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Use of the Biocartis Idylla™ Platform for the Detection of Epidermal Growth Factor Receptor, *BRAF* and *KRAS* Proto-Oncogene Mutations in Liquid-Based Cytology Specimens from Patients with Non-Small Cell Lung Carcinoma and Pancreatic Adenocarcinoma

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Abstract: The study aimed to demonstrate rapid and effective molecular testing on liquid-based cytology (LBC) samples for *EGFR*, *KRAS* and *BRAF* mutations using the Biocartis Idylla™. Rapid on-site evaluation (ROSE) LBC samples for patients with non-small cell lung carcinoma (NSCLC) or pancreatic ductal adenocarcinoma (PDAC) were tested for *EGFR*, *KRAS* and *BRAF* mutations based on the relevance to tumour subtype. The quantification values (Cq values) and mutation detection status were compared between LBC samples and routine formalin-fixed paraffin-embedded (FFPE) clot samples. ROSE LBC samples (n = 54) showed a higher yield of well-preserved tumour and wild type (WT) DNA, demonstrated by lower quantification cycles, no false positives or false negatives, and a higher sensitivity for low allele frequency mutations when compared with FFPE clot samples. The Biocartis Idylla™ provides highly sensitive, reliable and rapid testing for LBC samples for the detection of *EFGR* and *KRAS* mutations. *BRAF* mutations were not detected in the participant cohort; however, all LBC WT *BRAF* results correlated with the results from the FFPE clot samples. Access to rapid molecular testing using LBC samples can detect the most frequent driver mutations closer to the time of diagnosis, enabling the selection of the most effective first-line targeted therapy sooner, reducing delays or side effects from suboptimal treatments, patient anxiety and costs to healthcare systems, whilst improving patient outcomes.

Keywords: *KRAS*; *EGFR* (epidermal growth factor receptor); *BRAF*; liquid based cytology (LBC); non small cell lung carcinoma (NSCLC); formalin-fixed paraffin embedded (FFPE)

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1. Introduction

Worldwide, lung cancer is the leading cause of cancer-related death [1,2]. Prognosis remains poor, with average five-year survival rates of 16.2% [3]. Half of all lung cancers present stage 4 disease [1]. Non-small cell lung cancer (NSCLC) accounts for 85% of diagnosed lung cancer cases [2], and approximately 60% of advanced NSCLC subtypes have an actionable molecular target [4]. Current guidelines emphasise testing for epidermal growth factor receptor (*EGFR*), *ALK* receptor tyrosine kinase (*ALK*), Programmed death ligand 1 (*PD-L1*), ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*) and *B-Raf* proto-oncogene, serine/threonine kinase (*BRAF*) driver mutations prior to systemic anti-cancer treatment [5,6]. However, with the emergence of effective targeted therapies, this panel is expanding. *KRAS* proto-oncogene, GTPase (*KRAS*) mutations *G12C*, *G12V* and *G12D* are arguably the most prevalent gain-of-function alterations found in 20–40% of lung adenocarcinomas [7]. *G12C* mutations alone are found in 13% of NSCLC cases [8], and rates of concurrent mutations with other actionable molecular targets such as *EGFR*, *BRAF* and *ALK* are incredibly uncommon [9,10]. Agents to target *KRAS* mutations are desired and are

currently in development [11]. Sotorasib is a *G12C* GTPase inhibitor with a median overall survival of 12.5 months in patients with previously treated NSCLC harbouring the *G12C KRAS* mutation [12]. Sotorasib is approved by the National Institute for Health and Care Excellence (NICE) for early access to medicine on the National Health Service (NHS) [13].

Pancreatic adenocarcinoma (PDAC) carries a poor prognosis. Five-year survival for pancreatic cancer increased from 0.9% in 1975 to only 4.2% in 2011 in patients of all stages, while in surgically resected patients, it rose from 1.5% to 17.4% [14]. The disease often presents at an advanced and inoperable stage. Current treatment involves multi-agent combination chemotherapy regimens with limited targeted therapies to a small subset of patients; 1–2% of patients that have microsatellite instability (MSI)/mismatch repair deficiency (MMR) are eligible for immunotherapy [15]. Anti-*EGFR* therapies are used for patients presenting with *BRAF* mutations (11% of *KRAS* wildtype), and treatments for germline BRCA mutations (5–7% of patients) are also available; however, progression-free and overall survival rates remain poor [16].

