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Evaluation, Validation, and Implementation of the Idylla System as Rapid Molecular Testing for Precision Medicine

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The Idylla Mutation Test System is an automated, PCR-based mutation testing system. The advantages of this system can greatly impact the delivery of precision medicine. We describe our evaluation, validation, and implementation of this system for routine testing of *BRAF*, *EGFR*, *KRAS*, and *NRAS* using formalin-fixed, paraffin-embedded cancer samples. All four Idylla test systems showed excellent concordance with reference methods. The analytical sensitivity ranged from 94.66% to 100%, depending on the cartridge, and specificity was 100%. A few discordant results were noted and further investigated: *KRAS* Q61L was misclassified as Q61H; *KRAS* Q61R was not identified; there was a false-negative *EGFR* double mutation (L861Q and G719A); and there was a false-negative *EGFR* double mutation (T790M and exon 19 deletion). The limit of detection was determined to be 1% or 5% for the variants with available reference material. The turnaround time was shortened by 7 days on average. Idylla testing of a cohort of 25 non–small-cell lung cancer samples with insufficient material for next-generation sequencing testing delivered results for all cases and identified actionable results for eight cases. In addition, patient care would have been changed in four of these cases: targeted therapies were identified in two cases, and repeated biopsies would have been avoided in two cases. The Idylla molecular testing system is an accessible, rapid, robust, and reliable testing option for both routine and challenging formalin-fixed, paraffin-embedded specimens. *(J Mol Diagn 2019, 21: 862–872; https://doi.org/10.1016/j.jmoldx.2019.05.007)*

Optimal care of cancer patients requires precise pairing of chemotherapeutics with specific underlying genetic variants in their tumors. To achieve this, molecular testing is routinely performed as standard of care (SOC) to identify clinically actionable genomic biomarkers. Next-generation sequencing (NGS) and, to a lesser extent, Sanger sequencing are broadly used for this purpose. The list of actionable genetic variants linked to targeted therapy is relatively limited and includes *BRAF*, *KRAS*, and *NRAS* mutations in colorectal cancer,[1] *EGFR* mutations in lung cancer,[2] and *BRAF* and *NRAS* mutations in melanoma.[3] In recent years, the Idylla Mutation Test System (Biocartis, Mechelen, Belgium) has been introduced to specifically detect these mutations. Several studies, including multicentered research efforts, have been conducted in European countries and have demonstrated its effectiveness and efficiency on tumors with *KRAS*, *NRAS*, *BRAF*, and *EGFR* mutations.

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The Idylla system is a fully automated, PCR-based mutation testing system supporting hands-free processing of all steps, starting with tissue input to molecular results. We considered several promising advantages of this system. First, compared with the conventional SOC methods, which require considerable investments on instrumentation, trained laboratory personnel, and bioinformatics, the Idylla test is far easier to perform and interpret. Second, optimal tissue sampling and selection is required for most SOC testing, which pose challenges because of the type of procedure or the nature of the tissue. As there is less manipulation of the sample in the Idylla system, it may successfully process challenging samples. Third, the long turnaround time (TAT) for SOC testing is an obstacle preventing most molecular results from being finalized in a timely manner. The Idylla system only requires a few hours from tissue input to result, making it optimal for clinically urgent cases. Therefore, this system is poised to have a major impact on the current molecular testing process for precision medicine that we use today.

To date, there are few published studies describing routine testing of formalin-fixed, paraffin-embedded (FFPE) tissue samples with the Idylla system in the North American population.[4,5] An in-depth evaluation and validation of the Idylla system, targeting hotspot mutations, was performed in four clinically important genes: *KRAS*, *NRAS*, *BRAF*, and *EGFR*. As the largest single-center evaluation of the Idylla system, our study is among the first to systemically evaluate the performance of this rapid molecular testing platform and describe its potential clinical utility for delivering precision medicine.

Materials and Methods

Tissue Specimens

This study was approved by the Medical College of Wisconsin (Milwaukee, WI) Internal Review Board. FFPE tumor specimens were obtained from patients referred for routine molecular testing at the Medical College of Wisconsin Affiliated Hospitals between 2010 and 2018. Reference material with known variant allele frequency (VAF) was obtained from Horizon Discovery (Lafayette, CO). The study was conducted at the Medical College of Wisconsin Department of Pathology Clinical and Translational Research Lab, where the Idylla, NGS, and Sanger sequencing reference tests were performed. Hematoxylin and eosin–stained slides were reviewed by a board-certified pathologist (A.C.M.) to identify malignant tissue and ensure specimen adequacy. Several unstained slides (4 μm thick) or FFPE curls (10 μm thick) were prepared at the time of initial clinical testing, and additional leftover materials were subsequently tested with the appropriate Idylla assays. To compare the performance of the Idylla system as faithfully as possible with the reference tests, a similar-sized tissue fragment was tested on the Idylla. This was possible because extra slides and FFPE curls were cut when the sample was initially accessioned for clinical testing. For the Idylla testing, the adjacent unstained slide, which is the next level from the FFPE block, was aligned; and the same geographical area as was previously tested was selected. Consequently, the tissue used for the Idylla was as closely matched as possible to the tissue used for the reference testing.

