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Evaluation of the Idylla *KRAS* and *NRAS* mutation test in colorectal cancer tissue

Jamal Zekri[a,*], Mohammed A. Baghdadi[b], Hosam Alardati[c], Hamoud Khallaf[d], Juma H. Kabanja[d]

[a] King Faisal Specialist Hospital & Research Centre (Jeddah), College of Medicine, Alfaisal University, PO Box 40047, MBC: J-64, Jeddah 21499, Saudi Arabia
[b] Research Centre, King Faisal Specialist Hospital & Research Centre, Jeddah, Saudi Arabia
[c] Pathology Department, King Faisal Specialist Hospital & Research Centre, Jeddah, Saudi Arabia
[d] Department of Pathology & Laboratory Medicine, King Fahad Specialist Hospital, Dammam, Saudi Arabia

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ABSTRACT

Introduction: The currently approved techniques for *RAS* mutations testing in colorectal cancer (CRC) tissue are labor-intensive and time consuming. The Idylla technology (IT) is a rapid and fully automated diagnostics system. The primary aim of this study is to compare the Idylla performance against that of conventional techniques (CT).

Methodology: Archival CRC tumor samples from 2 hospitals were tested for *KRAS* and *NRAS* mutations using the IT. Results were compared to those obtained earlier by CT performed in accredited laboratories. Unexplained discordant results were verified locally by next generation sequencing (NGS) to ascertain the accuracy of IT.

Results: Forty five samples were processed. All samples underwent dual testing (CT & IT) for *KRAS* mutations. IT identified mutations in 2 samples that were not detected by CT. Primary concordance rate for *KRAS* was 93.3% and the accuracy rate improved to 100% after verification and explanation of discordant results. Only 18 samples underwent dual testing for *NRAS*. Primary concordance and accuracy rates for *NRAS* were 94.4%.

The mean time from dispatching the specimen for *RAS* testing by CT until receipt of results was 12 (7–28) days compared to few hours when IT was used.

Conclusion: IT provides a quick and reliable mean for *RAS* testing. In addition, it identifies mutations that are not detected by CT and thus may provide better guidance to treatment choices.

1. Introduction

Colorectal cancer (CRC) is the third most common diagnosed type of malignancy in the US. It is one of the leading causes of cancer mortality in men and women (Torre et al., 2015). The incidence of CRC may be slightly declining in some developed countries. However, it is on the rise in many others (Arnold et al., 2017). The most recently published Saudi Cancer Registry showed an 88% increase in the number of reported CRC cases in the year 2015 compared to the year 2005. CRC is the second most common cancer (after breast cancer) in the Kingdom of Saudi Arabia representing 12.9% of all cancer cases registered in the year 2015. The age standardized rate is 11.2/100,000 in males and 9.1/100,000 in females. At time of presentation, 26.3%, 38.6%, 28.1% and 7% of patients have localized, regional, metastatic and undocumented disease burden respectively (National Health Information Center. Saudi Cancer Registry, 2015).

The epidermal growth factor receptor (EGFR) and its downstream signaling pathways including the *RAS-RAF-MAPK* and the phosphatidylinositol 3-kinase (*PI3K)-Akt* pathways play an important role in the development and progression of CRC (Zenonos and Kyprianou, 2013).

Anti-EGFR monoclonal antibodies, such as cetuximab and panitumumab improve the outcome of patients with metastatic CRC (mCRC). This therapeutic benefit is limited to patient with wild type (WT) *RAS* tumors. Mutations in exons 2, 3 and 4 of *KRAS* and *NRAS* genes confer resistance to these antibodies (Bokemeyer et al., 2015; Douillard et al., 2014). Clinical practice guidelines mandate *RAS* testing for patients with mCRC and that only those with tumors expressing WT *RAS* genotype are candidates for anti-EGFR therapy. Such therapy is contraindicated in patients with mutant type (MT) *RAS* tumors (Van Cutsem et al., 2016).

Various molecular techniques exist to detect *RAS* mutations. However, only few methods are currently approved by the United

* Corresponding author.
E-mail addresses: jzekri@kfshrc.edu.sa (J. Zekri), mbaghdadi@kfshrc.edu.sa (M.A. Baghdadi), hal-aradati@kfshrc.edu.sa (H. Alardati), hamoud.khallaf@kfsh.med.sa (H. Khallaf), JumaH.Kabanja@kfsh.med.sa (J.H. Kabanja).