KRAS driver mutations are found in 90–93% of PDAC [16,17]. The most common mutations are *G12D*, *G12V*, *G12R*, *Q61H*, *Q61L* and *G12C*, respectively [17]. Unlike NSCLC, *G12C* mutations are rare in PDAC, presenting in roughly 1% of cases [18]. PDACs associated with *KRAS* mutations are aggressive [19], and improved survival rates have been seen in Q61 mutations compared with other *KRAS* mutations [20]. Therapeutic targets for *KRAS* isoforms are being researched with the aim to provide more effective personalised treatments. Positive outcomes have been observed with GTPases inhibitors (*KRAS G12C*) and lipid nanoparticle (LNP)-formulated mRNA-based cancer vaccines that elicit an adaptive immune response against *G12V* and *G12D* mutated PDAC. Oligonucleotide therapies targeted against *G12D* mutations are also in phase II trials [14].

As the need for diagnostic panels expands, contradicting the shift to minimally invasive procedures to obtain samples, maximising the efficiency of the diagnostic yield is paramount to preventing repetitive invasive procedures for patients [5]. Rapid on-site evaluation (ROSE)-assessed cytology samples from endobronchial ultrasound (EBUS), transbronchial needle aspiration (TBNA) and endoscopic ultrasound (EUS) are the first-line investigation for the sampling of mediastinal, perihilar lymph nodes, peribronchial or peritracheal masses, and pancreatic lesions [21,22]. Performing highly specialised molecular testing on residual ROSE-assessed liquid-based cytology (LBC) specimens could provide an efficient approach for meeting the competing aims of expanding testing requirements from smaller samples. Recent studies have shown that cytology samples in CytoLyt® solution can be used effectively for NGS [23]; however, NGS involves significant infrastructures, skills and resources. The Biocartis Idylla™ provides a highly sensitive and specific platform for local rapid molecular testing, which is vital for improved clinical outcomes in patients with advanced disease [24]. The Idylla™ is a fully automated, real-time polymerase chain reaction (PCR)-based molecular diagnostic system. Formalin-fixed paraffin-embedded (FFPE) biopsies and clots made from cytology samples are routinely used to detect *EGFR*, *KRAS* and *BRAF* mutations on the Idylla™ platform. Sample to result is obtained in 1.5 to 3 h. Initial multi-test studies have demonstrated the use of residual cytology specimens on the Idylla™ for *EGFR* [25,26]; however, with the recent developments in NSCLC and potential future requirements for *KRAS* in PDACs, a broader panel is needed for adoption into routine practice.

Objectives

The objectives are to determine whether LBC samples provide a feasible and reliable alternative to FFPE samples for detecting *EGFR*, *KRAS* and *BRAF* mutations using the Idylla™ platform.

2. Methods

Patients attending for EUS- or EBUS-guided fine-needle aspirations with a clinical suspicion of non-small cell lung carcinoma or pancreatic adenocarcinoma were included

for participation in the study (n = 55). Currently, *EGFR*, *KRAS* and *BRAF* testing can be conducted on the Idylla™ platform. Due to the high incidence of *KRAS* mutations in PDACs, and with *EGFR*, *KRAS* and *BRAF* being the most common driver mutations in NSCLC, both patient cohorts were recruited to increase the sample size and statistical validity of the study. Cytology samples were obtained with ROSE [27] for routine diagnostics. Diagnosis was carried out microscopically by a consultant pathologist as per RcPath guidelines for reporting NSCLC and PDAC by means of an interpretation of morphology (PAP/HE) and was supplemented with immunohistochemistry where applicable. The inclusion criteria was based on the appropriate diagnosis and available residual samples for testing. Cases were excluded from the study if material was exhausted following routine diagnostics. Molecular analysis (*EGFR*, *KRAS* and *BRAF* for the lung and *KRAS* for the pancreas) was conducted on both FFPE clot samples and LBC samples.

Preparation of Samples

Cytology samples were transported to the laboratory in CytoLyt® Solution for the preparation of a Thinprep PAP slide and a thrombin and plasma clot for diagnosis, routine molecular testing and additional molecular testing. From the FFPE clot samples, two sections were cut at a 10 µm thickness using the Leica RM2235, and the samples were tested using Idylla™ cartridges as per individual manufacturers' instructions for *EGFR*, *KRAS* and *BRAF*.