Assessment of Tumor Content

All patient samples in this study were previously tested as part of routine patient care using laboratory-developed NGS-based assays. Previous validation of the NGS-based assays demonstrated variants with a variant allele frequency >3.5% could be detected >95% of the time using macrodissected tissue, 3 to 5 mm^2 in area (eg, a 2-mm diameter circle), containing at least 10% malignant nuclei. On the basis of this, 3 to 5 mm^2 was the minimum size of macrodissected tissue containing a minimum 10% malignant nuclei that was placed directly into the Idylla cartridge for testing. For some experiments, a tissue curl was tested. Compared with macrodissected tissue, FFPE curls generally have more total DNA. However, the percentage of malignant nuclei is entirely dependent on the content and distribution of malignant cells in the source FFPE block. Consequently, the percentage of tumor nuclei in an FFPE curl can range from being similar, such as a macrodissected region from the same block, to being much lower.

Mutation Test by Reference Methods

A laboratory-developed NGS clinical assay was used as the reference method on all of the cases to determine the mutation status. DNA from the specimen was sequenced using the Ion Torrent AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Waltham, MA). DNA and library concentrations were quantified by spectrofluorometry methods (Qubit; Thermo Fisher Scientific). Genomic DNA was used to prepare a library following the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific) paired with the Ion AmpliSeq Cancer Hotspot Panel v2 (207 primer pairs/pool) protocols. Ion Xpress barcode adapters (Thermo Fisher Scientific) were then ligated to the fragmented DNA on both 5′ and 3′ ends. Templates were prepared by emulsion PCR using the Ion OneTouch 2 system (Thermo Fisher Scientific). Libraries and ion sphere particles were added to the Ion OneTouch 2, and the ion sphere particles containing template were enriched. The quality of the enriched template-positive ion sphere particles was assessed using the Qubit 2.0 Fluorometer. Enriched ion sphere particles were then loaded onto a 316 or 318 chip for semiconductor sequencing of the targeted region. The version 5.2 of TMAP and Torrent variant caller informatics tools were used to identify variants. Validation studies conducted in the Medical College of Wisconsin Clinical and Translational Research Lab have demonstrated that the analytical sensitivity was >95% and specificity was 100% for detecting single bp substitutions and small insertions-deletions with variant allele frequencies >3.5%.

Sanger sequencing was performed on some of the specimens as the reference method using M13-tailed primers targeting the mutant variants. The PCR amplicons were confirmed by electrophoresis in 2% agarose gel. PCR products were sequenced in both directions using the BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific), according to the manufacturer's conditions. After the sequencing step, the BigDye Xterminator Purification Kit was used to clean up the dye excess. Sequence data were obtained with a 3500 genetic analyzer (Thermo Fisher Scientific). Sequencing results were analyzed using SeqScape version 2.7 (Thermo Fisher Scientific).

Mutation Detection by the Idylla Molecular Diagnostic System

The Idylla system uses ready-to-use test cartridges that target specific hotspot mutations within four genes. There are four unique, gene-specific test cartridges available targeting *KRAS*, *BRAF*, combined *NRAS* and *BRAF* (ie, *NRAS/BRAF*), and *EGFR* genes. The cartridges were obtained from Biocartis. The mutations detected for each target are summarized in Supplemental Tables S1, S2, S3, and S4. Tests were performed according to the manufacture's recommended protocol. Briefly, a tumor-rich portion of tissue was macrodissected from glass slides, or an FFPE curl/scroll was inserted into the Idylla cartridge. A combination of chemical reagents, enzymes, heat, and high-intensity focused ultrasound induced deparaffinization, disruption of the tissue, and lysis of the cells inside the Idylla cartridge. The nucleic acids were liberated and ready for subsequent PCR amplification. Real-time PCR was then performed using allele-specific primers. In addition, the simultaneous detection of an endogenous sample processing control was performed. Detection of these specific targets is performed using fluorescent-labeled probes. The process in the cartridge was performed automatically. This method has been described previously.[6]