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States Food and Drug Administration (FDA) to test for *RAS* mutations in formalin-fixed paraffin-embedded (FFPE) tissue. These include the therascreen *KRAS* RGQ PCR Kit (Qiagen Manchester Ltd., Manchester, UK), the cobas® *KRAS* Mutation Test (Roche, Branchburg, NJ, USA) and the next generation sequencing (NGS) Praxis Extended *RAS* Panel (Illumina, Inc.). These methods are labor-intensive and time consuming requiring tissue de-paraffinization, DNA extraction and quantitative polymerase-chain reaction (qPCR) among many other steps.

The Idylla System (Biocartis, Mechelen, Belgium) is a CE-IVD (Conformité Européene in Vitro Diagnostics) labelled, fully automated, real-time PCR-based molecular diagnostics system. The system can be operated without the need of highly skilled staff. A single cartridge can test one or more genes for specified target mutations with results reported in about 120 min. The system allows characterization of predefined hot-spot mutations in exons 2, 3 and 4 of *KRAS* and *NRAS* genes.

The primary aim of this study is to compare Idylla performance for *KRAS* and *NRAS* mutations testing of archival CRC FFPE samples against that of the conventional techniques (CT). A secondary aim is to assess the average time required to receive the results using the CT.

2. Material and methods

In routine clinical practice setting, testing for *KRAS* and more recently *NRAS* mutations are requested by medical oncologists to guide treatment decisions for individual patients with mCRC. Subsequently, the pathology department outsources the testing process by sending the samples to overseas accredited laboratories. Ultimately, the results are electronically conveyed back to the pathology department. Time from dispatching the specimen for *RAS* testing by CT until receipt of results was calculated retrospectively for the purpose of this project.

These overseas laboratories utilized Sanger sequencing, NGS or qPCR which are labelled as CT for the purpose of this study. The Idylla technique (IT) uses microfluidics processing with all reagents on board. The operator is required to install a specific cartridge and places the macro-dissected tissue to be tested. The remaining process, including nucleic acid extraction, is fully automated. The results are ready in approximately 2 h, presented on the screen and can be printed. Results indicating WT *KRAS* mandate testing for *NRAS* mutations using another specific cartridge that tests for *BRAF* mutations at the same time.

The Idylla performance evaluation process was a joint project of 2 laboratories that included 45 FFPE tissue samples of CRC. The sample were selected through search in the archival material for the years 2016 and 2017. The specimens are routinely fixed in 10% neutrally buffered formalin for duration that ranges between 24 and 72 h and processed routinely for paraffin embedding. For the purpose of this project, archival tissue for each case was examined for selection of an appropriate tissue block that include at least 25% of tumor cellularity. Samples contained limited cellularity were excluded. Five paraffin rolls of 10 µm thick-section of the selected block was prepared to be tested by IT.

All specimens have already underwent prior testing by CT for routine clinical indications. One laboratory examined 15 specimens for *KRAS*. The other examined 30 specimens for *KRAS* with subsequent testing for *NRAS* only if *KRAS* was WT. Unexplained discordant results were planned to be verified locally by Ion Torrent Proton NGS.

3. Results

Collectively, 45 samples were processed. All samples underwent dual testing (CT & IT) for *KRAS* mutations. Table 1 illustrates the collective results of *RAS* mutation analysis by CT and IT. Twenty three out of 45 (51%) tumors were MT by CT of which 22 had mutations detected by IT. Thus the positive concordance rate for detecting *KRAS* mutations is 22/23 (95.7%). This discordant result (MT by CT but WT by IT) was verified by additional NGS and the result was similar to that obtained by IT.

KRAS was WT in 22/45 (48.9%) tumors tested by CT of which 20 were WT by IT yielding a negative concordance rate of 20/22 (91%). In these 2 discordant tumors, mutations were detected by IT due its wider spectrum for mutation testing (exon 4/codon 146 and exon 3/codon 61) compared to the CT. Table 2 illustrates the mutational analysis results by CT and IT for each individual sample.

