Improving Adequacy of Small Biopsy and Fine-Needle Aspiration Specimens for Molecular Testing by Next-Generation Sequencing in Patients With Lung Cancer

A Quality Improvement Study at Dartmouth-Hitchcock Medical Center

Vijayalakshmi Padmanabhan, MD; Heather B. Steinmetz, BS; Elizabeth J. Rizzo, BS; Amber J. Erskine, BS; Tamara L. Fairbank, BS; Francine B. de Abreu, PhD; Gregory J. Tsongalis, PhD; Laura J. Tafe, MD

• *Context.*—At our medical center, cytopathologists perform rapid on-site evaluation for specimen adequacy of fine-needle aspiration and touch imprint of needle core biopsy lung cancer samples. Two years ago the molecular diagnostics laboratory at our institution changed to next-generation sequencing using the Ion Torrent PGM and the 50-gene AmpliSeq Cancer Hotspot Panel v2 for analyzing mutations in a 50-gene cancer hot spot panel. This was associated with a dramatic fall in adequacy rate (68%).

***Objective.*—To improve the adequacy rate to at least 90% for molecular testing using next-generation sequencing for all specimens collected by rapid on-site evaluation by the cytology laboratory.**

***Design.*—After baseline data on adequacy rate of cytology specimens with rapid on-site evaluation for molecular testing had been collected, 2 changes were implemented. Change 1 concentrated all the material in one block but did not produce desired results; change 2, in addition, faced the block only once with unstained slides cut up front for molecular testing. Data were collected in an Excel spreadsheet and adequacy rate was assessed.**

***Results.*—Following process changes 1 and 2 we reached our goal of at least 90% adequacy rate for molecular testing by next-generation sequencing on samples collected by rapid on-site evaluation including computed tomography–guided needle core biopsies (94%; 17 of 18) and fine-needle aspiration samples (94%; 30 of 32).**

***Conclusion.*—This study focused on factors that are controllable in a pathology department and on maximizing use of scant tissue. Optimizing the adequacy of the specimen available for molecular tests avoids the need for a second procedure to obtain additional tissue.**

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Lung cancer is the most common malignancy worldwide and is the major cause of cancer related death.[1] The highest incidence of lung cancer (>100 cases/100 000 population) has been reported from the United States.[1] Also, globally and within the United States, although some types of lung cancer, like squamous cell carcinoma and small cell neuroendocrine carcinoma, are decreasing, there has been an increase in the incidence of adenocarcinoma (ADC) of the lung among both men and women.[2] Recently, there have been some major advances in our understanding of the pathogenesis and management of lung cancers, ADC in particular.[3] Specifically, the discovery of the biological and therapeutic importance of somatic alterations in 2 genes that encode pharmacologically targetable tyrosine kinases involved in growth factor receptor signaling, epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*), has changed the way these cancers are diagnosed and treated.[3]

Historically, the most important decision for pathologists when evaluating small biopsies and cytology samples was to distinguish between small cell and non–small cell lung cancer.[4] In the past it was not necessary to separate squamous cell carcinoma from ADC and other histologic types among non–small cell lung cancer.[5] This paradigm has changed dramatically in recent years.[4] Now it is essential for the pathologist to differentiate squamous cell carcinoma from ADC of the lung.[4] *EGFR* mutation is strongly associated with ADC histology, and patients with advanced non–small cell lung cancer and *EGFR* mutations have a better outcome and response to tyrosine kinase inhibitors as a first-line therapy, whereas patients without *EGFR* mutations seem to have a better outcome with chemotherapy.[6] Patients with advanced lung cancer treated with bevacizumab are at increased risk for life-threatening hemorrhage if

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From the Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire. Dr Padmanabhan is now with the Department of Pathology, Baylor College of Medicine, Ben Taub General Hospital, Houston, Texas.

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Reprints: Laura J. Tafe, MD, Department of Pathology, Dartmouth-Hitchcock Medical Center, One Medical Center Dr, Lebanon, NH 03756 (email: Laura.J.Tafe@Hitchcock.org).

they have squamous cell carcinoma.[7] Therefore, differentiating the type of cancer is crucial for patient management. In addition to differentiating the type of lung cancer, molecular characterization of lung carcinoma contributes valuable information in terms of the patient's diagnosis and prognosis and the potential for treatment with targeted therapy.[8] Cytology material is increasingly being used to diagnose lung cancer and triage material for molecular testing.[5,9,10]

