KRAS mutation analysis by PCR: a comparison of two methods.

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Introduction: KRAS mutation analysis is a companion diagnostic for the use anti-EGFR antibodies in colorectal cancer, and there is evidence to suggest that detection of BRAF mutation is also important in these patients. The methods used to establish KRAS mutation are essentially divided into sequencing and PCR based methods. While sequencing can lack sensitivity, particularly in the presence of large amounts of wild-type DNA from infiltrating cells, it does have the ability to find many more mutations than most commercially available PCR methods. In contrast, PCR methods often have better sensitivity, but poorer coverage of all possible gene mutations. Direct comparison of newer diagnostic methods with existing methods is an important part of validation of any new technique. In this this study, we have compared the Therascreen (Qiagen) assay with the new Taqman® Mutation Detection Assays powered by castPCR™ technology (Life Technologies) to determine equivalence for KRAS mutation analysis.

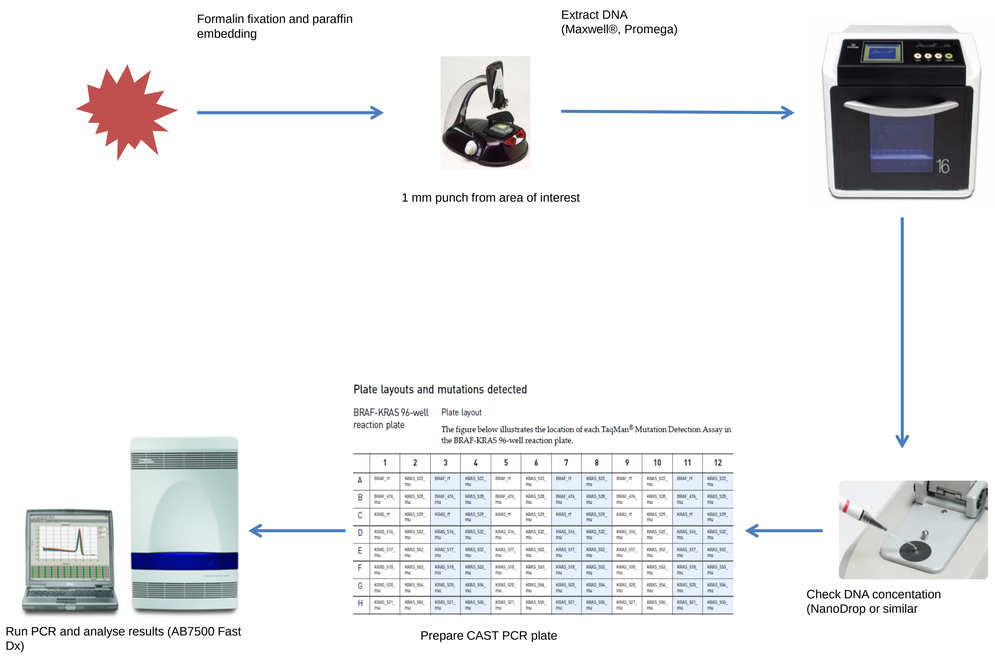

Figure 1: Sample flow for CAST PCR using automated extraction to allow results to be generated in <24 hours.

Methods: DNA was extracted by Maxwell® (Promega) from two punches obtained from areas of colorectal cancer identified by a pathologist in blocks of formalin-fixed paraffin-embedded tissue in 93 cases (fig. 1). The ARMS-based Therascreen assay was performed according to the manufacturer's instructions, as was the castPCR™ method. All assays were performed on an Applied Biosystems 7500 Fast Dx real-time PCR machine (Life Technologies). The data were collected and discrepant results retested with newly extracted DNA from the same blocks in both assay types.

Table 1. Summary of results. Therascreen does not include Q61 mutations or BRAF. One case had KRAS p.G13D and p.Q61R mutations, while a further case had BRAF p.V600E and KRAS p.G12V mutations. This table includes retesting results.

	CAST PCR	Therascreen
BRAF c.1799T>A p.V600E	10	0
KRAS c.34G>T p.G12C	4	4
KRAS c.34G>A p.G12S	2	1
KRAS c.34G>C p.G12R	3	3
KRAS c.35G>T p.G12V	10	10
KRAS c.35G>A p.G12D	9	9
KRAS c.35G>C p.G12A	1	1
KRAS c.37G>A p.G13S	0	0
KRAS c.37G>C p.G13R	0	0
KRAS c.38G>A p.G13D	5	5
KRAS c.182A>G p.Q61R	1	0
KRAS c.182A>T p.Q61L	0	0
KRAS c.183A>C p.Q61H	0	0
KRAS c.183A>T p.Q61H	0	0
Total KRAS mutant	33	33
Total BRAF mutant	10	0
Total wild-type	50	60
Total patients	93	93

Results: Of the 93 cases included, 47 were wild-type (WT) for KRAS, and 33 had KRAS mutations. The initial runs identified just three cases with different results between the two assay types, with complete concordance in 90/93 cases. One sample was negative in Therascreen and borderline positive (ΔCt = 9.41) by castPCR™, and was retested as WT in both assays. One sample was WT on retesting by both Therascreen and castPCR™, but had been called mutant by the first Therascreen. The third sample was mutant in Therascreen and borderline WT in castPCR™, but on retesting mutant in both assays. Ten cases showed BRAF mutation (V600E is not included in Therascreen) and in one of these there was also a KRAS mutation (table 1). The castPCR™ Ct values were on average 0.8 cycles lower than Therascreen, suggesting marginally greater sensitivity (figs 2 and 3).