Only 18 samples were previously tested by CT for *NRAS* mutations in routine clinical setting and were subsequently tested by IT in the context of this project. Results showed WT *NRAS* in 16/18 (88.8%) by both CT and IT thus yielding a negative concordance rate of 16/16 (100%). Two cases showed MT *NRAS* by CT of which one was also mutant by IT. However, the other was MT (exon 2, codon 12, G12D) by CT and was WT by IT. This discordant result was not verified due to consumption of all archival tissue.

The collective preliminary concordance rate for all tests (*KRAS* and *NRAS*) was 93.65% (59/63). However, the final concordance rate after verification and explanation was 98.4% (63/64).

Time from dispatching the specimen for *RAS* testing by CT until receipt of results was obtainable from one of the laboratories (30 cases). The mean time was 12 (7–28) days in comparison to few hours when the test was performed using IT.

4. Discussion

Large number of mutations have been identified in *KRAS* ($n = 663$) and *NRAS* ($n = 246$) and are documented in the catalogue of somatic mutations in cancer (COSMIC) list. However, only limited number of mutations in exons 2, 3 and 4 of *KRAS* and *NRAS* genes have been studied in clinical setting and were found to be associated with resistance to anti-EGFR monoclonal antibodies (Bokemeyer et al., 2015; Douillard et al., 2014).

The Idylla *RAS* mutation testing on the molecular diagnostics Idylla platform is a simple, and reliable in vitro diagnostic solution. The system has gained attention due to its practicality and convenience as it can be operated without the need of highly skilled staff and with < 2 min hands-on time. In addition, it provides results within few hours and thus allows oncologist to choose the most appropriate therapy without delay. Results of our study support the latter notion as we observed that the average time to obtain the mutation result with CT is 12 (up to 28) days compared to only few hours when the IT is employed.

The ability of the Idylla platform to reliably detect *RAS* mutations has been reported in recent validation studies (Al-Turkmani et al., 2018; Colling et al., 2017). Solassol J et al. reported a 98.9% concordance between Idylla and standard reference Sanger sequencing among 374 CRC FFPE samples (Solassol et al., 2016).

In our study, results from the Idylla *KRAS/NRAS* mutation test were compared with results previously obtained by other approved and established techniques performed in internationally reputable laboratories. *KRAS* mutations were detected in 23 tumors by CT but in 22 of these 23 by IT (i.e. one tumor was MT: exon 2, G12A by CT and WT by IT). This one discordant result was locally verified by NGS and the result confirmed the absence of *KRAS* mutation supporting the result of IT. These findings confirm the reliability of IT to detect *KRAS* mutations at least as reliably as other established techniques with a suggestion of even better performance. Weyn C et al., reported a 96.7% overall agreement between IT and therascreen technology for the detection of *KRAS* mutations among 182 CRC samples. Similar to our findings, they reported one specimen labelled as MT (12 ALA) using therascreen but was found to be WT using IT. An alternative confirmatory technique supported the IT result (Weyn et al., 2017). The Idylla *KRAS* mutation test covers 21 *KRAS* mutations in exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) providing a broader mutation screening platform compared to other commercially established tests (Table 3). For example, among PCR methods, therascreen (Qiagen) covers only codons 12 and 13 and Cobas (Roche) covers only codons 12, 13 and 61 (Cree,

Table 1
Results of *RAS* mutation analysis by conventional and Idylla techniques.

	MT *KRAS*	WT *KRAS*	MT *NRAS*	WT *NRAS*
Conventional techniques	23/45 (51%)	22/45 (48.9%)	2/18 (11.1%)	16/18 (88.8%)
Idylla technique	22/23 (95.7%)	20/22	1/2	16/16
Discordant cases	1	2	1	0
Preliminary concordance rate	95.7%	91%	50%	100%
Preliminary concordance rate per gene	42/45 (93.3%)		17/18 (94.4%)	
Preliminary concordance rate for all 64 tests	60/64 (93.8%)			
Verification and/or explanation of discordant results	Confirmed the Idylla results by NGS	Idylla detected mutations in codons not screened by CT	Not verified due to inadequate tissue	No discordance
Verified/explained concordance rate	100%	100%	Verification not possible	100%
Verified/explained concordance rate per gene	45/45 (100%)		18/19 (94.7%)	
Verified/explained concordance rate for all 64 tests	63/64 (98.4%)			

2016).