Cytology material from the lung commonly used in the diagnosis and/or staging of lung cancer includes touch imprint cytology of minute needle core biopsies (NCBs)—usually transthoracic computed tomography (CT)–guided NCBs—and bronchoscopy-guided fine-needle aspiration (FNA) cytology smears and endobronchial ultrasound–guided FNA cytology smears with or without a formalin-fixed, paraffin-embedded cell block.[11–20] Additional types of material from the lung that are used include bronchoscopic brushing or washes.[18] In many institutions cytopathologists collect material on site at the CT and/or endoscopic suites and perform a rapid on-site evaluation (ROSE) for specimen adequacy for a diagnosis, and in addition they triage the specimen for other studies like flow cytometric analysis and microbiologic culture studies.[21–25] Rapid on-site evaluation for specimen adequacy is performed on the vast majority of the lung cancers diagnosed at our institution. Of late, most cytopathologists at our institution noticed that assuring collection of adequate material for molecular testing for lung cancer had crept into this ROSE paradigm. Rapid on-site evaluation at our institution includes evaluation of air-dried Diff-Quik stained FNA smears from bronchoscopically acquired specimens and evaluation of air-dried, Diff-Quik stained touch imprint cytology of NCB for CT-guided acquisition of minute NCBs. Additional passes were routinely collected by placing and rinsing needle aspirates or NCB directly in normal saline (the collection medium used in our laboratory) with the precollected sample. Furthermore, the collection tube with the sample was grossly assessed to ensure that there are some tissue fragments for the block. Rapid on-site evaluation included evaluation of only the touch imprint of NCB from CT-guided specimens (FNA is not routinely performed for the CT-guided cases) and only FNA smears for bronchoscopic specimens. All of these samples collected by ROSE (FNA or NCB) are further processed by the cytology laboratory and hence called cytology specimens. A cell block (vide infra) was prepared from all bronchoscopically acquired specimens and a tissue block was prepared from all CT-guided NCB specimens.

Next-generation sequencing (NGS) allows identification of clinically actionable genetic variants across many genes in a single test. Detection of multiple somatic cancer alterations—including single-nucleotide variants, small insertions and deletions (indels), copy number variations, and chromosomal structural variants (including rearrangements, translocations, and inversions)—can be performed efficiently and cost-effectively using NGS.[26,27] Next-generation sequencing–based diagnostics offer an improvement over other molecular methods, such as Sanger sequencing and other polymerase chain reaction–based assays, by which only a limited spectrum of mutations can be identified at a single genomic locus.[28] Though there are data on the use of cytology material for molecular testing for *EGFR* and *KRAS* gene mutation and for *ALK* rearrangement by fluorescence in situ hybridization, data on the use of cytology material for NGS suggest that scant cytology samples can be studied by NGS.[3,29–41]

In our institution molecular testing for ADC or poorly differentiated carcinomas of the lung is performed as a reflex test. The cytopathologist orders the molecular test from a list of test options in the laboratory information system (LIS). Insufficient material for molecular testing could result in having to bring some of the patients back for repeat sampling, as this information is required for various treatment options and protocols. Increasingly, in 2013 we received complaints from the clinicians that molecular testing was not successful on an increasing number of cytology samples because of inadequate material. Our preliminary data collected as part of this study showed that our adequacy on some cytology specimens was down to less than 60% (see below). A cursory look at our internal LIS data showed that more samples that were diagnosed by cytology were being called inadequate for molecular testing than a year or two prior. However, during this same time period, the molecular testing strategy had changed from analyte-specific polymerase chain reaction–based testing for a small set of mutations in 3 genes (*EGRFR*, *KRAS*, and *BRAF*) to NGS with testing for a 50-gene panel with *ALK* rearrangement analysis by fluorescence in situ hybridization.[33] It was determined that the current process for collecting and processing these specimens that were once adequate for the polymerase chain reaction–based testing was insufficient for NGS.

The aim of this quality improvement (QI) project, based on lean principles, was to improve sample adequacy rate of cytology specimens collected by ROSE for molecular testing by NGS to at least 90%.

MATERIALS AND METHODS

Our problem was to determine potential causes for inadequate specimens and improve processes that are controlled by the laboratory.

An overview of our design is as follows:

1. Collect baseline data from January 2014–August 2014 from LIS in an Excel spreadsheet.
2. Brainstorm the process with key personnel in the laboratory involved with the sample preparation and reporting, including people from molecular pathology, histology, LIS, cytology, and laboratory QI.
3. Identify steps in the process in the pathology department with a thorough review of each step for a shared cytology/molecular pathology specimen.
4. Prepare a cause-and-effect diagram with input from all key personnel to determine all potential causes of an inadequate specimen.
5. Based on expert consensus, make a change in processing the specimen in histology (vide infra).
6. Collect data from before and after the change was made in an Excel spreadsheet.