Vendor-supplied software (BRAF_RUO/2.0 - Release v2.0, Recipe v2.0, Product v2.0, NRAS3_RUO/1.1 - Release v2.1, Recipe v6.8, Product v1.1, KRAS_RUO/2.2 - Release v2.4, Recipe v21.18, Product v2.2, EGFR_RUO/1.0 - Release v1.2, Recipe v1.18, Product v1.0; Biocartis) automatically analyzed the collected fluorescent signals. Results were presented on the Idylla Console. The obtained fluorescent signals were evaluated for PCR curve validity. A cycle of quantification value (Cq) was calculated for every valid curve. The presence of a mutant genotype was determined by calculating the difference between the sample processing control Cq and the Cq obtained for the mutant signal(s). This difference between the control signal and the mutant signal was defined as the ΔCq. The mutant signal was considered valid if the ΔCq value was within a predefined range established by the vendor and the detected variant was reported. At the end of the run, a final report indicated the presence or absence of a specific codon mutation in the targeted gene.

Results

Final Data Set

The specimen type selected for each Idylla cartridge was from lesions that warrant the detection of the targeted mutations for prognostic or therapeutic purposes. Most specimens were colorectal cancer for *KRAS*, *NRAS*, and *BRAF*; melanoma for *BRAF* and *NRAS*; and lung cancer for *EGFR*. The number of unique patients or reference samples used in the study included 52, 68, 43, and 47 samples for the *KRAS*, *NRAS-BRAF*, *BRAF* and *EGFR* Idylla cartridges, respectively, and are summarized in Table 1. Supplemental Tables S5, S6, S7, and S8 summarize the number of samples for each mutant variant and the number of replicate tests that were performed.

Test Performance

Tables 2, 3, and 4 show the accuracy of Idylla *KRAS*, *NRAS/BRAF*, and *EGFR* mutation results, respectively, compared with the results obtained by the reference method. Overall, Idylla results showed excellent concordance with the results obtained from the reference method. A few discordant results were noted: *KRAS* Q61L was misclassified as Q61H; *KRAS* Q61R was not identified by Idylla; there was a false-negative *EGFR* double mutation (L861Q and G719A); and there was a false-negative *EGFR* double mutation (T790M and exon 19 deletion). These were further investigated and are discussed below. The concordance between the Idylla system and the SOC method was 100% for the *BRAF*-only and *NRAS/BRAF* combined cartridges, 95.8% for *KRAS*, and 97.2% for *EGFR*. Collectively, the concordance rate for all the Idylla tests was 97.6%.

To determine the precision of the four Idylla mutation tests, samples were tested three different times by three different technologists. The samples included patient samples or commercially available reference material at 1% or 5% VAF and included the following: seven samples with unique genotypes run on the *KRAS* cartridge, three samples

Table 1 Summary of Specimen Types for Each Idylla Test

Specimen type	*KRAS*	*NRAS/BRAF*	*BRAF*	*EGFR*
Melanoma	1	16	22	1
Colorectal adenocarcinoma	24	22	5	3
Non—small-cell lung cancer	12	4	0	28
Endometrial adenocarcinoma	4	4	1	4
Thyroid tumors	0	4	0	0
Brain tumors	0	2	4	0
Other	0	2	3	0
Reference specimens	11	14	8	11
Total	52	68	43	47

Data are expressed as *n*.

Table 2 Accuracy of Idylla *KRAS* Mutation Tests Compared with Reference Tests

Idylla KRAS mutation test	Reference test															
	G12C	G12D	G12V	G12R	G12A	G12S	G13D	A59T	Q61H	Q61K	Q61L	Q61R	K117N	A146T	WT	Total
G12C	13															13
G12D		11														11
G12V			5													5
G12R				4												4
G12A					3											3
G12S						3										3
G13D							4									4
A59E/G/T								1								1
Q61H/H2									6		2*					8
Q61K/K2										0						0
Q61L/R											1					1
K117N/N2													0			0
A146T/V/P														1		1
WT												1*			17	18
Total	13	11	5	4	3	3	4	1	6	0	3	1	0	1	17	72

Data are expressed as *n*.
*Discordant result.
WT, wild type.

with unique genotypes run on the *BRAF*-only cartridge, seven samples with unique genotypes run on the *NRAS/BRAF* cartridge, and six samples with unique genotypes run on the *EGFR* cartridge. The mutant variants used for the testing are shown in Supplemental Table S9. All samples were successfully detected and identified by different technologists.