Although the Idylla *KRAS* mutation platform tests for all the common and some of the rarer *KRAS* mutations, some rare mutations such as those in G13C are not covered. The therapeutic implications of these rare mutations are unknown (Colling et al., 2017; Tong et al., 2014).

In our study, 2 tumors were labelled as *KRAS*-WT by CT but were found to harbor mutations detected by IT. This observation does not constitute a real discordance but rather a design difference where the CT screens for less mutations than IT and thus further verification was not required.

The broader spectrum of mutation detection provided by IT will guarantee more accurate selection of patients to anti-EGFR therapy. This will be translated to cost effective use of resources, less toxicity and better clinical outcome by avoiding the administration anti-EGFR antibodies to patients with MT *KRAS* (Bokemeyer et al., 2015).

The Idylla system is designed to initially test for *KRAS* mutations through a specific cartridge. Only cases that lack of mutation (WT) will be subjected *NRAS* and *BRAF* testing through a different cartridge. This design is cost effective and time efficient because it appropriately limits the testing process to one stage in a significant proportion of cases as about half of CRC tumors harbor *KRAS* mutations (Ibrahim et al., 2010; Peeters et al., 2015; Zekri et al., 2017).

The Idylla *NRAS* platform tests for 18 mutations in exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) of the *NRAS* gene (Table 3). Only 18 tumor samples (out of 45) were tested for *NRAS* by CT in routine clinical setting and 16 of them were found to be WT. Using the CT as a reference, we report a 100% (17/17) concordance between IT and CT for identification of WT *NRAS* genotype. There were 2 MT-*NRAS* results reported by CT. One of them was found to be WT when tested by IT. Unfortunately, we were unable to verify this discordant result due to consumption of all archival tissue. A large study of 414 specimens reported a 97.8% (405/414) total concordance of *NRAS* results. The discordance was bidirectional, including 6 cases found to be MT by CT but WT by IT and 3 cases in the opposite direction (Prieto-Potin et al., 2018).

Colling et al., reported a 94% (17/18) concordance between Idylla and Ion Torrent for *NRAS*. There was one discordant case which appeared mutated by IT but WT by Ion Torrent. They further verified this case by droplet digital PCR (ddPCR) and this confirmed a Q61L mutation in *NRAS* similar to that detected by IT (Colling et al., 2017). A study of 242 samples reported a similar trend when a G12D *NRAS* mutation was detected by IT but not by the comparator reference test. This result was confirmed to be a true positive by ddPCR analysis (Johnston et al., 2018).

There is mounting evidence that *BRAF* mutations are associated with shorter survival in early stage and mCRC (Roth et al., 2010; Venderbosch et al., 2014). The ability of *BRAF* status to predict benefits from systemic anti-cancer therapies is unconfirmed. Nevertheless, and based on limited evidence, some clinical guidelines suggest considering triplet chemotherapy combined with bevacizumab and avoiding anti-EGFR antibodies to manage selected patients with these aggressive tumors (Loupakis et al., 2014; Pietrantonio et al., 2015).

For the above reasons, assessment of *BRAF* is not carried out routinely at our institutions and thus we were unable to include it in this study. However, few samples ($n = 9$) were tested by CT for *BRAF* mutation of which 2 showed mutations. In one sample, the mutation was c.1406G > A (p.G496E) in exon 11 but was WT by IT. The other sample showed mutation in c.1799 T>A (p.V600E) in exon 15 which was also detected by IT (Table).

Eighteen samples were tested for *BRAF* by IT of which 16 were WT and 3 MT. This is in line with previous reports in the literature considering the small sample size tested for *BRAF* mutations in our study (Tie et al., 2011).