Both the PAP slide from LBC and the HE slide from the FFPE clot sample were assessed microscopically to obtain a count of neoplastic cells and the neoplastic cell content (%).

The residual LBC samples were tested for an additional molecular analysis (*EGFR/KRAS/BRAF*). Pellets were formed by centrifuging the remaining sample and processed by placing them straight onto the lysis pad of the FFPE Idylla™ cartridges (20 µL).

The Idylla™ *EGFR* mutation test (Biocartis, Mechelen, Belgium) is an in-vitro diagnostic (IVD) test that uses real-time PCR to qualitatively detect exon 18 (G719A/C/S), exon 21 (*L858R*, *L861Q*), exon 20 (*T790M*, *S768I*) mutations, exon 19 deletions and exon 20 insertions in the *EGFR* oncogene. The testing platform has been IVD-validated by the manufacturer for FFPE human tissue samples of NSCLC. The test is also validated at the Royal Cornwall Hospital for use on FFPE sections from human NSCLC against Sanger Sequencing [26]. The limit of detection of this assay is between 1–5% (mutation-dependent) with a background of genomic wild type DNA, the local sensitivity being 90% and specificity 100%.

The *KRAS* assay is used to detect mutations in codons 12, 13, 59, 61, 117 or 146 of the *KRAS* oncogene, verified for use at RCHT by comparison Pyrosequencing (100% specificity and sensitivity) technology.

The *BRAF* assay identifies V600E/E2/D and V600K/R/M mutations in codon 600 of the *BRAF* gene, verified locally against the Cobas Roche platform 4800 (100% specificity and sensitivity).

Each assay (*EGFR/KRAS/BRAF*) independently performs liquefaction, cell lysis, DNA extraction and amplification of the target regions. The testing package includes a software analysis of the DNA primer amplification, which will differentiate between wild-type and mutated amplicons. This is achieved by the dissociation of the annealed fluorescent beacons using heat. The fluorescent profile is then interpreted by the in-built algorithm so as to display any identified mutation(s) on the console.

The results of the mutations (Idylla™ output) and integrity of the DNA were collected to study the limit of detection between LBC and FFPE clot samples per assay type. For each valid PCR curve, a cycle of quantification value (Cq) is calculated. Cq values are inverse to the amount of target nucleic acid that is in the sample and correlate to the number of target copies. It is a pseudo value that can be used to estimate the integrity of the DNA that is being sequenced. Samples for which the wild-type Cq was within a predefined range were given a genetic call [no mutation or a group of mutations]. A call of an INVALID result is where the output was not able to be determined. The delta Cq value relates to the relative gene expression value. The lower the value, the higher the amount of targeted nucleic acid

in the sample. The Cq values for both methodologies were put through a *t*-test to establish whether there was a significant difference in the cycles of PCR (Cq) on the Idylla platform.

This study received medical ethical approval on 18 June 2020. IRAS Number: 275530.

3. Results

55 patients were recruited, 45 had both LBC and Clot FFPE samples for analysis. From these, a total of 59 assays were carried out, as shown in Table 1.

Table 1. Number of LBC samples tested for each cancer type.

	KRAS	*EGFR*	*BRAF*	TOTAL
PANCREATIC ADENOCARCINOMA	8	-	-	8
LUNG ADENOCARCINOMA	8	29	8	45
LUNG SQUAMOUS CELL CARCINOMA	6	-	-	6
TOTAL	22	29	8 *	59

* There were fewer numbers of *BRAF* tests due to expiration of the cartridges as a result of fewer cases during the pandemic.

The 29 LBC samples tested for *EGFR* mutations demonstrated 26 WT DNA and three *EGFR* exon 19 mutations. The 22 LBC samples tested for *KRAS* showed 13 WT and nine *KRAS* mutations. 7/9 *KRAS* mutations were detected in the PDAC cohort (87.5%), three *G12D*, two *Q61H* and two *G12R* mutations. 2/9 *KRAS* mutations were detected in the NSCLC (Adenocarcinoma) cohort (25%), one *G12C* and one *G12V* (Table 2). 0/6 *KRAS* mutations were detected in the lung Squamous cell carcinomas. The eight LBC samples tested for *BRAF* showed eight WT DNA results.