The study setting involved the radiology, bronchoscopy and pathology departments. The pathology department included cytology, histology, and molecular diagnostics.

The study subjects included all patients who had lung nodule(s) and/or mediastinal (or other) lymph node enlargement and underwent CT-guided NCB and/or endobronchial/endoscopic ultrasound–guided FNAs with a pathological diagnosis of ADC or poorly differentiated carcinoma of the lung. Only specimens that were by cytology with ROSE were evaluated in this study. All cytology specimens were submitted for molecular testing for gene

Table 1. Process Map With Review of Each Step for a Shared Cytology/Molecular Pathology Specimen Collected by Rapid On-Site Evaluation

Process Step	Deliverable	Observations/Findings
Contact laboratory	Person responsible: provider 1. Pages FNA pager	• Documentation of possible cases noted in electronic health record but communication with laboratory occurs on a stat basis with cytology No defined form of communication with molecular laboratory • Care plan is not known till cytopathologist determines type of tumor
Sample collection	Person responsible: cytotechnologist 2. Collect core biopsies	• Multiple persons collecting samples (trainees, attendings) • Gauge of needle is small (20 g or more) • Slides brought to cytology technologist (usually multiple cores on 1 slide) • Patient factors—ability to withstand procedure • Pneumothorax • Accessibility of nodule • Size of nodule • Skill level of collector • Tissue factors: fibrotic/necrotic, etc • Sampling normal tissue instead of tumor
Touch preparation slides	Person responsible: cytotechnologist and cytopathologist 3. Touch preparation slides made 4. Biopsies into specimen container 5. Touch preparations stained 6. Adequacy assessment made at collection 7. Usually additional tissue requested and cores that were not evaluated for adequacy placed in separate block	• Disposable device used to transfer cores from slides to specimen container • Stains filtered and the containers cleaned after each use • Variable number of cores requested—pathologist comfort level • Unsure of tissue sampled in block with additional samples that were not evaluated by pathologist
Molecular order request	Person responsible: provider or cytopathologist 8. Laboratory test orders for collection	• Molecular testing ordered by collecting provider or by cytopathologist
Specimen preparation	Person responsible: pathology technician 9. Tissue description 10. Cassettes	• Specimen spun • Specimen filtered • Specimen put in agar • Size and type of specimens determine processing
Processing	Person responsible: tissue express processor 11. Tissue ready for embedding	• Processor loaded with cassettes • Automated, proprietary system
Embedding	Person responsible: histology technician 12. Tissue paraffin block or blocks made as determined by the person grossing the specimen in	• Processed core biopsies (wrapped) removed from cassette • Biopsies unwrapped from tissue paper • Biopsies transferred into block mold with forceps or scalpel • Paraffin added to fill out block
Sectioning/slide preparation	Person responsible: histology technician 13. Slides with thin sections	• Water bath cleared with Kim-wipe • Microtome used for cutting several thin sections • Sections floated on water bath and captured on printed slide
Case review	Person responsible: resident or pathologist 14. Review slides 15. Orders immunohistochemistry	• Morphology interpreted based on several slides • Immunohistochemistry ordered; then, after determination of diagnosis, molecular tests ordered
Case verified	Person responsible: pathologist 16. Generate patient report 17. Orders molecular panel on laboratory information system	• Report reviewed in laboratory information system, edited for wording or content • Case verified
NGS	Person responsible: molecular pathology technologist	• Molecular testing may be ordered by cytopathologist at the same time or after immunohistochemistry • Tumor content determined by separate pathologist
Communication	Person responsible: molecular laboratory technologist	• NGS runs repeated for insufficient DNA and delay provider communication

Abbreviations: FNA, fine-needle aspiration; NGS, next-generation sequencing.

mutation analysis by NGS using the Ion Torrent PGM and the 50-gene AmpliSeq Cancer Hotspot Panel v2 (both from Thermo Fisher Scientific Inc, Waltham, Massachusetts).

A detailed process map with deliverables and observations is given in Table 1. Touch imprint cytology was evaluated only on air-dried Diff-Quik smears, which were brought back to the laboratory and coverslipped for a final read. All FNA smears had an alcohol-fixed smear made per pass; the mirror-image, air-dried Diff-Quick stained smear was initially evaluated for ROSE and the alcohol-fixed smear was brought back to the laboratory and stained with Papanicolaou stain. Both of these smears were examined for the final read by the pathologist. Only tissue block or cell block material was submitted for molecular testing. Material on the smears was not submitted for molecular testing.