The sensitivity and specificity of each Idylla mutation test are presented in Table 5. The overall performance was high in each of the tests, with sensitivity ranging from 94.55% to 100%, and specificity was 100% for all four tests. The analytical performance of the *KRAS* test was calculated on the basis of Q61L misclassified as Q61H in two replicated tests (false negatives). Because the presence of the clinically actionable *KRAS* mutation was successfully identified, the clinical sensitivity and negative predictive value of the *KRAS* test is higher: 98.18% and 94.44%, respectively (Supplemental Table S10).

LOD Data

The limit of detection (LOD) is defined as the lowest allelic frequency at which the mutant alleles can consistently be detected in ≥95% of the test cases. To estimate the LOD for the mutations detected by the Idylla tests, a set of commercially available FFPE reference samples with 1%, 5%, and

Table 3 Accuracy of Idylla *NRAS/BRAF* Mutation Tests Compared with Reference Tests

Idylla NRAS/BRAF mutation test	Reference test													
	G12D	G12A/V	G13D	G13R/V	A59T	Q61K	Q61R	Q61L	Q61H/H2	K117N/N2	A146T/V	V600E/D	V600K/R	WT	Total
G12D	5														5
G12A/V		1													1
G13D			1												1
G13R/V				3											3
A59T					1										1
Q61K						4									4
Q61R							13								13
Q61L								7							7
Q61H/H2									1						1
K117N/N2										1					1
A146T/V											1				1
V600E/D-BRAF												25			25
V600K/R-BRAF													5		5
WT														22	22
Total	5	1	1	3	1	4	13	7	1	1	1	25	5	22	90

Data are expressed as *n*.
WT, wild type.

Table 4 Accuracy of Idylla *EGFR* Mutation Tests Compared with Reference Tests

	Reference test							
Idylla EGFR mutation test	G719A	G719S	E746_A750	E746_A750	E746_S752delins	L747_A750delinsP	L747_P753	S752_I759del
G719A	6							
G719S		2						
E746_A750 (c.2235_2249del15)			9					
E746_A750 (c.2236_2250del15)				2				
E746_S752delins					3			
L747_A750delinsP						2		
L747_P753							3	
S752_I759del								2
S768_D770dup								
S768I								
V769_D770insASV								
T790M								
L858R								
L861Q								
T790M, exon 19 deletion								
L861Q/G719A								
L861Q/E746-A750								
L858R/T790M/G719S								
WT								
Total	6	2	9	2	3	2	3	2

(*table continues*)

Data are expressed as *n*.
*Discordant result.
WT, wild type.

50% known mutant VAF were tested to verify the LOD for each mutation. For variants without a commercial reference sample with a VAF <10%, patient samples with a low VAF (<10%) determined by NGS were used. The results are shown in Table 6, with two patient samples included.

Variants were successfully identified in all the *KRAS*, *NRAS*, and *BRAF* and 9 of 10 *EGFR* LOD samples that were tested. The results indicated that the assay could detect an LOD of 1% with 100% CI for the following genotypes: *BRAF* V600E and V600K; and *EGFR* L861Q, E746 to A750, L858R, and G719S. The LOD is 5% for *KRAS* G12A, G12C, G12D, G12R, G12S, G12V, and G13D and *EGFR* T790M. In addition, the LOD is at least 6% for *KRAS* 61H and 2.9% for *NRAS* G12D. These results indicate that the Idylla assay can detect variants with these tested genotypes when the VAF is at least as listed. However, it does not rule out a lower LOD for these variants. Determination of the true LOD requires samples with known lower VAF, which were not available to us. One sample with 1% *EGFR* T790M showed a negative result by Idylla. However, another specimen containing 5% T790M and a specimen with 5% multiplex mutations including T790M both showed positive results. Therefore, it was concluded that the LOD for *EGFR* T790M is 5%. The LODs of 10 *KRAS*, 10 *NRAS*, 3 *BRAF*, and 50 *EGFR* variants were not determined because of the lack of material with known VAF. This does not indicate that the Idylla system is incapable of detecting these variants, but rather the performance of the Idylla system was not assessed for these variants because of the lack of availability of reference standards.

Discordant Results

Overall, four samples demonstrated discordant results and are indicated with an asterisk in Tables 2 and 4. Two *EGFR* mutations and one *KRAS* mutation were detected by the reference method and missed by Idylla, and one *KRAS* variant was misclassified by Idylla. These discordant samples were further investigated.

