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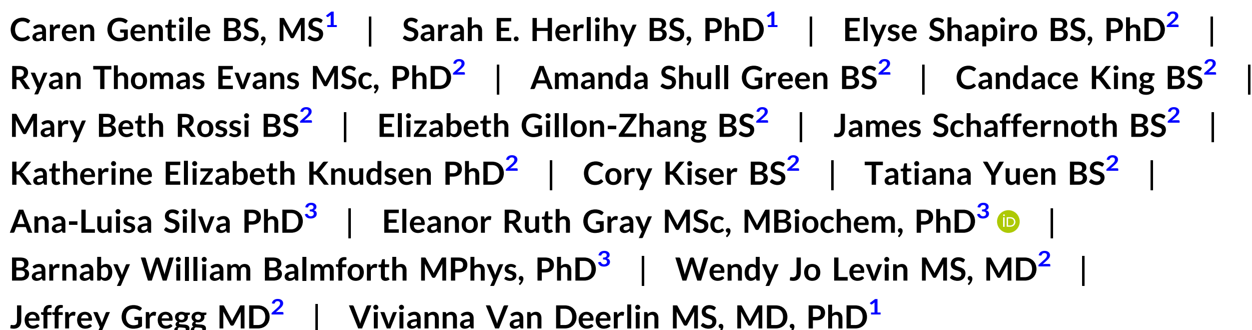
ORIGINAL ARTICLE

Successful simplified genomic profiling of cytology specimens using Aspyre Clinical Test for Lung (Tissue)



Caren Gentile BS, MS[1] | Sarah E. Herlihy BS, PhD[1] | Elyse Shapiro BS, PhD[2] | Ryan Thomas Evans MSc, PhD[2] | Amanda Shull Green BS[2] | Candace King BS[2] | Mary Beth Rossi BS[2] | Elizabeth Gillon-Zhang BS[2] | James Schaffernoth BS[2] | Katherine Elizabeth Knudsen PhD[2] | Cory Kiser BS[2] | Tatiana Yuen BS[2] | Ana-Luisa Silva PhD[3] | Eleanor Ruth Gray MSc, MBiochem, PhD[3] | Barnaby William Balmforth MPhys, PhD[3] | Wendy Jo Levin MS, MD[2] | Jeffrey Gregg MD[2] | Vivianna Van Deerlin MS, MD, PhD[1]

[1]Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA

[2]Biofidelity Inc, Morrisville, North Carolina, USA

[3]Biofidelity Ltd, Cambridge, UK

Correspondence

Barnaby William Balmforth.
Email: b.balmforth@biofidelity.com

Funding information

Biofidelity Ltd

Abstract

Background: Testing patients with non–small cell lung cancer for actionable variants is essential for guiding treatment decisions in accordance with established cancer care guidelines, though limited quantity and quality of tumor tissue often leaves insufficient material for comprehensive testing. Cytopathology specimens obtained through minimally invasive techniques are a potential source of diagnostic material for genomic profiling, though typically challenging to analyze.

Methods: A total of 85 DNA or total nucleic acid non–small cell lung cancer samples derived from 45 fine-needle aspirate rinse or pleural fluid samples from the Hospital of the University of Pennsylvania archive were tested using the Aspyre Clinical Test for Lung (Tissue) in Biofidelity's CAP/CLIA laboratory. All samples were previously characterized by the Oncomine Precision Assay Genexus assay (the orthogonal reference method).

Results: Eighty-four of 85 passed Aspyre Lung quality control, one failed. Twenty-six samples were positive for variants in the Aspyre Lung panel: 17 for single nucleotide variants (*KRAS*, *EGFR*), three for *EGFR* insertions/deletions, two for *MET* exon 14 skipping, and five for gene fusions. Eighty-two of 85 samples were run at standard input levels; three of 85 were run at low input but passed Aspyre Lung controls and include one *EGFR* exon 20 insertion variant-positive. All results were concordant between methods. Positive Percent Agreement and Negative Percent Agreement were 100%.

Conclusions: Aspyre Clinical Test for Lung performs effectively on samples derived from fine needle aspirate rinses and pleural fluid. Using these cytology-based

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specimens for biomarker testing enables pathologists to perform simplified genomic profiling while preserving valuable tissue specimens, potentially reducing the need for additional invasive procedures.