The study showed a 50% invalid rate in the *BRAF* FFPE clot samples (Table 3). Four cases did not have sufficient samples remaining in the block, a reflection of having exhausted the samples due to the clot samples routinely having immunocytochemistry (*TTF1, P40, ROS1, PDL* and *ALK*) prior to the molecular testing for the study. *BRAF* was run last after *EGFR* and *KRAS*. There were no invalids in any of the LBC samples.

There was a 97% (28/29) correlation of LBC with Clot FFPE results for EGFR testing. An *S768I* mutation (L015) (false positive) was erroneously detected in the FFPE sample as a result of over-amplification (Table 2). This has been previously observed in other studies for this mutation on the Idylla^{TM} platform [28] and is being addressed in an updated version of the Idylla^{TM} *EGFR* test. The sample was referred for next-generation sequencing (NGS), and by this method no mutation was identified. Retrospectively, comparing the sample size and DNA integrity can help with identifying the rationale behind the discordant result. The clot WT *EGFR* Cq (26.1) required more PCR cycles to amplify the DNA that was present in the sample, and though the mutation call was made, it was very near the cut-off of a true mutation call (26), highlighting the importance of scrutinising the Cq values. The LBC Cq value of 18.8 indicated a high level of well-preserved DNA and a high tumour yield in the LBC sample, confirming a reliable WT result.

The analysis of samples for *KRAS* demonstrated a 90% (20/22) correlation of LBC with the Clot FFPE results. One case that was discordant (L038) demonstrated a *KRAS G12V* mutation with a WT DNA Cq value of 18 and a high delta Cq of 8.4 detected in the LBC sample, which was not detected in the FFPE sample (Table 2). The green circle in the graph on the left in Figure 1 shows the LBC sample *G12V* mutation as being well within the threshold of detection. The initial FFPE clot sample (red circle) and the repeat FFPE clot run (red square) are both outside the Biocartis algorithm cut-off for detection. Despite there being a sufficient neoplastic cell content (HE confirmed 80% and >200 cells), the call on the Idylla was 'no mutation'. The lack of available DNA in the sample meant that the limit of detection of the test was reduced and that the mutation detected call was just missed in both cases. The study demonstrated the detection for low allele frequency mutations in the LBC sample.

Table 2. Comparison of LBC and Clot FFPE mutations detected.

Study Number	LBC Slide Tumour %	LBC Result	LBC WT Cq Value	LBC Mutation Cq Value	LBC Delta Cq Value	Clot Slide Tumour %	Clot Result	Clot WT Cq Value	Clot Mutation Cq Value	Clot Delta Cq Value
Panc 1	5% >50 cells	*KRAS* Q61H mutation detected	21.74	31.68	9.73	<100 cells <5% tumour	*KRAS* Q61H mutation detected	25.39	29.93	4.35
Panc 2	5% >100 cells	*KRAS* Q61H mutation detected	22.35	30.12	7.65	100 cells 10% tumour	*KRAS* Q61H mutation detected	25.53	29.23	3.8
Panc 3	50% >100 cells	*KRAS* G12D mutation detected	25.15	28.03	3.04	<100 cells 5% tumour	*KRAS* G12D mutation detected	25.45	30.85	5.67
Panc 5 A	Slide not found	*KRAS* G12R mutation detected	19.8	23.41	3.59	>100 cells 60% tumour	*KRAS* G12R mutation detected	24.30	31.12	6.86
Panc 5 B	40% >100 cells	*KRAS* G12R mutation detected	20.24	24.89	4.77	<50 cells 5% tumour	No mutation detected	28.36	n/a	n/a
Panc 6 A	10% >100 cells	*KRAS* G12D mutation detected	18.9	22.9	4.05	>100cells 80% tumour	*KRAS* G12D mutation detected	21.64	25.39	3.75
Panc 6 B	10% >100 cells	*KRAS* G12D mutation detected	18.37	21.34	3.03	<100 cells <5% tumour	*KRAS* G12D mutation detected	25.39	29.93	4.35
Lung adeno L021	40%>20 cells	*KRAS* G12Cmutation detected	22.41	27.61	6.24	<200 cells >50% tumour	*KRAS* G12Cmutation detected	23.8	28.08	5.00
Lung SCC L038	90% >100 cells	*KRAS* G12V mutation detected	18.21	26.72	8.44	>200 cells >80%	No mutation detected	24.55	n/a	n/a
Lung adeno L002	50% >200 cells	*EGFR* Mutation detected Exon 19	15.4	20	4.87	80% 500 cells	*EGFR* Mutation detected Exon 19	21	26	5.66
Lung adeno L012	30% >200 cells	*EGFR* Mutation detected Exon 19 deletion	20.7	25.2	4.18	80% 100 cells.	*EGFR* Mutation detected Exon 19	21.5	26.32	4.62
Lung adeno L015	90% >200 cells	No mutation detected	18.8	n/a	n/a	90%. >200	*EGFR* Mutation detected S768I * false positive	26.1	28.24	2.94
Lung adeno L044	5%>100 cells	*EGFR* Mutation detected Exon 19	19.15	27.38	7.85	75% >200 cells	*EGFR* Mutation detected Exon 19	25.2	29.01	3.80