Cell Block Preparation

The FNA specimen, which included needle rinses and additional passes collected in normal saline, was poured into a 50-mL tube labeled with the patient's demographics. CytoRich Red (Becton, Dickinson and Company, Franklin Lakes, New Jersey) was added to make up the volume to 45 mL. The tube was centrifuged at 600*g* for 5 minutes. The supernatant was pipetted and used for a standard liquid-based preparation; the remnant cell particles were stained with a drop of eosin. In case the material was deemed minute by the technician, Trypticase Soy Agar (Thermo Fisher Scientific) was heated in a microwave oven until it melted (typically within 1 minute) and added to the specimen in a ratio of 1 part melted agar to 2 parts specimen. Following gentle vortexing, the specimen was centrifuged at 600*g* for 5 minutes. The solidified gel button was gently dislodged and the specimen end of the gel button was cut and placed in Histo-Wrap (Obex Industries, LLC, Chagrin Falls, Ohio) in a tissue cassette. For larger specimens, the button was directly placed in Histo-Wrap and placed in a cassette. This was formalin fixed and processed.

Tissue Block Preparation

The needle core biospies were gently placed in Histo-Wrap in a cassette. The container of normal saline was filtered and any material that was identified was also placed in the cassette. This was formalin fixed and processed. The paraffin-embedded blocks were sectioned and stained with routine hematoxylin-eosin (H&E) stains.

Tissue Processing for NGS Molecular Analysis

An H&E-stained slide and eight 4-μm unstained slides were submitted for molecular analysis. The H&E-stained slide was reviewed by an attending pathologist who circled the area of tumor and provided an estimate of tumor cellularity. Macrodissection of the circled area was performed and DNA was extracted from unstained formalin-fixed, paraffin-embedded slides with a minimum tumor cellularity of 10%. DNA was quantified using the PicoGreen method (Thermo Fisher Scientific). Bar-coded libraries were prepared using 10 ng of DNA; sequencing was performed on the Ion Torrent PGM (318 chip; Life Technologies, Rockville, Maryland). Variants were identified using the Ion Torrent Variant Caller Plugin and reference genome hg19. SVS software (Golden Helix, Bozeman, Montana) was used for annotation was prediction of the significance of the variants.[33]

Baseline

Multiple cell blocks were prepared with 2 core biopsies per cassette/block. For cell blocks, if the technician determined that the amount of tissue was abundant, multiple cassettes/blocks were made. The H&E-stained sections were reviewed by the pathologist and the block with maximal amount of tumor identified was used for molecular testing.

We measured adequacy rates (number of cases adequate for molecular testing/total number of cases tested) at baseline, following change 1, and following change 2 for CT-guided cases and for endobronchial/endoscopic ultrasound–guided cases. We decided to focus on variables within the laboratory that we could control. Preanalytic variables, including patient selection, gauge of needle used for the biopsy, radiologist/pulmonologist level of expertise (trainee versus radiologist), and specimen characteristics (fibrotic/necrotic, low tumor volume, etc), were beyond the control or the scope of this pathology QI project. Data collected from the LIS included type of procedure (CT/FNA), number of passes performed, pathologist who signed out the case, number of immunohistochemical (IHC) markers studied, tumor content on block used for NGS studies, and whether the sample was adequate for molecular testing or not.

Process Change 1

This change (instituted week of September 11, 2014; data collected September through December 2014) was based on expert consensus among the 4 cytopathologists. Two senior cytopathologists had noted that in the past, when all the material was collected as one block, the block (cell block/tissue block) showed abundant, concentrated material. Thus, we would (1) use one block with all tissue in it to concentrate tumor instead of 2 or more blocks (regardless of the number of NCBs or amount of FNA material); and (2) order IHC and molecular diagnostics when the pathologist reviewed the H&E–stained section of the block.

Process Change 2

This change (instituted week of December 11, 2014; data collected December 2014 through May 2015) was a modification of change 1 and was based on expert consensus opinion from the laboratory personnel. It included ordering unstained slides for molecular studies at the time of determining specimen adequacy. This order by the cytopathologist would then initiate precutting slides up front by histology. The first 12 precut slides would be used for molecular diagnostics, the 13th slide for H&E, and the next 10 slides for IHC. Slides for molecular testing would have multiple sections on each slide. The intent was to concentrate the DNA and not waste any tissue while avoiding refacing the block.