One *KRAS* Q61L sample was misclassified as Q61H by Idylla on two replicate runs. NGS sequencing identified a two-nucleotide insertion-deletion (c.182_183AA>TT) in this sample. Considering that this variant was not described in the Idylla Technical Data Sheet, and Idylla detected a variant but misclassified it at Q61H, the likely explanation is that the Idylla system detects this specific variant but is not capable of differentiating it from Q61H (c.183A>C). In another sample, the *KRAS* Q61R mutation identified by NGS was not identified by Idylla. NGS sequencing identified a second variant in this sample that is located two nucleotides away in the 3′ direction. It is possible that this second mutation interferes with the Idylla detection system, leading to the false-negative result.

Table 4 (*continued*)

Reference test											
S768_D770dup	S768I	V769_D770insASV	T790M	L858R	L861Q	T790M, Ex19 deletion	L861Q/G719A	L861Q/E746-A750 L858R/T790M/G719S	WT	Total
										6
										2
										9
										2
										3
										2
										3
										2
4										4
	1									1
		1								1
			1							1
				12						12
					5					5
						3				3
							1			1
								4		4
						1*	1*		9	11
4	1	1	1	12	5	4	2	4	9	72

Two false-negative *EGFR* samples were identified. Both of these were from patient samples, and each sample had two mutations: one with an L861Q and a G719A double mutation and the other with a T790M and an exon 19 in-frame deletion mutation. The mutations were identified using FFPE curls as starting material, but they were not detected when macrodissected FFPE tissue was used as input. We attribute this to insufficient amounts of starting material secondary to the microdissection.

QNS Specimens

As demonstrated above, testing of small microdissected tissue samples can result in the isolation of insufficient

Table 5 Variants Tested, Results, and Analytical Performance for Each Idylla Test

			Reference						
Mutation	Idylla cartridge	Idylla	Mutant variant, *n*	Wild type, *n*	Total, *n*	Sensitivity, %	Specificity, %	PPV, %	NPV, %
KRAS	KRAS	Positive	52	0	52	94.55	100.00	100.00	85.00
		Negative	3	17	20				
		Total	55	17	72				
NRAS	NRAS-BRAF	Positive	40	0	40	100.00	100.00	100.00	100.00
		Negative	0	52	52				
		Total	40	52	92				
BRAF	NRAS-BRAF	Positive	30	0	30	100.00	100.00	100.00	100.00
		Negative	0	62	62				
		Total	30	62	92				
BRAF	BRAF	Positive	33	0	33	100.00	100.00	100.00	100.00
		Negative	0	10	10				
		Total	33	10	43				
EGFR	EGFR	Positive	61	0	61	96.83	100.00	100.00	81.82
		Negative	2	9	11				
		Total	63	9	72				

NPV, negative predictive value; PPV, positive predictive value.

Table 6 LOD for Each Mutation Variant Tested by Idylla

Idylla cartridge	Mutation variant	LOD, %	Result
KRAS	G12A	5.0	Positive
	G12C	5.0	Positive
	G12D	5.0	Positive
	G12R	5.0	Positive
	G12S	5.0	Positive
	G12V	5.0	Positive
	G13D	5.0	Positive
	Q61L	50.0	Positive
	Q61H*	6.0	Positive
	Q61H	50.0	Positive
	A146T	50.0	Positive
BRAF only	V600E	1.0	Positive
	V600E	5.0	Positive
	V600E	20.0	Positive
	V600E	50.0	Positive
	V600K	1.0	Positive
	V600K	5.0	Positive
	V600K	50.0	Positive
	V600R	50.0	Positive
NRAS/BRAF combined	G12A/V	50.0	Positive
	G12D*	2.9	Positive
	G13D	50.0	Positive
	A59T	50.0	Positive
	Q61K	50.0	Positive
	Q61H	50.0	Positive
	K117N	50.0	Positive
	A146T	50.0	Positive
EGFR	L861Q	1 Multiplex	Positive
	E746-A750		Positive
	L858R		Positive
	G719S		Positive
	L861Q	5 Multiplex	Positive
	E746-A750		Positive
	L858R		Positive
	T790M		Positive
	G719S		Positive
	E746_A750	5.0	Positive
	G719S	5.0	Positive
	L858R	5.0	Positive
	L861Q	5.0	Positive
	S768I	50.0	Positive
	V769_D770insASV	50.0	Positive
	T790M	5.0	Positive
	T790M	1.0	Negative

*Patient sample with <10% variant allele frequency was used.
LOD, limit of detection.

quantity of DNA and thereby introduce the risk of false-negative results. Consequently, a fraction of all samples submitted for clinical molecular analysis by SOC methods is rejected after pathology review, library preparation, or quality control measures. This becomes particularly important when small biopsies with scanty tissue or a sample with low percentage of tumor is submitted for molecular testing. We speculated that Idylla testing would be a useful alternative to the current SOC (ie, NGS- or Sanger-based) testing methods for these challenging samples. To test this