The financial implications of implementing the Idylla technology in molecular diagnostics laboratories is not well studied. At the current currency exchange rates, the local costs of the *KRAS* and *NRAS/BRAF* cartridges are £242 and £272 respectively. Our local hospital guidelines do not mandate *BRAF* testing and thus the detection of a mutant *KRAS* will not be proceeded by *NRAS/BRAF* testing. For every 100 cases, it is expected that about 50 cases will have a mutation in *KRAS* (£242 × 50 = £12,100) and will not need *NRAS* testing. The other 50 cases will undergo additional *NRAS* mutation testing (£272 × 50 = £13,600), costing in total £25,700 (£12,100 + £13,600). According to the above calculations, testing of 100 cases using the IT will cost £37,800. The most recent cost of collective *KRAS*, *NRAS* and *BRAF* mutations testing in the contracted overseas accredited laboratory (through an intermediary agent) using NGS is £220 Accordingly, testing of 100 cases using the CT will cost £22,000. Similar to our calculations, a cost analysis performed by a group from the Oxford University Hospitals NHS Trust concluded that a move to IT in an established molecular diagnostics laboratory may not be cost-effective (Colling et al., 2017). However, the fast turnaround times (few hours) is attractive and can justify the use of IT in institutions with no molecular testing facilities. A group from France have acknowledged the additional cost incurred by the implementation of the IT and they suggested a system of partial implementation of IT to minimize this cost. They proposed initial *KRAS* testing using the specific cartridge which will identify mutations in about 50% of cases and negates further testing in these cases. Only the other 50% of cases (*KRAS*-WT) will undergo NGS mutational analysis (Le Flahec et al., 2017). At our institution, tumors from patients who underwent curative intent resection for stage III CRC undergo pre-emptive *RAS* testing as they are at high risk of developing mCRC in the future. As time is not a pressing issue for these patients, we propose that these tumors are to be tested for *RAS* mutations by CT to reduce cost. Meanwhile, IT can be implemented for tumors from patients with metastatic disease who need immediate therapeutic decisions and have not had prior *RAS* testing. In laboratories with limited molecular diagnostic facilities, such strategy will reduce the total cost of *RAS* testing without compromising the promptness of treatment

Table 2
KRAS, NRAS and *BRAF* results of individual samples showing the sites of mutations when identified.

Sample number	Results of conventional methods			Results of Idylla		
	KRAS	*NRAS*	*BRAF*	*KRAS*	*NRAS*	*BRAF*
Validating laboratory A						
1[b]	W	W	ND	W	W	W
2[b]	W	W	ND	W	W	W
3[b]	W	W	ND	W	W	W
4[b]	W	W	ND	A146P/T/V (c.436G>C; c.436G>A; c.436C>T)	W	W
5[b]	W	W	ND	W	W	W
6[c]	W	W	ND	W	W	W
7[b]	W	W	ND	W	W	V600E/D (c.1799 T>A; c.1799_1800delinsAA/ c.1799_1800delinsAC)
8[b]	W	ND	ND	W	W	W
9[b]	W	W	ND	W	W	V600E/D (c.1799 T>A; c.1799_1800delinsAA/ c.1799_1800delinsAC)
10[b]	W	W	ND	W	W	W
11[c]	G12A (c.35G>C)	ND	ND	G12A (c.35G>C)	ND	ND
12[b]	G12C (c.34G>T)	ND	ND	G12C (c.34G>T)	ND	ND
13[c]	G12 V (c.35G>T)	ND	ND	G12 V (c.35G>T)	ND	ND
14[c]	G13D (c.38G>A)	ND	ND	G13D (c.38G>A)	ND	ND
15[c]	G12 V (c.35G>T)	ND	ND	G12 V (c.35G>T)	ND	ND
16[c]	G12A (c.35G>C)	ND	ND	G12A (c.35G>C)	ND	ND
17[b]	G12 V (c.35G>T)	ND	ND	G12 V (c.35G>T)	ND	ND
18[b]	G12 V (c.35G>T)	ND	ND	G12 V (c.35G>T)	ND	ND
19[b]	G12D (c.35G>A)	ND	ND	G12D (c.35G>A)	ND	ND
20[b]	G12D (c.35G>A)	ND	ND	G12D (c.35G>A)	ND	ND
21[a]	W	W	W	W	W	W
22[a]	W	W	W	W	W	W
23[a]	G12D (c.35G>A)	ND	W	G12D (c.35G>A)	ND	ND
24[a]	W	G12D (c.35G>A)	W	Q61H (c.183A>C; c.183A>T)	W	W
25[a]	W	W	G496E (c.1406G>A)	W	W	W
26[a]	W	G13R (c.37G>C)	W	W	G13R/V (c.37G>C/ c.38G>T)	W
27[a]	W	W	W	W	W	W
28[a]	G12A (c.35G>C)	W	W	W (confirmed M by NGS)	W	W
29[a]	W	W	W	W	W	W
30[a]	W	W	V600E (c.1799 T>A; c.1799_1800delinsAA)	W	W	V600E/D (c.1799 T>A; c.1799_1800delinsAA/ c.1799_1800delinsAC)
Validating laboratory B						
31[b]	G12 V (c. 35G>T)	ND	ND	G12 V (c. 35G>T)	ND	ND
32[b]	G13D (c.38G>A)	ND	ND	G13D (c.38G>A)	ND	ND
33[b]	G12A (c.35G>C)	ND	ND	G12A (c.35G>C)	ND	ND
34[b]	G12D (c.35G>A)	ND	ND	G12D (c.35G>A)	ND	ND
35[b]	W	ND	ND	W	ND	ND
36[b]	W	ND	ND	W	ND	ND
37[b]	G13D (c.38G>A)	ND	ND	G13D (c.38G>A)	ND	ND
38[b]	G13D (c.38G>A)	ND	ND	G13D (c.38G>A)	ND	ND
39[b]	G12 V (c. 35G>T)	ND	ND	G12 V (c. 35G>T)	ND	ND
40[b]	G13D (c.38G>A)	ND	ND	G13D (c.38G>A)	ND	ND
41[b]	G12D (c.35G>A)	ND	ND	G12D (c.35G>A)	ND	ND