Monitoring

After the process improvement had been put into place, the change was monitored from May through December 2015 to ensure expectations were still being met.

RESULTS

Review of Processes

Figure 1 shows a flow chart of the process at baseline for CT-guided NCB collection (using a 20-g needle) by the intervention radiologist and preparation by the pathology technician and histology staff followed by sign-out by the cytopathologist. Table 1 provides more details of this process map. At baseline some pathologists ordered molecular testing at the time of ordering IHC whereas others ordered it after finalizing the case. Figure 2 shows a detailed analysis of the process for CT-guided NCB, prior to any changes from collection of the specimen at ROSE to sign out of the case.

The process differs a little for FNA cases where the pulmonologists perform EBUS-guided FNA of central lung masses or lymph nodes to stage and diagnose the patient. Smears were made on site followed by adequacy assessment using telepathology.[42] Needle rinses and additional passes were collected in a transport tube in normal saline and brought to the laboratory and processed without any delays. A formalin-fixed, paraffin-embedded cell block was made for each case from the concentrate of the material collected in normal saline. Sections from the cell block were used for IHC and molecular testing. Figure 2 shows a cause-and-effect diagram that was made to determine reasons for inadequate samples.

At baseline, 51 cases collected by CT-guided NCB and touch imprint cytology between January and August 2014 were analyzed by NGS, and 35 cases (69%) were adequate for molecular studies (Table 2). During the same period, 28 cases were collected by FNA and 25 of these cases (89%) were adequate for molecular studies (Table 3). The FNA samples were consistently more adequate for molecular studies than the CT-guided NCB samples.

We looked at multiple variables comparing adequacy rates with tumor content, number of passes, and number of IHC stains ordered (Tables 2 and 3). Numerous inconsistencies were discovered in the count of cores (some pathologists

