

Notification: Bleach protocol recommendation for CytoScan assays

An internal Thermo Fisher Scientific study was designed to assess the effect of potential carryover of previously run DNA and/or staining reagents on the performance of Applied Biosystems™ CytoScan™ assays within a fluidics module bleach cycle. A bleach cycle is considered to be 4 runs processed on a single fluidics station module per week.

A total of 8 sequential runs were performed on each fluidics module over a total of 12 modules across 3 fluidics stations. One sample of Applied Biosystems™ Reference Genomic DNA 103 (REF 103, Cat. No. 900421) was run, followed by 3 successive aberrant samples (same aberrations) and another REF 103 sample, the goal being that any potential carryover of aberrant DNA would be assessed in run 5 by analyses of percentage of markers correctly called (PMC), median of the absolute values of all pairwise differences (MAPD), SNPQC (a measure of

how well SNP alleles are resolved in microarray data), and waviness SD (a global measure of variation of microarray probes, which is insensitive to short-range variation and focuses on long-range variation).

Results of the study showed that there was no significant carryover effect caused by genomic DNA samples or staining reagents on the array performance after 5 runs (Tables 1 and 2). The goal of runs 6–8 was to look further at whether carryover of staining reagents would lead to significant performance changes. The only metric that showed any significant change compared to run 1 was median raw intensity (MRI), which showed a drop in performance after run 6 (Figure 1).

From this study, it can be concluded that the recommended bleaching of the fluidics stations each week will suffice as long as no more than 6 arrays are processed in a module per week.

Table 1. Summary statistics for the primary and secondary metrics for runs 1 through 5 (N = 12).

Run ID	PMC (%)		MAPD		SNPQC		Waviness SD		Median raw intensity		Antigenomic ratio	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0	0.02	0.18	0.01	22.21	2.49	0.08	0.01	960.5	102.84	0.13	0.01
2	99.94	0.08	0.22	0.02	18.98	2.47	0.12	0.02	1016.17	143.48	0.13	0.02
3	99.91	0.13	0.23	0.03	18.32	2.34	0.12	0.02	893.25	98.42	0.13	0.01
4	99.94	0.09	0.22	0.02	19.19	2.89	0.12	0.02	946.25	109.16	0.13	0.01
5	0.03	0.08	0.19	0.02	23.3	3.01	0.08	0.01	993.17	129.85	0.13	0.01

Table 2. PMC (%) of the arrays in the first 5 fluidics runs, in which runs 1 and 5 were of REF 103 samples and runs 2–4 were of aberrant samples.

Module	Run 1	Run 2	Run 3	Run 4	Run 5
1	0.000	99.990	99.981	99.981	0.000
2	0.000	99.914	100.000	99.966	0.000
3	0.000	100.000	100.000	100.000	0.000
4	0.000	100.000	100.000	100.000	0.000
5	0.055	100.000	99.994	99.994	0.048
6	0.000	99.995	99.995	99.995	0.000
7	0.000	100.000	100.000	100.000	0.283
8	0.000	99.833	99.624	99.833	0.000
9	0.000	100.000	99.910	100.000	0.000
10	0.000	99.899	99.899	99.899	0.000
11	0.000	99.746	99.750	99.700	0.000
12	0.000	99.929	99.789	99.960	0.000

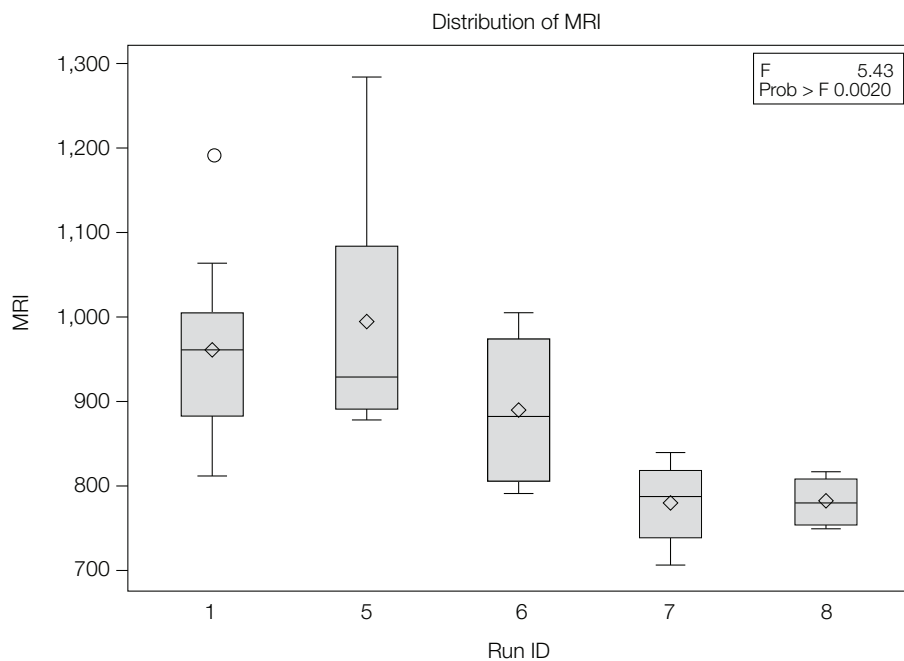


Figure 1. Box plots showing a decrease in MRI after more than 6 arrays are processed on the same fluidics module within a bleach cycle.

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