Green represents correlation of results between LBC and FFPE. Orange highlights where non correlations occurred.

Table 3. The number of mutations detected and the invalid rate for each test type.

	Total Tests	Number of Mutations Detected	Invalids LBC	Invalids Clot FFPE
KRAS LBC	22	9	0/22 (0%)	0/22 (0%)
KRAS Clot	22	7		
EFGR LBC	29	3	0/29 (0%)	0/29 (0%)
EGFR Clot	29	3		
BRAF LBC	8	0	0/8 (0%)	4/8 (50%)
BRAF Clot	8	0		

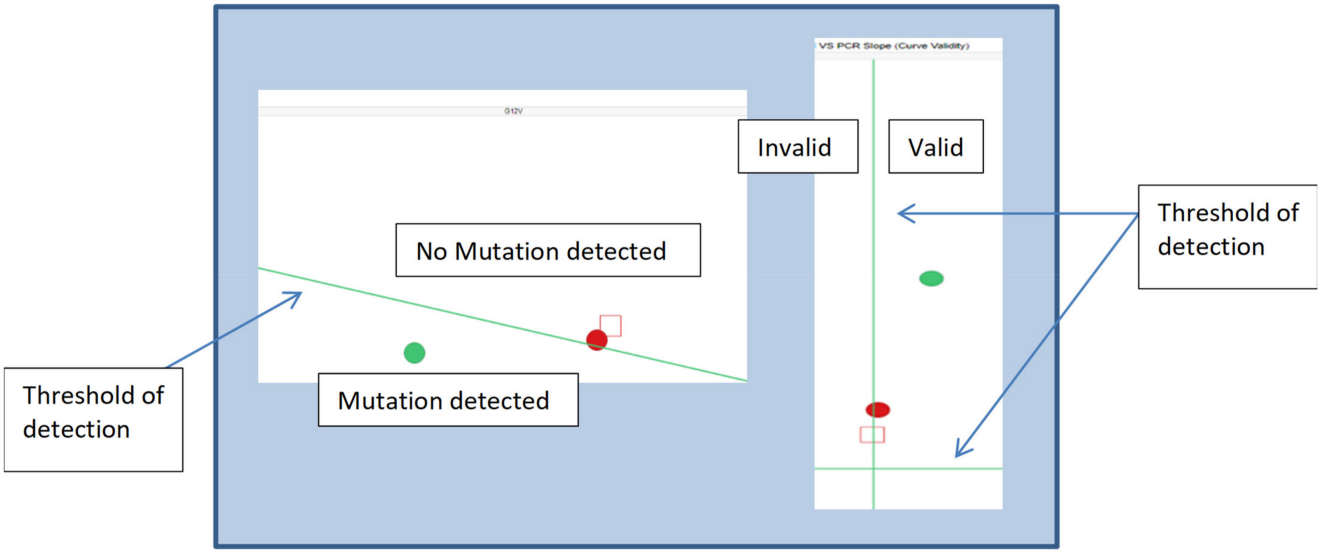

Figure 1. Output supplied by Biocartis following the review of the discordant FFPE result (L038). The graph on the left shows the threshold for positive and negative detection. The green circle is LBC sample. Red circle and red square are FFPE clot samples (one repeat). The graph on the right demonstrates the validity of the runs. The initial FFPE run was just inside the threshold for a valid result. The repeat run (red square) was considered invalid. The green circle (LBC) is well within range.