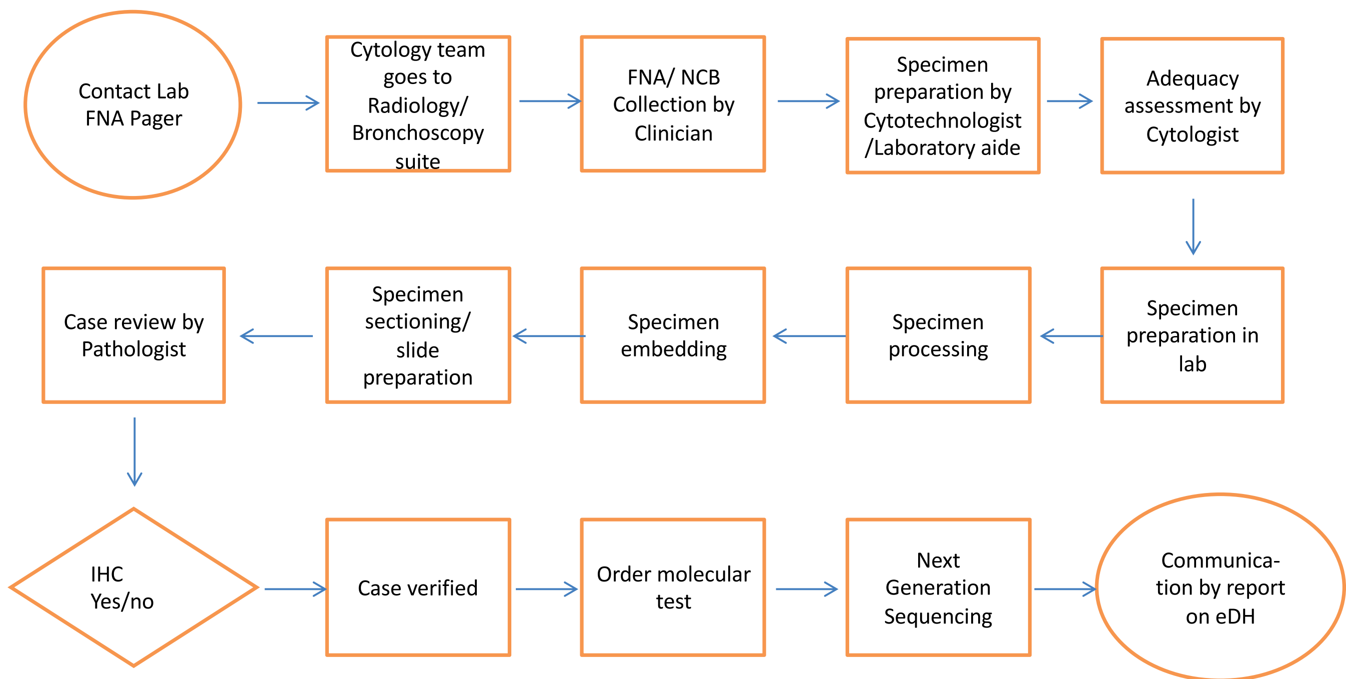

Figure 1. *Process map of cytology specimens collected by rapid on-site specimen evaluation and submitted for molecular testing prior to any changes in the process. Abbreviations: eDH, Dartmouth-Hitchcock's electronic medical record; FNA, fine-needle aspiration; IHC, immunohistochemistry; NCB, needle core biopsy.*

counted only the passes and some counted each core) and number of IHC tests ordered. These parameters were deemed important and will be pursued as separate QI projects.

At baseline (Tables 2 and 3), the average tumor content on the slide (as determined by the pathologist) for cases that were adequate for molecular testing was 36.7% (range, 20%–80%) for CT cases and 45% (range, 10%–80%) for FNA cases whereas it was 28.4% (range, 10%–75%) and 33% (range, 30%–40%), respectively, for cases inadequate for molecular testing. Following change 1, adequacy rate decreased for both CT and FNA cases to 50% (5 of 10) and 81% (13 of 16) from a baseline adequacy rate of 69% (35 of 51) and 89% (25 of 28) for CT and FNA cases, respectively. This phase of the study had not included all the people responsible for the specimen. Brainstorming for change 1 was initially performed by the 4 cytopathologists, and the histology supervisor had not been involved with this process. Prior to change 2, technical expertise was sought and all the stakeholders were present. One of the options we considered for the decreased adequacy following change 1 was the possibility of tissue loss from facing into the block multiple times. Following change 2, adequacy rate increased to 94% (17 of 18) for CT cases and 94% (30 of 32) for FNA cases. By this time, the project was becoming well known in the department, with multiple aspects of the laboratory joining in. It is likely that involvement of the supervisor and the technical staff, in

Table 2. Computed Tomography–Guided Needle Core Biopsy Specimen Adequacy for Next-Generation Sequencing Testing at Baseline (Before Process Change) and Following Process Changes 1 and 2[a]

	Baseline (January–August 2014)	After Process Change 1—Week of September 11, 2014 (September–December 2014)	After Process Change 2—Week of December 11, 2014 (December 2014–May 2015)
Total No. of cases	51	10	18
Inadequate/insufficient cases, No. (%)	16 (31)	5 (50)	1 (5)
Adequate cases, No. (%)	35 (69)	5 (50)	17 (95)
Average No. of IHC stains (range)			
Adequate cases	3.4 (0–7)	1.2 (0–5)	7.3 (1–13)
Inadequate cases	2.8 (1–8)	1.2 (1–2)	7
Average number of passes (range)			
Adequate cases	4.17 (2–9)	5.8 (5–6)	6 (5–8)
Inadequate cases	5.5 (3–9)	3.5 (0–6)	5
Average tumor content on block, % (range)			
Adequate cases	36.7 (20–80)	37.5 (10–60)	30 (30–60)
Inadequate cases	28.4 (10–75)	43.3 (20–70)	30

Abbreviation: IHC, immunohistochemistry.

[a] Percentages have been rounded up for whole numbers.