(*continued on next page*)

Table 2 (*continued*)

Sample number	Results of conventional methods			Results of Idylla		
	KRAS	*NRAS*	*BRAF*	*KRAS*	*NRAS*	*BRAF*
42[b]	G12 V (c. 35G>T)	ND	ND	G12 V (c. 35G>T)	ND	ND
43[b]	W	ND	ND	W	ND	ND
44[b]	G12S (c.34G>A)	ND	ND	G12D (c.35G>A)	ND	ND
45[b]	W	ND	ND	W	ND	ND

W: Wild Type
M: Mutant Type
ND: Test Not Done
[a] Next generation sequencing, Lab 21.
[b] Sanger sequencing, Neogenomics Laboratories.
[c] RT-PCR, Clarient Diagnostic.

Table 3
KRAS, NRAS and *BRAF* mutations detected by Idylla technique.

KRAS	
Codon 12 (exon 2)	G12C (c.34G > T)
	G12R (c.34G > C)
	G12S (c.34G > A)
	G12A (c.35G > C)
	G12D (c.35G > A)
	G12 V (c.35G > T)
Codon 13 (exon 2)	G13D (c.38G > A)
Codon 59 (exon 3)	A59E (c.176C > A)
	A59G (c.176C > G)
	A59T (c.175G > A)
Codon 61 (exon 3)	Q61K (c.181C > A; c.180_181delinsAA)
	Q61L (c.182A > T)
	Q61R (c.182A > G)
	Q61H (c.183A > C; c.183A > T)
Codon 117 (exon 4)	K117 N (c.351A > C; c.351A > T)
Codon 146 (exon 4)	A146P (c.436G > C)
	A146T (c.436G > A)
	A146V (c.437C > T)
NRAS	
Codon 12 (exon 2)	G12C (c.34G > T)
	G12S (c.34G > A)
	G12D (c.35G > A)
	G12A (c.35G > C)
	G12 V (c.35G > T)
Codon 13 (exon 2)	G13D (c.38G > A)
	G13 V (c.38G > T)
	G13R (c.37G > C)
Codon 59 (exon 3)	A59T (c.175G > A)
Codon 61 (exon 3)	Q61K (c.181C > A)
	Q61L (c.182A > T)
	Q61R (c.182A > G)
	Q61H (c.183A > C; c.183A > T)
Codon 117 (exon 4)	K117 N (c.351G > C; c.351G > T)
Codon 146 (exon 4)	A146T (c.436G > A)
	A146V (c.437C > T)
BRAF	
Codon 600	V600E (c.1799 T > A; c.1799_1800delinsAA)
	V600D (c.1799_1800delinsAC)
	V600K (c.1798_1799delinsAA)
	V600R (c.1798_1799delinsAG)

provision.

In conclusion: The Idylla platform offers a compact and reliable alternative to conventional assays and does not require specialist training to operate. The system also offers a case by case on-demand service with minimal turnaround time. This system should be appreciated as an appropriate solution for *RAS* mutational testing in smaller laboratories. In addition, it can complement available testing modalities and provide rapid results in larger laboratories.

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Declaration of Competing Interest

All authors declare no conflict of interest.

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