KEYWORDS
cytology, diagnostic accuracy, FNA rinse, molecular profiling, NSCLC, somatic variants

Introduction

Patients diagnosed with non–small cell lung cancer (NSCLC) now have access to a range of targeted therapeutics that are highly effective with minimal side effects; however, timely molecular profiling is essential. Rapid testing is needed to guide patients into appropriate treatment pathways because initiating chemotherapy before targeted therapy can compromise outcomes even if targeted therapy is introduced later.[1,2] Simplified Genomic Profiling provides targeted multigene biomarker characterization beyond the capabilities of single-gene testing but without the complexity and high sample quality requirements of comprehensive genomic profiling.[3]

In a study tracking the care pathways of more than 38,000 patients with actively managed NSCLC, 84.6% of patients received a tissue biopsy of some kind, with nearly 80% fine-needle aspirates (FNA), and approximately 10% core needle biopsies.[4] After most of the diagnostic tissue is collected from the FNA and core needle biopsy samples, needle rinses offer a valuable source of additional material for biomarker analysis.[5–7] FNA rinse samples, obtained by washing residual material from the needle after primary biopsy preparation, have gained traction as a way to recover additional cells for ancillary testing. Material thus obtained can be used in a variety of assays, including testing for molecular, cellular and immunological biomarkers.[2,7–9] Material may be used fresh or processed into a cell block; fresh samples have the advantage of a more rapid turnaround time.[10]

Aspyre Clinical Test for Lung (hereafter Aspyre Lung) has previously been validated for formalin-fixed, paraffin-embedded (FFPE) tissue[11] and initial testing of 16 cytology specimens provided early evidence that these specimens may also be compatible with the assay.[12] Following initial assay validation, additional studies demonstrated equivalent sensitivity and specificity at inputs as low as 5 ng DNA and 1.5 ng RNA.[13] These findings support the feasibility of using Aspyre Lung with low input or nontraditional samples, potentially expanding access for patients who cannot undergo resection or more invasive biopsy procedures.[7]

Cytology samples at the Hospital of the University of Pennsylvania comprise approximately half of the samples received and are routinely analyzed using next-generation sequencing (NGS)–based methods.[6,10] A collaboration enabled us to test residual samples that already had biomarker profiles. Archived samples were selected based on the availability of orthogonal test results from the Oncomine Precision Assay Genexus kit (hereafter Oncomine Precision) and sufficient DNA and/or total nucleic acid (TNA) to run on Aspyre Lung. A total of 85 samples from 45 patients were selected: 81 were derived from 43 FNA rinse specimens, and four were derived from two pleural fluid specimens (Figure 1), yielding 44 DNA and 41 TNA samples in total.

In this study, we aimed to assess the diagnostic accuracy of the Aspyre Lung on FNA rinse samples by comparing results to NGS-based molecular profiling. Aspyre Lung had previously been validated on FFPE lung tissue; these samples were extracted from fresh material that had not been fixed or embedded, and thus represent an opportunity to assess assay performance.

MATERIALS AND METHODS

Ethical approval

For samples retrospectively identified for this study, the study was approved by the Institutional Review Board of the University of Pennsylvania Institutional Review Board #2 Protocol 854192 and individual consent for this retrospective analysis was waived.

Clinical samples

FNA samples were collected into CytoLyt or saline using standard procedures. Needles were then rinsed with the corresponding collection media to collect residual material, which was transferred to PreservCyt media (Hologic) for storage.

Nucleic acid extraction

Vials with FNA rinse specimens in PreservCyt media were spun down and the cell pellet was processed for nucleic acid extraction. DNA and TNA were extracted from samples in parallel using the Agencourt FormaPure kit (Beckman, Brea, CA, USA) without the deparaffinization and decrosslinking steps.

Next-generation sequencing

Nucleic acid sequencing was performed using the Genexus Platform with the NGS-based Oncomine Precision Assay Genexus kit (Thermo Fisher). Inputs used for sequencing were 13.4 ng of DNA and 13.4 ng of TNA. These Oncomine Precision results were used as the reference dataset for comparison with Aspyre Lung.