The second case that was non-correlated was the case of Panc 5B. Figure 2 demonstrates high levels of WT *KRAS* with amplification at a Cq of 20 with a G12R mutation detected at a Cq of 24 in the LBC sample. The tumour cell count was 40% > 100 cells. The Clot FFPE sample that was reported as no mutation detected (WT only) had only 5% tumour and <0 cells. The *KRAS* mutation was detected after a Cq of >33, past the Idylla internal cut-off threshold, highlighting that 5% and <50 cells is too low for a reliable detection in FFPE clot samples.

The lowest tumour yield in LBC samples for the detection of a mutation was 5% neoplastic cell content and >100 tumour cells for *EGFR* WT with a Cq of <26, and 5% neoplastic cell content and >50 tumour cells for *KRAS* WT with a Cq of <33 for WT (Table 2). Necrosis or excessive amounts of inflammatory cell populations did not impact the results, provided that sufficient tumour cells were present.

The 8/8 WT *BRAF* results for LBC and FFPE showed a concordance.

Using the criteria in Table 4 provides an effective way of assessing sample suitability and an analysis for an assurance of the reliability and integrity of the results. The Idylla™ test cartridges have thresholds that are applied to determine if a PCR curve is a valid (true) amplification curve and whether a mutation is detected, and these are based on a number of parameters within the software decision tree and may vary depending on factors such as the total DNA input.

