.74	0.80	0.71	0.81
2	0.61	0.61	0.61	0.70	0.56	0.66
1	0.52	0.51	0.50	0.70	0.49	0.55

Table 3: Mean Intensity Index

	All Probe Sets		PB Lymphocytes Only		Bone Marrow Only	
Hyb Time	Mean	SD	Mean	SD	Mean	SD
ON	1.00	-	1.00	-	1.00	-
4	0.92	0.15	0.94	0.07	0.91	0.19
3	0.83	0.12	0.83	0.10	0.83	0.14
2	0.82	0.12	0.84	0.10	0.79	0.14
1	0.76	0.19	0.71	0.20	0.80	0.18

Table 4: Sensitivity Index

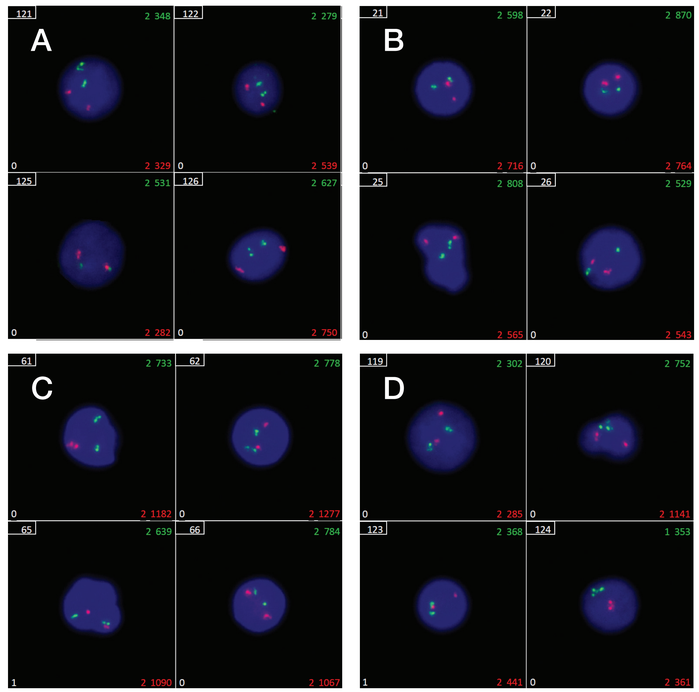

Figure 1: Comparison of hybridisation results. LPH007 probe set at A overnight, B four hours, C three hours, D one hour. Output from Metafer at 60x

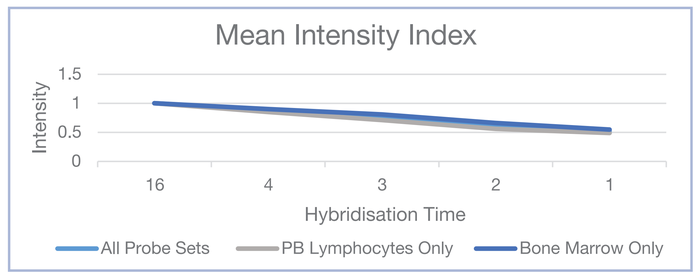

Figure 2: Mean Intensity Index

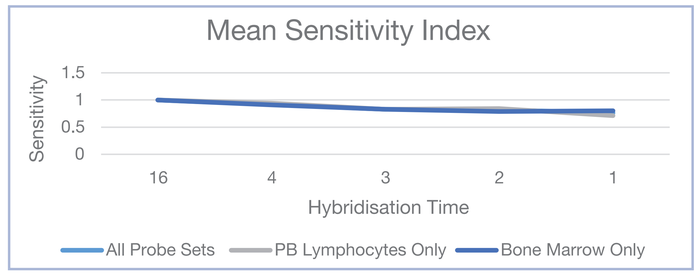

Figure 3: Mean Sensitivity Index

Conclusions

These data show that, for a range of probe and specimen types, the hybridisation time for standard CE IVD-marked Cytocell Aquarius probes may be shortened from overnight to four hours or fewer, whilst retaining excellent signal sensitivity and intensity. This gives laboratories the flexibility to validate and use off-the-shelf Cytocell probes with reduced hybridisation times, facilitating same-day reporting, should this be required.