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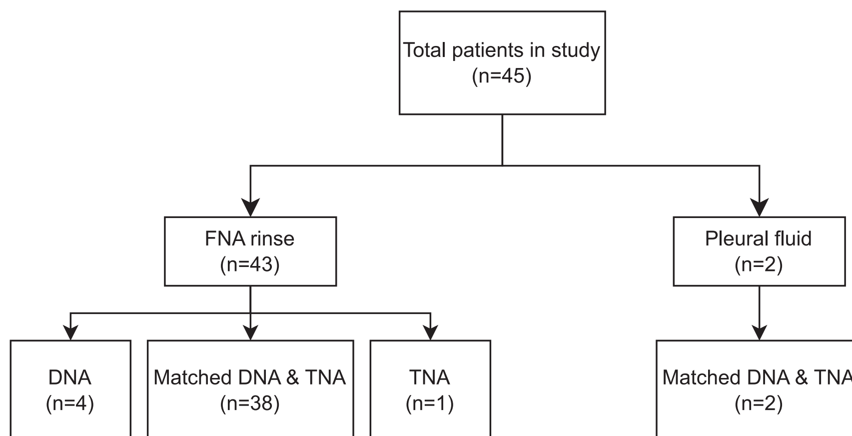

FIGURE 1 Samples analyzed in this study by sampling type and nucleic acid. A breakdown of sample type and the orthogonal test result is in Supplementary Table 1. FNA indicates fine needle aspirate; TNA, total nucleic acid.

Aspyre Clinical Test for Lung (Tissue) runs and analysis

After assessment of the concentration of each sample at Biofidelity Inc Laboratory (Morrisville, NC, USA) using the Qubit 1X dsDNA HS kit or Qubit 1x RNA HS kit, no further controls were applied. Aspyre Lung runs were performed as previously described[11] except for two TNA samples with RNA concentrations below the quantification limit (<4 ng/μL), which were processed using the maximum possible input volume.

Data analysis

The raw file generated by the Design and Analysis 2 software (v2.7, Thermo Scientific, Waltham, MA, USA) was run through AspyreLab software v1.3.1. This software analyzes the raw fluorescent output curves, applying quality checks, corrections, and normalizations to generate variant calls of "detected," "not detected," or "undetermined" for each variant in the panel.[14] A sample was considered concordant if both assays produced the same qualitative result for all shared targets. All analysis of Aspyre Lung runs and variant calling was performed blinded to orthogonal test results and clinical information.

Statistical analysis

Descriptive statistics were used; positive percent agreement and negative percent agreement were calculated using 2×2 tables.

RESULTS

There are currently no Food and Drug Administration-approved tests for NSCLC cytology specimens; however, local validated tests for liquid or tissue specimens may be used. A pilot study using three FNA rinse samples (among other cytological preparations) provided preliminary evidence that Aspyre Lung could generate biomarker data using this material.[12] To consolidate this finding and assess the performance of Aspyre Lung on a larger number of cytology samples spanning a broader range of variants, 85 previously characterized DNA or TNA samples derived from FNA rinses or pleural fluid from the Hospital of the University of Pennsylvania were sent to the Biofidelity Clinical Laboratory for analysis using Aspyre Lung. These samples had already been extracted and stored at −20°C for up to 14 months, but otherwise samples were run according to standard protocols.

The provenance and characterization of these samples, including whether samples were matched DNA and TNA, or unmatched is shown in Figure 1. The results from Oncomine Precision runs and the corresponding results from Aspyre Lung are shown in Figure 2, grouped according to nucleic acid and the original sample type.

