BRAF Mutation Cross-Contamination Is Not Identified In A Large Series Of Slides Sectioned Using A Fully Robotic Microtome (Tissue-Tek Smartsection®)

Regan Fulton[1, 2], Scott Crawford[1], Kelly Turner[2], Sandra Bohling[2], Cathy Natividad[2], Hanli Liu[2], Alyicia Rios[3], Erico Von Beuren[3], Allen Gown[2], and Harry Hwang[2]

Array Science[1], Sausalito, CA; PhenoPath[2], Seattle, WA ; Sakura Finetek[3], Torrance, CA

INTRODUCTION

An increasing number of molecular diagnostic tests are performed on patient tissue scraped from glass slides sectioned on a manual microtome. In the case of RNA-based assays, extensive cleaning of instruments and apparatus are undertaken to prevent cross-contamination of specimens or the introduction of RNAses. Little is known about the frequency of carry-over contamination between positive and negative samples during microtomy. Now that fully-robotic microtomy stations are becoming available, we asked whether the Tissue-Tek SmartSection was capable of producing a large number of sections without cross-contamination in a real-time PCR assay. During the production of slide sections from FFPE blocks, a robotic microtome interacts with the surface of each block and section: cooling and robotic handling of blocks, determining block height, humidifying and trimming the block surface, cutting sections and transferring them through the circulating water bath to the roller and finally placement on the slide by a second robot.

MATERIALS AND METHODS

A 2-core cell culture microarray (Horizon Discovery, UK) containing a single BRAF (V600E)+ cell line and a second microarray with an EGFR+ cell line were alternately sectioned, one-by-one, for total of 99 slides. Slides were cut at 4um and blades were replaced after each section. BRAF studies were performed on pooled extractions from 5 slides. Positive controls consisted of BRAF+ samples from the start and end of sectioning. Testing of BRAF-/EGFR+ samples was performed on 5 pooled samples taken at intervals spanning the series of 93 slides. The presence or absence of the BRAF mutation was detected using a real time PCR lab developed test method.

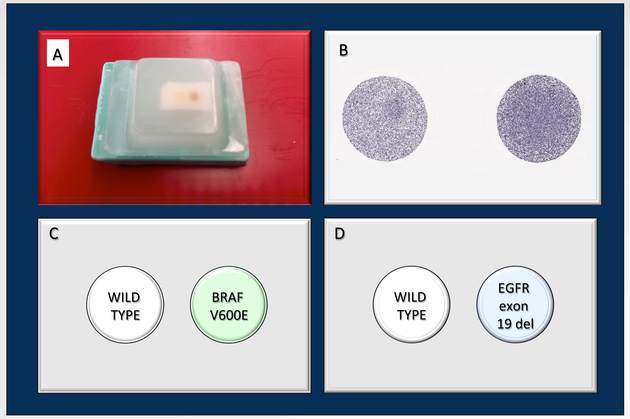

Figure 1. A) Example two-core cell culture array block, B) H&E section (20X), C) schematic design of BRAF (V600E), and D) EGFR exon 19 deletion arrays.

Figure 2. Fully Automated Robotic Microtomy Instrument (Tissue-Tek SmartSection).

Table 1. (Right) Sectioning sequence showing results from BRAF analysis on pooled DNA extraction samples.

Figure 3. (Upper Right) BRAF V600 mutational analysis using a real time PCR method. Ct curve RFU tracings (log scale) for the internal sample control reaction (M1) and the V600 mutation reaction (M2), and the threshold RFU (bar). Positive, negative, and no template controls are included in the run. The threshold delta Ct for this assay is <9.0.

Slide #	Microarray Block	Reaction Number	BRAF Result
1	EGFR		
2	EGFR		
3	EGFR		
4	EGFR		
5	EGFR		
6		BRAF 1	
7		BRAF 1	POSITIVE
8		BRAF 1	
9		BRAF 1	
10		BRAF 1	
11	EGFR	2	
12		BRAF 2	
13	EGFR	2	NEGATIVE
14		BRAF 2	
15	EGFR	2	
16		BRAF 2	
17	EGFR	2	
18		BRAF 2	
19	EGFR	2	
20		BRAF	
21	EGFR		
22		BRAF	
23	EGFR		
24		BRAF	
25	EGFR		
26		BRAF	
27	EGFR		
28		BRAF	
29	EGFR	3	
30		BRAF	
31	EGFR	3	
32		BRAF	NEGATIVE
33	EGFR	3	
34		BRAF	
35	EGFR	3	
36		BRAF	
37	EGFR	3	
38		BRAF	
39	EGFR		
40		BRAF	
41	EGFR		
42		BRAF	
43	EGFR		
44		BRAF	
45	EGFR		
46		BRAF	
47	EGFR	4	
48		BRAF	
49	EGFR	4	NEGATIVE
50		BRAF	
51	EGFR	4	
52		BRAF	
53	EGFR	4	
54		BRAF	
55	EGFR	4	
56		BRAF	
57	EGFR		
58		BRAF	
59	EGFR		
60		BRAF	
61	EGFR		
62		BRAF	
63	EGFR		
64		BRAF	
65	EGFR		
66		BRAF	
67	EGFR	5	
68		BRAF	
69	EGFR	5	
70		BRAF	NEGATIVE
71	EGFR	5	
72		BRAF	
73	EGFR	5	
74		BRAF	
75	EGFR	5	
76		BRAF	
77	EGFR		
78		BRAF	
79	EGFR		
80		BRAF	
81	EGFR		
82		BRAF	
83	EGFR		
84		BRAF	
85	EGFR	6	
86		BRAF	
87	EGFR	6	
88		BRAF	NEGATIVE
89	EGFR	6	
90		BRAF	
91	EGFR	6	
92		BRAF	
93	EGFR	6	
94		BRAF	
95		BRAF 7	
96		BRAF 7	
97		BRAF 7	POSITIVE
98		BRAF 7	
99		BRAF 7	
100	EGFR		
101	EGFR		
102	EGFR		
103	EGFR		
104	EGFR		

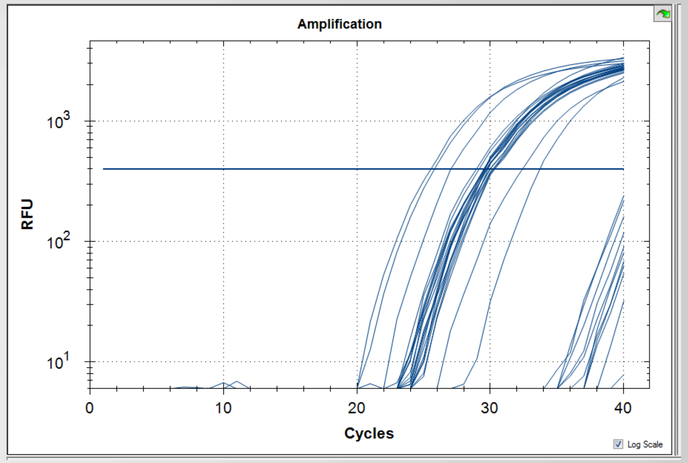

Figure 3. (Legend to left.)

RESULTS

BRAF positive samples displayed the expected positive result at the beginning (slides 6-10) and end (slides 95-99) of sectioning, while the BRAF-/EGFR+ samples spanning slides 11-93, at intervals, remained negative.

CONCLUSIONS

No specimen cross-contamination was found using molecular testing on sections cut from cell culture microarray blocks on the Tissue-Tek SmartSection Fully Robotic Microtome. Future studies using limiting dilution and RNA-based assays are warranted.