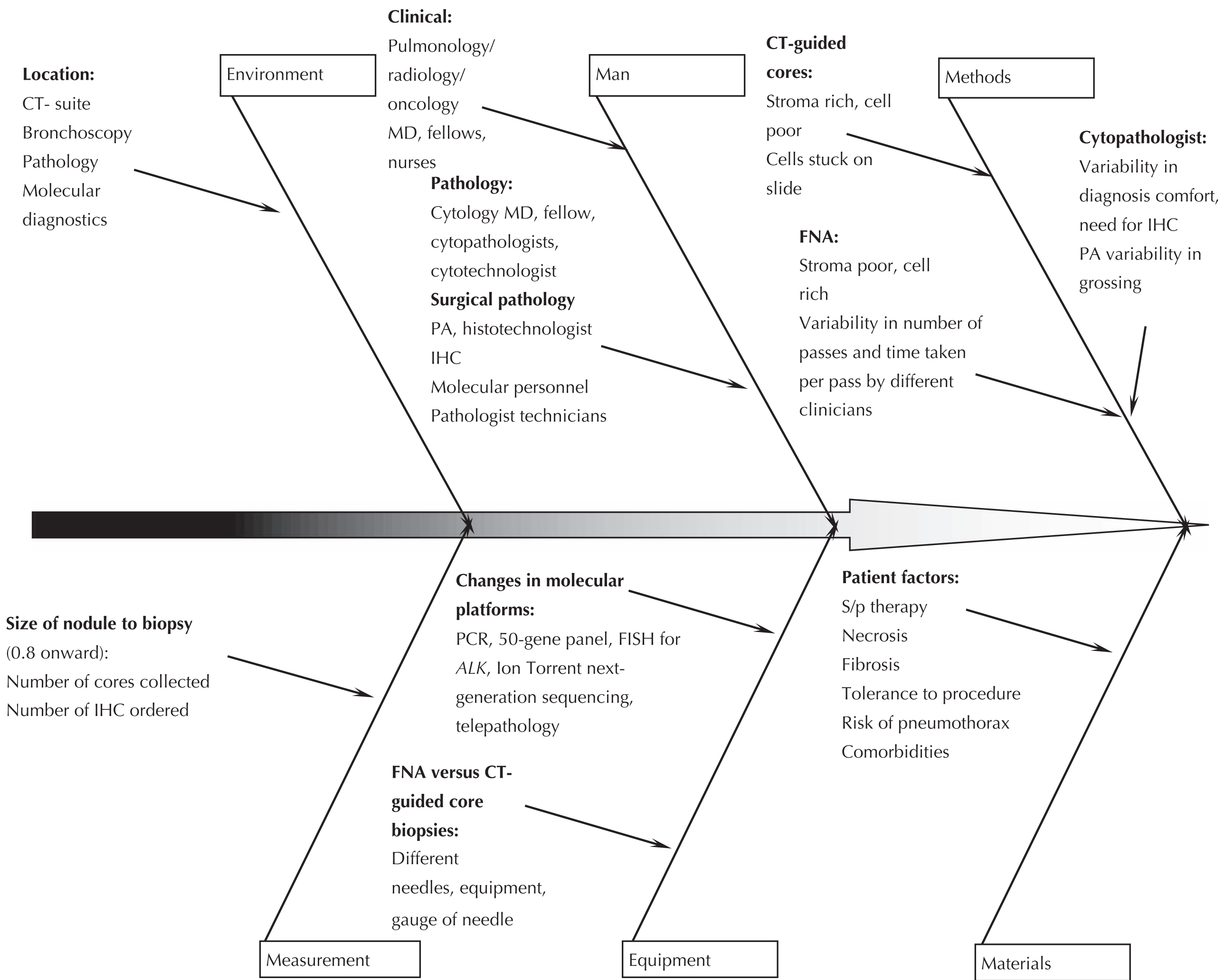

Figure 2. *Use of cytology (cell block from fine-needle aspiration [FNA] and tissue blocks from skinny needle core biopsies) material for molecular testing—reasons for inadequate samples cause-and-effect fishbone diagram. Abbreviations:* ALK, *anaplastic lymphoma kinase; CT, computed tomography; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; PA, pathologists' assistant; PCR, polymerase chain reaction; S/p, status post.*

Table 3. Fine-Needle Aspiration Specimen Adequacy for Molecular Testing at Baseline (Before Process Change) and Following Process Changes 1 and 2[a]

	Before Change (January–August 2014)	After Change 1 (Week of September 11, 2014)	After Change 2 (Week of December 11, 2014)
Total No. of cases	28	16	32
Inadequate/insufficient cases, No. (%)	3 (11)	3 (19)	2 (6)
Adequate cases, No. (%)	25 (89)	13 (81)	30 (94)
Average No. of IHC stains (range)			
Adequate cases	3.4 (1–8)	2.3 (0–5)	7 (1–8)
Inadequate cases	5.6 (5–7)	7.6 (1–16)	2 (2)
Average No. of passes (range)			
Adequate cases	4.6 (2–8)	3.8 (3–6)	7 (1–7)
Inadequate cases	2.75 (1–4)	4 (2–6)	4 (4–4)
Average tumor content on slide, % (range)			
Adequate cases	45 (10–80)	46.25 (15–70)	81 (10–90)
Inadequate cases	33 (30–40)	22.5 (10–30)	20 (10–30)

Abbreviation: IHC, immunohistochemistry.

[a] Percentages have been rounded up for whole numbers.

addition to concentration of all the sample on one block, helped impart the importance of being extremely careful while processing minute specimens.