DNA samples

There were 44 DNA samples analyzed in this study, of which 42 were derived from FNA rinse samples and two from pleural fluid. One FNA-rinse DNA sample failed Aspyre Lung quality control (QC) and was excluded from concordance analysis. Among the remaining 43 evaluable DNA samples, concordance with Oncomine Precision was 100% (43/43). The positive concordant calls included 15 samples with a single nucleotide variant in the KRAS proto-oncogene, GTPase (*KRAS*) gene, two samples with a single nucleotide variant in the epidermal growth factor receptor (*EGFR*) gene, and three samples with deletions or insertions in *EGFR*. There were 23 DNA samples negative for all DNA targets in the Aspyre Lung panel. Two of these had MET proto-oncogene, receptor tyrosine kinase (*MET*) exon 14 skipping reported by Oncomine from DNA; because Aspyre Lung evaluates *MET* exon 14 skipping via RNA, these calls were assessed in the TNA/RNA analysis (see the next section). Six samples were positive by Oncomine for genomic alterations not covered by Aspyre Lung; one *EGFR* exon 19 deletion, one *EGFR* exon 19 insertion, and four *EGFR* exon 20 insertions (Supplementary Table 2). Of these, two of the six had been previously profiled by COSMIC,[15] and three of

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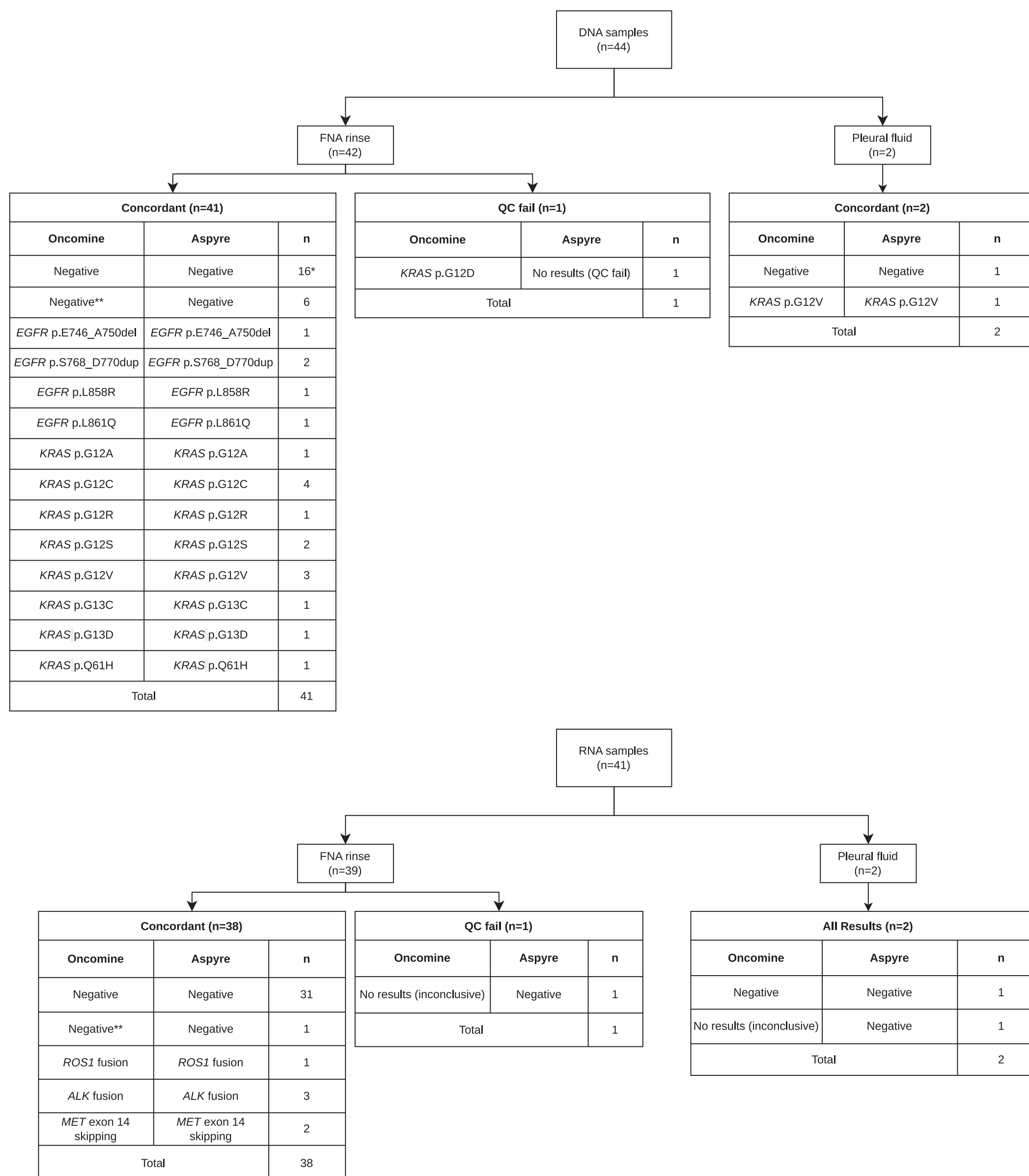