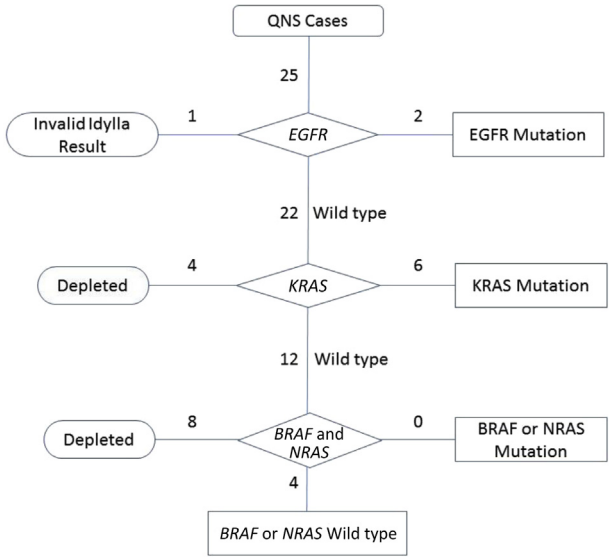

Figure 1 Flow chart describing the testing strategy of the 25 quantity not sufficient (QNS) cases. The flow chart is organized from top to bottom with diamond shapes indicating the order of testing that was performed. The numbers next to or above each line represent the number of cases. Oval shapes represent testing in which a result was not obtained because of either failed testing or depleted specimens. Rectangular shapes describe test results in which a mutation was detected. If a mutation was detected in a case, no further testing was performed. If a mutation was not detected, then subsequent testing was performed until either a mutation was identified or the specimen was depleted.

hypothesis, 25 lung FFPE samples were identified with a histologic diagnosis of non–small-cell lung cancer (NSCLC) that were rejected for NGS testing because of quantity not sufficient (QNS), and they were tested for *EGFR* mutations. To minimize the possibility of false-negative results, additional molecular testing was performed for *KRAS*, *BRAF*, and *NRAS* mutations (Figure 1). Two of the 25 (8%) tested positive for *EGFR* mutation by Idylla. Eighteen *EGFR*-mutation negative specimens with residual tissue were tested for *KRAS*, and *KRAS* mutations were identified in 6 of 18 specimens. Residual tissue from four of the remaining *EGFR*- and *KRAS*-negative cases tested negative for both *BRAF* and *NRAS* mutations. In summary, Idylla provided actionable clinical results for 8 of 25 NSCLC cases that were rejected by NGS because of QNS. This indicates that the Idylla mutation system is a robust alternative assay that can successfully analyze suboptimal samples not amenable to other sensitive methods of testing, including NGS-based assays.

Of these 25 QNS cases, clinical follow-up data were available for 20 patients. Retrospectively testing the residual QNS material allowed us to determine that Idylla testing would have significantly changed patient care if these results had been available at the time of the initial test request (Table 7). Of the 20 QNS cases, seven patients received standard cytotoxic therapy and did not undergo any further molecular testing or repeat biopsies. In patient #1, an activating *EGFR* mutation was identified by Idylla on the QNS

Table 7 Clinical Impact of Idylla Testing on Patients with QNS Specimens

Patient	Initial NGS result	Initial management	Idylla result on QNS material	Affect on management
1	QNS	Standard Therapy	*EGFR L858R*	EGFR Targeted therapy
2	QNS	Standard Therapy	*EGFR T790M* not detected	Avoid T790M targeted therapy
3	QNS	Standard Therapy	*KRAS G12C*	Standard therapy
4	QNS	Standard Therapy	*KRAS A146*	Standard therapy
5	QNS	Standard Therapy	*EGFR, KRAS, BRAF* WT	Standard therapy
6	QNS	Standard Therapy	*EGFR, KRAS* WT	Standard therapy
7	QNS	Standard Therapy	*EGFR* WT	Standard therapy
8	QNS	Repeat Biopsy	*KRAS G12D*	Avoid repeat biopsy
9	QNS	Repeat Biopsy	*KRAS Q61H*	Avoid repeat biopsy
10	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
11	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
12	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
13	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
14	QNS	Repeat Biopsy	*EGFR* WT	Repeat biopsy performed
15	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
16	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
17	QNS	Repeat Biopsy	Failed Testing	Repeat biopsy performed
18	QNS	Repeat Biopsy	*EGFR* WT	Repeat biopsy performed
19	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed
20	QNS	Repeat Biopsy	*EGFR, KRAS* WT	Repeat biopsy performed