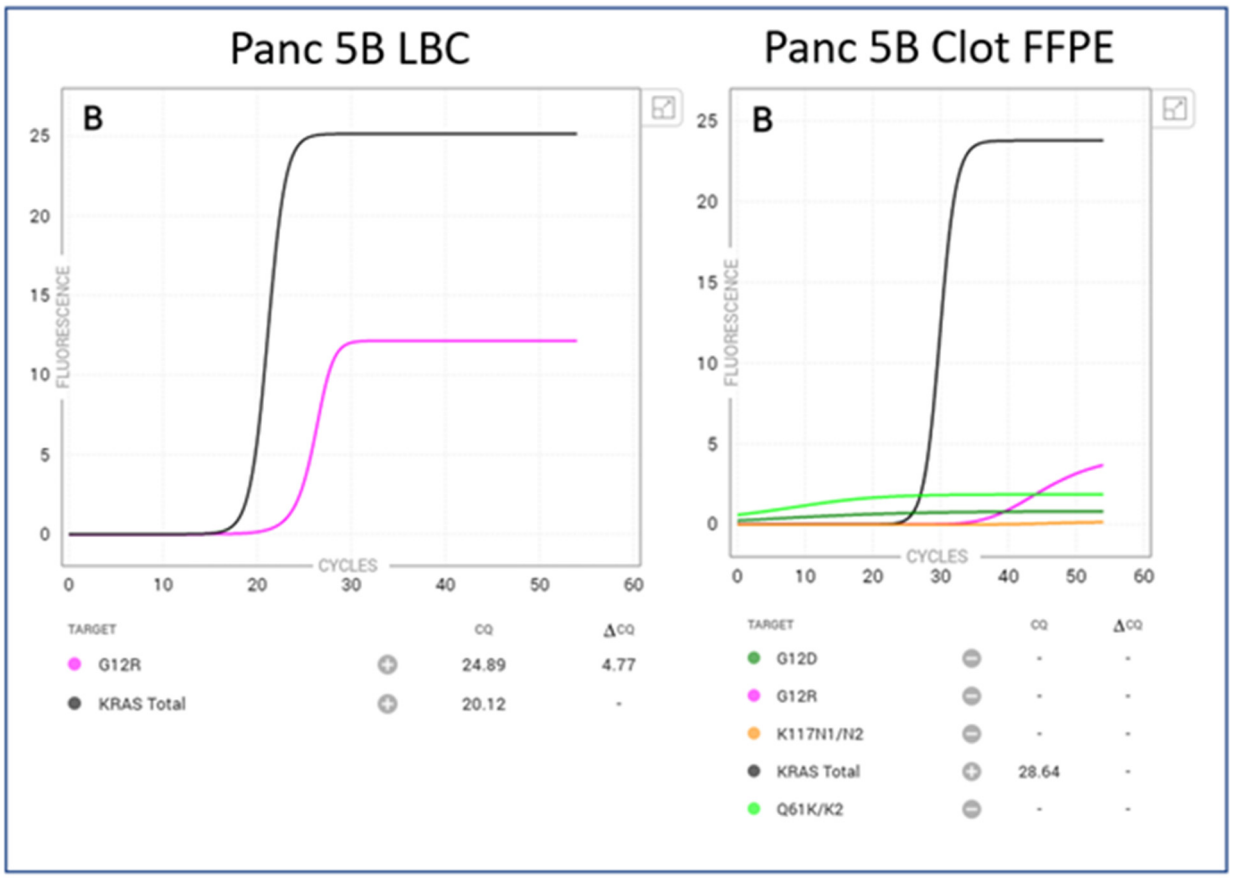

Figure 2. Comparison of the Idylla output PCR graphs LBC vs. Clot FFPE for the case of Panc 5B. *KRAS* wild-type amplification is indicated by the black curves. *KRAS G12R* mutation is indicated by the magenta curves.

Table 4. LBC sample prerequisites and data scrutiny criteria.

Test	Neoplastic Cell Content (LBC PAP)	Pellet Volume	WT Cq Value	Delta Cq	Valid Amplification Curve
KRAS	5%>50 cells	20 µL	<33	Low	✓
EGFR	5%>100 cells	20 µL	<26	Low	✓
BRAF	>50%	20 µL	<33 *IFU	Low	✓

* Instructions for use (IFU) for BRAF recommend 50% tumour for BRAF as macro section cannot be per-formed.

The results (Table 2) demonstrate the limit of detection for the techniques used with LBC samples and are summarised in Table 4. *BRAF* mutations were not detected in this study, and thus the thresholds provided by the manufacturer for FFPE were used for the assessment of WT *BRAF*.

The LBC samples demonstrated higher levels of well-preserved DNA. Lower Cq values were consistently seen in the LBC samples when compared with the Clot FFPE samples (Table 5).

The difference between the LBC *KRAS* WT and Clot *KRAS* WT Cq values was significant at $p < 0.01$ with a *t*-value of −6.42925 ($n = 22$). The difference between the LBC *EGFR* WT and Clot *EGFR* WT Cq values was significant at $p < 0.01$ with a *t*-value of −10.92458 ($n = 29$). The difference between the LBC *KRAS* WT and Clot *KRAS* WT Cq values was

significant at $p < 0.01$ with a t-value of −4.00241 ($n = 7$). The difference between the LBC *BRAF* WT and Clot FFPE *BRAF* WT Cq values was significant at $p < 0.01$ with a t-value of −4.00241 ($n = 7$).

Table 5. Comparison of average Cq values by test type.

	LBC Average Cq Value	Clot FFPE Average Cq Value	LBC Average Mutation Cq Value	Clot FFPE Average Mutation Cq Value
EGFR	17.6	22.4	24.1	27.3
KRAS	21.4	24.5	25.8	28.7
BRAF	30.9	34.29	-	-

The difference between the Cq values for WT DNA in all the LBC samples and Clot FFPE samples was significant at $p < 0.01$. ($n = 54$) The t-value was −5.62499. The p-value was <0.00001. There were significantly higher levels of well-preserved DNA in the LBC samples for each test type.

4. Discussion

The sample recruitment was lower than anticipated due to a reduced patient presentation during the coronavirus pandemic, leading to the *BRAF* cartridges expiring, which resulted in fewer tests being performed (Table 1). The initial study focused on *EGFR* in LBC samples only. The expansion of the project resulted in *KRAS and BRAF* for LBC samples, resulting in seven more *EFGR* cases than *KRAS* cases. In cases where ROSE deemed the sample insufficient, the entire sample was made into a clot to preserve material for the diagnosis, resulting in a PAP not being made for a comparison.

Using the LBC PAP slides provides an indication of the neoplastic cell count; however, this must be considered against the remaining samples in the vial after processing, as paucicellular samples will appear more cellular on the slide.