Concordant (n=41)		
Oncomine	Aspyre	n
Negative	Negative	16*
Negative**	Negative	6
EGFR p.E746_A750del	*EGFR* p.E746_A750del	1
EGFR p.S768_D770dup	*EGFR* p.S768_D770dup	2
EGFR p.L858R	*EGFR* p.L858R	1
EGFR p.L861Q	*EGFR* p.L861Q	1
KRAS p.G12A	*KRAS* p.G12A	1
KRAS p.G12C	*KRAS* p.G12C	4
KRAS p.G12R	*KRAS* p.G12R	1
KRAS p.G12S	*KRAS* p.G12S	2
KRAS p.G12V	*KRAS* p.G12V	3
KRAS p.G13C	*KRAS* p.G13C	1
KRAS p.G13D	*KRAS* p.G13D	1
KRAS p.Q61H	*KRAS* p.Q61H	1
Total		41

QC fail (n=1)		
Oncomine	Aspyre	n
KRAS p.G12D	No results (QC fail)	1
Total		1

Concordant (n=2)		
Oncomine	Aspyre	n
Negative	Negative	1
KRAS p.G12V	*KRAS* p.G12V	1
Total		2

Concordant (n=38)		
Oncomine	Aspyre	n
Negative	Negative	31
Negative**	Negative	1
ROS1 fusion	*ROS1* fusion	1
ALK fusion	*ALK* fusion	3
MET exon 14 skipping	*MET* exon 14 skipping	2
Total		38

QC fail (n=1)		
Oncomine	Aspyre	n
No results (inconclusive)	Negative	1
Total		1

All Results (n=2)		
Oncomine	Aspyre	n
Negative	Negative	1
No results (inconclusive)	Negative	1
Total		2

FIGURE 2 Variant calls from Oncomine Precision and Aspyre Lung, grouped by nucleic acid and sample type. *Includes two samples positive for *MET* exon 14 skipping, which is detected by Aspyre Lung through RNA. No further variants were detected from DNA for these samples. **Includes calls made by Oncomine Precision that are not in the Aspyre Lung panel (Supplementary Table 2).

four variants with unique protein changes were not found in cohorts included in cBioPortal.[16,17] These six variants were outside the Aspyre Lung panel and therefore excluded from concordance calculations.

RNA

There were 41 TNA samples analyzed in this study, of which two were derived from pleural fluid, and the remaining 39 from FNA rinse samples. Two samples, one derived from each sample type, failed internal RNA expression controls for Oncomine Precision and yielded inconclusive results. 39 variant calls were concordant; of these, 33 samples were negative for all biomarkers on the Aspyre Lung panel. Six samples were positive by both assays for variant calls: one ROS proto-oncogene 1, receptor tyrosine kinase (*ROS1*) fusion, three ALK receptor tyrosine kinase (*ALK*) gene fusions, and two with *MET* exon 14 skipping events. One sample was positive for a rare *HIP1-ALK* gene fusion, which is not covered by the Aspyre Lung panel (Supplementary Table 2).

Overall

Overall concordance between Aspyre Lung and Oncomine Precision across all evaluable DNA and TNA samples was 100% (n = 82,

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TABLE 1 Summary of concordance for samples tested on both the Oncomine Precision and Aspyre Lung.

	Aspyre +	Aspyre –
Oncomine +	26	0
Oncomine –	0	56
PPA - 100%; NPA - 100%		

Note: Eighty-five DNA or TNA samples were tested; three that failed quality control of either test were excluded. Each DNA or TNA sample is considered independently, regardless of whether they are from matched or unmatched samples.