NGS, next-generation sequencing; QNS, quantity not sufficient; WT, wild type.

specimen, and if this had been known at the time of the initial testing, *EGFR* targeted therapy would have been used. In patient #2, the goal of the initial test request was to identify the *EGFR* T790M variant because the patient was known to have an activating *EGFR* variant and was no longer responding to therapy. Idylla testing of the initial QNS specimen did not identify the T790M variant, and this information would have been used to define a treatment plan for this patient. The remaining 13 patients all underwent a second biopsy along with repeat NGS testing. In 2 of these 13 patients, Idylla testing identified *KRAS* variants on the initial QNS specimens (patient #8 and #9), and had these results been available at the time of initial testing, neither of these two patients would have had a second biopsy or further molecular testing. Taken together, in 4 of 20 patients whose initial tissue samples were unsuitable for NGS testing, Idylla testing on these same samples would have significantly affected treatment decisions and patient management. Therefore, the Idylla platform satisfies an unmet clinical need as a useful option for molecular analysis of problematic tissue samples not otherwise suitable for clinical NGS testing.

TAT Data

The TAT for molecular testing of pathology specimens may vary depending on the testing method. To compare the TAT between Idylla and the existing SOC assays that are currently used, the TAT of the cases submitted during the previous 2 years (2017 and 2018) was determined. The TAT is defined as the time from when the sample was received in the laboratory to when the results became available to the clinician. The TAT includes sample preparation, DNA extraction, sample loading, test performance, data analysis, and report. The SOC methods included both in-house, laboratory-developed assays and send-out testing to various reference laboratories. The average SOC TAT of these cases is 8.3 days ($n = 55$; SD = 3.3 days), ranging from 2 to 15 days, depending on the site and method. As comparison, the typical TAT for Idylla mutation test is within 24 to 48 hours. The rate-limiting step for the Idylla assay is the time required to cut the block and prepare the FFPE curls or unstained slides in histology. Once the tissue was prepared, the testing time for the Idylla test is 1.5 to 3 hours from sample loading to the availability of test results.

Discussion

The increasing number of studies evaluating the performance of the Idylla system in the clinical laboratory setting demonstrates that it is a quick, on-demand system that permits rapid analysis of hotspot mutations with LOD as low as 1%, depending on the specific mutation. It has been shown that Idylla is highly concordant with SOC tests for mutations in *KRAS*,[7–9] *NRAS*,[10,11] *BRAF*,[12–18] and *EGFR*[9,19–21] on different types of specimens, while also being highly sensitive and specific. The goal of our study was to evaluate, validate, and implement four different cartridges targeting common, clinically actionable mutations in frequently encountered solid tumors. In addition to the recent retrospective study by Al-Turkmani et al,[4,5] who

evaluated the Idylla *KRAS* and *NRAS-BRAF-EGFR* S492R cartridges, our study is among the first evaluations of this system described in a North American clinical laboratory. It is also a single-institution study with one of the largest cohorts of samples described to date. Our data show that the Idylla system is highly comparable with the current SOC methods in analytical performance, and yet it has the significant advantages of expanding the types of samples that can be routinely tested (ie, small/scanty biopsies), as well as the turnaround time for the molecular results.

The current SOC testing methods require sufficient number and/or percentage of tumor tissue for successful library preparation or sufficient sequencing metrics for mutation detection. Suboptimal specimens (ie, small biopsies with scanty tissue, cytology specimens with small number and/or percentage of malignant cells, or decalcified bone specimens) are often labeled QNS during pathologist review and rejected for NGS testing. Previous work by De Luca et al[19] reported 20 of 25 (80%) of their NGS-invalid cases were successfully amplified with the Idylla *EGFR* assay. Our finding that the Idylla system provided *EGFR* results for 96% (24 of 25 cases) of the QNS NSCLC specimens that were previously rejected or unsuccessful for NGS testing is consistent with these results. Taken together, these findings indicate that Idylla has the potential to significantly influence treatment decision at the time of initial testing and reduce the number of repeated biopsies and repeated molecular testing.

Unlike molecular-based infectious disease testing, FFPE specimens have limited biological content. Consequently, determining the number and types of samples to run during validation represents a balance between availability of comparative specimens, accessibility of low-frequency variants, and laboratory economics. In conjunction with these measures, laboratory directors should develop an Individualized Quality Control Plan that incorporates risk and validation assessment. Risk assessment includes specimen handling, training of testing personnel, assay quality control measures, laboratory environmental conditions, and instrumentation calibration and maintenance. It is critical to perform preanalytical quality control through careful histologic assessment of each specimen for the presence of malignant cells. The internal amplification control on each Idylla cartridge serves as a quality control indicator for successful specimen processing and DNA yield after the onboard extraction.