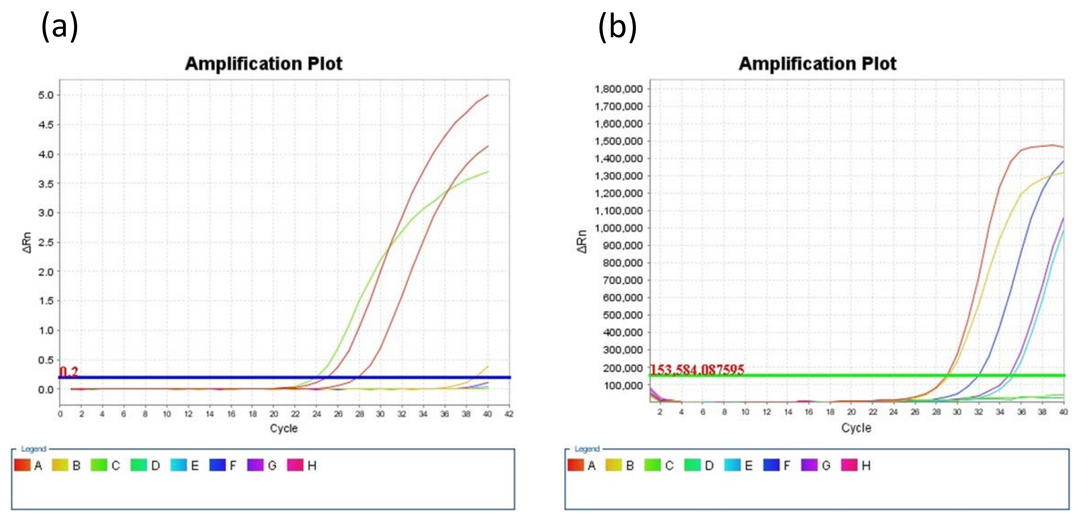

Figure 2: (a) castPCR™ (b) Therascreen for a case with KRAS mutation (p.Gly12Ala c.GGT>GCT) by both methods.

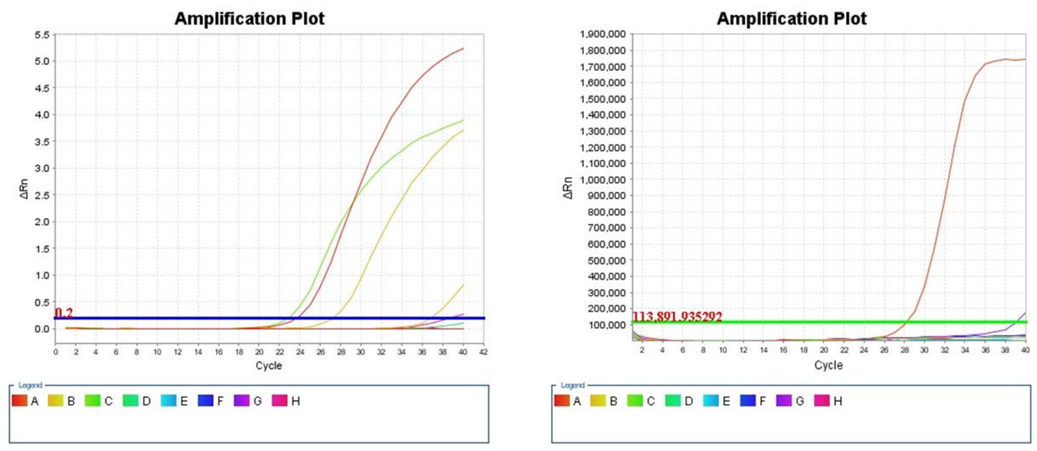

Figure 3: (a) castPCR™(b) Therascreen for a case with BRAF mutation (p.V600E, c.GTG>GAG) by CAST alone as BRAF not included in Therascreen assay (control positive).

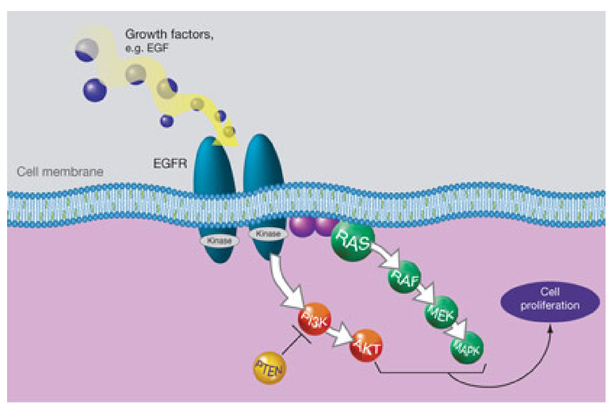

Fig. 4: EGFR pathway.

Conclusion: There was excellent correlation between the two methods, although castPCR™ includes BRAF. castPCR™ shows slightly better sensitivity than Therascreen, this is unlikely to be clinically significant. However, castPCR™ does include both BRAF and Q61 mutations giving greater coverage of the pathway (fig 4).