Abbreviations: NPA, negative percent agreement; PPA, positive percent agreement.

positive percent agreement [PPA] = 100%, negative percent agreement [NPA] = 100%; Table 1). When results are considered on a per-patient basis, 45 patients have evaluable results as QC failures occurred solely in DNA or TNA samples from matched pairs, and PPA and NPA remain 100% (Supplementary Table 3).

DISCUSSION

As the number of approved targeted therapies increases, so too does the number of patients who could potentially benefit from these drugs, but only if they receive timely and appropriate biomarker testing. Despite the availability of established assays, clinical practice gaps have remained in ensuring NSCLC testing is performed early and fast enough to inform treatment decisions. Aspyre Lung was developed to close these gaps, offering a rapid 2-day turnaround time and full coverage of National Comprehensive Cancer Network-recommended first-line genes (v3.2025). The assay has now been validated for use in blood samples (at 20/5 ng cell-free DNA and 42/6 ng cell-free RNA for standard-/low-input levels[18]), and in tissue samples down to 5 ng DNA and 1.5 ng RNA input, with no loss of sensitivity or specificity.[13] In this study, we sought to expand a preliminary sample set of cytological specimens[12] to assess the feasibility of using nucleic acid derived from FNA rinse samples (which are not always routinely processed) for molecular testing along with other available nucleic acid derived from pleural fluid. Making the most of samples that are currently underused would enhance testing options for patients.[7]

In this study, we conducted concordance testing on 85 DNA or TNA samples from 45 patients, all of which had orthogonal testing results from the Oncomine Precision. There were 21 samples with DNA-detectable variants and six samples with RNA-detectable variants. Three samples failed QC; two from the Oncomine Precision and one from Aspyre Lung. The latter was a run-level failure as the internal positive control failed checks, rendering the results from DNA invalid (but not TNA). Concordant variant calls were obtained from 20/20 evaluable DNA samples with positive variant calls (the remaining 21st DNA variant-positive sample was the QC failure) and from all evaluable TNA samples, resulting in a PPA and NPA of 100% for this sample set.

One TNA sample yielded a concordant negative result for both assays, but was subjected to further investigations due to a known history of a gene fusion in *ALK* for the patient. Manual review of the Oncomine Precision result identified 17 sequencing reads consistent with an *EML4-ALK* gene fusion (E6ins33:A20, COSF474). No other *ALK* fusion variants were found, and the call from the Oncomine Precision was negative. The low read count in Oncomine Precision may have fallen below the calling threshold. Given that this fusion is included in the Aspyre Lung panel,[19] we reexamined the results from Aspyre Lung. Although the assay does not generate quantitative fluorescent data with cycle threshold values, visual inspection of the output curves showed this sample lying between known *ALK* positive and *ALK*-negative controls (intermediate space), suggesting a borderline signal. An *ALK* fusion–positive clinical sample from a previous study, also COSF474 and called positive by Aspyre Lung served as a reference.[3] There was an additional eight months of storage, and the sample underwent at least two freeze–thaw cycles between the two tests; a 19% drop in measurable nucleic acid was noted before the Aspyre Lung run, which could have degraded RNA and copy numbers such that the resulting signal fell below the detection threshold.

The cellular material found in fixed pleural fluid and FNA rinse samples will differ from material that has been formalin treated, as fixation engenders fragmentation and cross-linking; the latter generally being reversed during nucleic acid extraction. This reversal often involves a high heat step (in excess of 90°C), which can damage nucleic acid. By contrast, untreated cellular material is more likely to remain intact and is suitable as a source material for molecular diagnostics, as long as sufficient material is available and the specificity of the test is unaffected. Aspyre Lung has been validated for use on FFPE tissue (which may include treated cytology samples) and blood plasma samples, which are highly fragmented.[20] Although a full validation on nonfragmented samples such as fresh cytology specimens has not yet been completed, in-house testing has long used high-quality genomic DNA during assay development without any observed increase in false positives (equivalent studies for RNA have not been performed).