Because amplification on the Idylla system occurs with sufficient numbers of nonmalignant cells, it is extremely challenging to precisely determine the false-negative rate for variant detection. This is a particularly important issue when testing small samples not otherwise suitable for NGS or other SOC-based methods. The inherent challenge of characterizing and/or determining the false-negative rate was considered to be a critically important component of the validation plan. It was reasoned that performing all available Idylla assays in a serial and deliberate manner on the QNS samples provided the best assessment of the false-negative rate of variant detection. Furthermore, analyzing QNS cases ensured the highest stringency. Considering the number of specimens and the low incidence of *BRAF* V600 in NSCLC, our observation of actionable variants in 32% (8 of 25 cases) of QNS NSCLC cases is in line with the expected rate of approximately 40%. These results strongly suggest that the false-negative rate of variant detection with the Idylla system is not a significant source of risk and, therefore, indicate that Idylla is a suitable testing option for tissue samples that are insufficient or inadequate for NGS testing.

The TAT for Idylla is significantly shorter than the reference methods. In our experience, Idylla results are available to the clinician 3 to 10 days earlier than SOC NGS testing. Other studies have demonstrated that the Idylla clinical report could be obtained in 2 to 3 hours, allowing initiation of treatment to be accelerated by 10 days.[9,16] The fact that the Idylla system provides molecular testing results within 1 to 2 days has several advantages that have been encountered since validation and implementation of the platform. For example, an acutely ill patient for whom targeted therapy was a last option and the TAT for NGS-based testing was too long was tested. Another example frequently encountered is test requests for patients whose follow-up clinic visits are scheduled before SOC molecular results are available, and the clinician needs results to initiate the appropriate therapy.

The simple processing steps and the small size of the instrument strongly support the use of Idylla system on-site in the histology laboratories rather than being located in an off-site clinical laboratory to optimize TAT. It has been shown that the Idylla system can be successfully implemented in clinical laboratories where the staff is less experienced with molecular technology.[21] A cytopathologist, for example, could easily integrate molecular findings with the morphologic diagnostic report because of the rapid testing afforded by Idylla.[22] Compared with transporting the specimens off-site for Idylla testing via a courier, placing an Idylla system on-site accompanied by a trained technician and pathologist has the potential to shorten the TAT by an additional day.

A limitation of the Idylla system is its inability to discern true-negative results from false-negative results. Previous studies have shown that Idylla false-negative results are attributed to VAF below the threshold detectable by Idylla, rare mutations not included in the Idylla test reference range, and technical problems related to malfunctioning cartridges.[23] In particular, Idylla may not detect some low amounts of *EGFR* T790M mutations,[9] as confirmed by our study. Our study also indicated that false-negative results may also be caused by secondary mutations that can affect the PlexZyme/PlexPrime technology[6] used by the cartridge. In addition, our experience suggests that inserting FFPE curls in Idylla is superior to small, macrodissected FFPE tissues, which can be attributed to superior PCR because of a larger amount of input DNA. Therefore, further study and

characterization is warranted to specify the sample input limit of this assay using well-characterized reference specimens. Molecular testing laboratories should include as many different variants and replicates as possible during validation and the ongoing quality control process. In addition, laboratory directors need to develop and implement operating policies and procedures for confirmation testing. False-positive results, on the contrary, were not observed in this study; no published reports of false-positive test results were encountered.

Another potential limitation of the Idylla system is the scalability of the platform. Only one sample can be run at a time on one instrument, limiting its potential for large-volume testing. Therefore, for practical reasons, the specimens coming to the laboratory should be triaged, and samples with higher clinical priority should be directed to the Idylla system first. Considering the increasing number of new genetic targets that are in development for the Idylla system, an investment on multiple instruments may be considered. The financial implication is dependent on the type of specimen. A French financial review indicated that using Idylla as a first-line analysis is a cost-effective approach in melanoma and colorectal cancer, but combining Idylla with NGS was not cost-effective in NSCLC.[24] Further evaluations are needed to confirm and quantify the medicoeconomic benefits of Idylla testing.

In summary, the Idylla molecular testing system is an accessible, rapid, and reliable testing option for both routine and challenging FFPE specimens. It can serve well as an alternative or a complementary molecular diagnostic tool in most pathology laboratories. It is relatively low throughput, and false-negative results due to off-target variants or secondary mutations must also be taken into consideration.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2019.05.007*.

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