Cytology samples are often the only modality to obtain diagnostic material. Using residual LBC samples provides an advantageous medium for molecular testing (Figure 3), demonstrating increased sensitivity compared to FFPE clot samples, with no invalids. LBC Cq values were lower, illustrating high levels of well-preserved DNA. The use of whole cells fixed in alcohol, rather than sections of cells fixed in formalin, results in a higher nucleic acid yield, superior DNA preservation and the removal of the formalin fixation artefact [24]. This in turn reduces the likelihood of false positive or false negative results, increasing the sensitivity for detecting low allele frequency mutations. Using LBC samples eliminates concerns about erroneous DNA contamination associated with thrombin and plasma clots and removes multiple processing steps, reducing the likelihood of cross contamination. ROSE is recommended to ensure that sufficient material is obtained and that the DNA is preserved optimally to effectively cover the full scope of testing requirements.

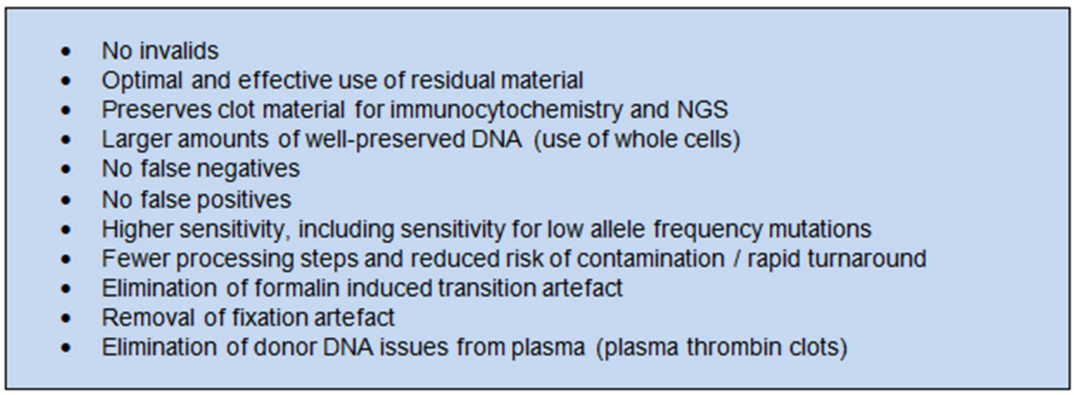

Figure 3. Benefits of utilising LBC samples compared with FFPE clot samples.

KRAS testing in NSCLC has recently been FDA approved and is available to a subset of patients on an early access scheme. Due to the mutual exclusivity of *EGFR*, *KRAS* and *BRAF*, laboratories can streamline workflows by testing the most common mutation first (*KRAS*), and if only WT *KRAS* is detected they can then proceed to *EGFR* and *BRAF* testing, optimally preserving samples and resources.

5. Conclusions

The Biocartis Idylla™ provides a highly sensitive, reliable and rapid testing platform for LBC samples for the detection of *EGFR* and *KRAS* mutations. *BRAF* mutations were not detected in the participant cohort; however, all LBC WT *BRAF* results correlated with the results from the FFPE clot samples.

Using the criteria demonstrated in Table 4 provides an effective way of assessing sample suitability and an analysis for the assurance of the reliability and integrity of the results. The Idylla™ cartridges are currently not IVD-labelled for the use of liquid-based cytology, but our research showed that they had an enhanced performance when compared with FFPE clot samples.

The benefits of utilising residual LBC samples are demonstrated in Figure 3.

Using the Idylla™ to conduct rapid molecular testing on LBC cytology samples enables localised rapid testing without the need for significant infrastructures, skills and resources. The study recognises the need for NGS at larger centres, but turnaround times are often prolonged (several weeks), which has significant consequences for patients [29]. Testing locally on residual LBC samples for the most common mutation facilitates timely first-line treatments for the majority of patients, reserving the FFPE samples for NGS testing at centralised sites. Detecting the most common mutations sooner helps to reduce patient anxiety, and, in cases where a mutation is detected, the best treatment plan can commence, thereby removing unnecessary delays as well as suboptimal or unnecessary treatments [30].

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Royal Cornwall Hospital NHS Trust and HRA and Health and Care Research Wales (HCRW) protocol code 275530 and date of approval 18/06/2020 for studies involving humans.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that supported the findings in this study is available on reasonable request from the corresponding author. The data is not publically available due to ethical restrictions.

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Conflicts of Interest: The authors declare no conflict of interest.

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