All samples in this study were taken from an archive and preselected based on sufficiency of material (both total quantity and concentration, as well as quality to yield results for DNA or TNA), and prior results available from an NGS-based assay. It is therefore not possible to conclude how successful Aspyre Lung might be on an unselected sample set. Cytology samples received at the Hospital of the University of Pennsylvania are normally processed for nucleic acid extraction and testing on receipt, with surplus nucleic acid frozen at −20°C (DNA) or −80°C (TNA). All samples were less than 15 months old between receipt at the Hospital of the University of Pennsylvania and the Aspyre Lung runs; however, samples had undergone at least two freeze-thaws before the Aspyre Lung run. It is not known what effect these freeze-thaw cycles may have had on the nucleic acid. Finally, at the time of this study, Aspyre Lung was validated for use with RNA, not TNA. TNA is a more complex sample type than pure DNA or

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mRNA, being composed of both DNA and highly heterogeneous RNA comprising the targeted mRNA but also tRNA, rRNA, and many smaller species.[21] TNA is commonly extracted by clinical laboratories due to workflow efficiency, and so is an attractive analyte in real-world practice. Since this study, use of TNA for both the DNA and RNA parts of the Aspyre Lung assay has been validated, thus enabling simplified workflows. While we sought to compose a test set that contained both samples negative for all markers in the panel and a spread of different variant-positive samples, a key determinant of inclusion was sample sufficiency which limited the available pool and the resulting diversity, thus this cannot be considered a formal assay validation.

Because Aspyre Lung is a targeted panel assay, focused on actionable or prognostic variants in guideline-recommended genes, it has the limitation that it does not detect some low prevalence variants, for example uncommon *EGFR* exon 20 insertions, and rare gene fusions. Additionally, genes that currently fall outside of recommendations could determine eligibility for clinical trials. Patient demographics at clinical care centers differ such that there is no single diagnostic assay that is appropriate for every setting. The assay has a low failure rate and a rapid turnaround time,[3] which make it suitable as a first-line test, with the option for reflex to more comprehensive genomic profiling in the case of a negative result. Rather than a single diagnostic assay being appropriate for all patients, targeted and comprehensive assays can serve complementary roles within a tiered testing strategy.

Laboratories have an obligation to conserve tissue which may be needed for multiple different preparations and a variety of tests. Using portions of sample preparations that would otherwise be discarded increases the number of tests that can be performed and enhances the information available to guide clinical care. Nonetheless, these findings are encouraging for laboratories aiming to maximize the utility of cytology samples.

AUTHOR CONTRIBUTIONS

Caren Gentile: Conceptualization; data curation; formal analysis; investigation; methodology; resources and writing–review and editing. **Sarah E. Herlihy**: Conceptualization and resources. **Elyse Shapiro**: Conceptualization; data curation; formal analysis; methodology; project administration; and supervision. **Ryan Thomas Evans**: Data curation and investigation. **Amanda Shull Green**: Supervision. **Candace King**: Investigation. **Mary Beth Rossi**: Investigation. **Elizabeth Gillon–Zhang**: Investigation. **James Schaffernoth**: Investigation. **Katherine Elizabeth Knudsen**: Investigation. **Cory Kiser**: Investigation. **Tatiana Yuen**: Investigation. **Ana–Luisa Silva**: Investigation. **Eleanor Ruth Gray**: Data curation; formal analysis; writing–original draft; writing–review and editing and visualization. **Barnaby William Balmforth**: Funding acquisition and supervision. **Wendy Jo Levin**: Conceptualization; funding acquisition; methodology; and supervision. **Jeffrey Gregg**: Writing–original draft and writing–review and editing. **Vivianna Van Deerlin**: Conceptualization; resources and supervision.

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CONFLICT OF INTEREST STATEMENT

The following authors are employees or consultants of Biofidelity Ltd or Biofidelity Inc and may also hold stock or stock options: E.S., R.T.E., A.S.G., C. King, M.B.R., E.G.Z., T.Y., J.S., K.E.K., C. Kiser, A.-L.S., E.R.G., B.W.B., J.G., W.J.L.

DATA AVAILABILITY STATEMENT

All patient data available within the restrictions of the ethical approval process are already presented in the manuscript.

ORCID

Eleanor Ruth Gray https://orcid.org/0000-0002-6515-839X

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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