Monitoring

Between the time that change 2 was instituted and December 2015, 212 samples that were collected by ROSE had the unstained slides ordered at the time of evaluation of specimen adequacy. Of these, 61 went on to have molecular analysis performed. Five cases had an insufficient amount of material to complete sequencing (4 cell blocks and 1 CT-guided CNB), for an overall adequacy of 92% (56 of 61).

DISCUSSION

Using NGS for clinical molecular testing is still relatively new in the United States. However, because our institution is a tertiary referral center, many patients are seen for primary diagnosis of lung masses or for targeted therapy. The cytopathologist is intimately involved in the diagnosis of these patients, as cytology is used extensively for this purpose. Accuracy for the classification of lung carcinoma, especially subtyping non–small cell lung cancer on cytology specimens, was 100% when combined with IHC in a study by Rekhtman et al.[9] Cytopathologists at our institution also use IHC markers to confirm their morphologic findings, and because these samples are often sparse, this exhausts much of the specimen if the block is refaced multiple times. In this QI project we maximized the use of the scant tissue by establishing a protocol that limits the number of times a block is cut, creating reserve unstained slides up front, thus allowing for preservation of adequate material for IHC and molecular testing. Concentrating all the tissue in one block and concentrating the specimen by having multiple sections on each slide submitted for molecular testing showed a trend in the right direction with improved adequacy rate.

We created a cause-and-effect fishbone diagram that identified several factors that pay into the adequacy of a sample: small size of the nodules and patient characteristics including prior therapy, necrosis, and/or fibrosis need to be considered in the reasons for inadequate samples. Though information on the size of the nodule, experience of the radiologist performing the procedure, etc, was not collected in this study, these are clearly important factors that may yield an inadequate sample.

At baseline, we identified that refacing the block multiple times for baseline H&E sections followed by IHCs at a later date and then followed by molecular testing was a significant source of waste. Process change 1, where we just concentrated all the cores into one block and ordered molecular testing up front, did not produce the desired results, as the block was being refaced when these were ordered at a later date after the initial H&E section. Process change 2 slides produced the desired results with a 90% specimen adequacy, which was our goal. Although precutting slides could be perceived as waste, this was felt to be an acceptable trade-off as compared with bringing back patients for additional invasive procedures, especially because most of the cases were now adequate for molecular testing; the precut slides were being used fully in the vast majority of the cases. Also, when cytopathologists perform ROSE, they have a fairly good idea of the tumor type and they can triage a case by ordering precut slides only when they think they are dealing with an ADC/poorly differentiated carcinoma. This, we felt, would help minimize additional work for the histotechnologists and minimize wastage. Ongoing monitoring of the systems shows consistently adequate samples to date.

During the study period, FNA samples were consistently more adequate for molecular testing than CT-guided NCB samples, as seen in Tables 2 and 3, unlike the study by Schneider et al.[32] However, this wasn't consistent throughout the current monitoring period. Many variables are likely to play a role, including type of lesion (central for FNA samples versus NCB); operator experience (fewer physicians at our institution perform FNA compared with NCB); and the material itself (FNAs are typically stroma poor and cell rich because of the nature of the procedure, which actively aspirates the cells, whereas NCB yields cells and the associated stroma).

One limitation of this study is that we evaluated only the intradepartmental processes within the pathology department. The reasons for an inadequate sample are many, as seen in the cause-and-effect diagram (Figure 2). It was beyond the scope of this project to examine the lung biopsy process in radiology, clinical decision making of patient selection, etc.

In summary, this study highlights the complexity of processes in laboratories. It was not determined up front that changing the molecular platform from polymerase chain reaction–based tests to NGS by the molecular department would be associated with decreased sample adequacy. The change made by just the cytopathologists (change 1) did not produce the desired result. Involvement of all the stakeholders and possibly the increasing amount of tissue collected (because the project was getting well known by then) likely helped in the success of this project. By concentrating all the material on one block and by ordering precut slides for IHC and molecular testing up front, we now ensure that no tissue is wasted in the process of cutting the formalin-fixed, paraffin-embedded block. We have eliminated refacing the block. In the baseline process the cell block was initially cut for an H&E section, followed by refacing the block for IHC, followed by again refacing and cutting for molecular testing, frequently exhausting the tissue by this time. There are currently no studies that have considered these variables for testing on the NGS, especially when dealing with scant tissue from 20-g NCB. Use of NGS and molecular testing, especially when it is therapeutically required, is only going to increase over time. Having a process, monitoring it routinely, and communication are key to successful testing.

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