Original Article

Evaluation of automated sample preparation system for lymph node sampling

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Background: Endobronchial ultrasound-guided transbronchial fine needle aspiration (EBUS-FNA) has revolutionized the diagnostic and staging approach to non-small cell carcinoma and thoracic lymphadenopathy. However, obstacles to efficacy of rapid on-site evaluation (ROSE) of the samples include variability in sample quality and slow and cumbersome process in the procedure room leading to extended procedure time. The purpose of this pilot study was to evaluate the feasibility and specimen quality of lymph node biopsies prepared through a novel automated system for automated fixation, drying and staining compared to standard slide preparation method.

Methods: We performed a prospective, single-center pilot feasibility study of patients undergoing EBUS. Samples were split into conventional standard of care (SOC) slide preparation and preparation using the device ("instrument"). Pathologists compared the SOC slides to the slides prepared by the automated system and assessed the following metrics: nuclear and cytoplasmic quality, presence of debris/artifact, staining quality, creation of a monolayer, and ease of adequacy/diagnosis assessment. A score between 1 (lowest quality) and 3 (highest quality) was assigned to the above metrics.

Results: Sixty patients were recruited. One to three lymph nodes were sampled for each patient for a total of 72 samples collected. The mean scores of each assessment category showed no statistical difference between the two preparation techniques except for improved monolayer creation in the instrument samples. Thirty of thirty-one (96.8%) paired samples in the final analysis showed diagnostic equivalency between the automated slides and conventional slides; the discordant pairing was reported to be suspicious on the instrument sample and atypical on the SOC.

Conclusions: Study results suggest that slides prepared by the automated system are of adequate quality for adequacy assessment with diagnostic concordance when compared to SOC slides.

Keywords: Lung cancer; biopsy; rapid onset evaluation; pathology; bronchoscopy

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Introduction

Background

Endobronchial ultrasound-guided transbronchial fine needle aspiration (EBUS-FNA) has revolutionized the diagnostic and staging approach to non-small cell carcinoma and thoracic lymphadenopathy. Studies highlighting the optimal parameters for diagnostic accuracy including the ideal number of passes and collected samples/slides subsequently followed (1,2). Similarly, the complementary introduction of rapid on-site evaluation (ROSE) in which aspirated samples are stained and screened for cytologic diagnostic tissue during the procedure further improved the diagnostic process. The ROSE method aids in assessment of sample adequacy and provides a preliminary diagnosis based on the morphologic criteria which can help prioritize the additional samples for adjuvant testing such as microbiology culture and flow cytometry analysis (3).

Knowledge gaps

ROSE has been shown to avert additional sampling without reducing diagnostic yield which likely leads to reduced procedural risk and improved patient care (4). Overall reduction in tissue sample processing and laboratory resources are financially advantageous despite limited impact on procedural time (4-8). Despite these advances, challenges of ROSE include: (I) high variability in sample quality; (II) cumbersome process in the procedure room; and (III) slow specimen preparation leading to extended procedure time.

We report the results of a novel automated sample preparation system by ASP Health (ASP Health, Chicago, IL, USA) that combines both specimen deposition and staining in a compact/mobile unit (*Figure 1*). The ASP device is an instrument that distributes cells from a FNA sample onto a glass slide and stains in less than two minutes. The automated system uses an integrated heating strip to heat the specimen slide and a series of pumps to dispense milliliters of fresh stain reagent onto the slide to stain the cells. Currently, the system is configured to perform modified Giemsa staining, which is commonly used in the ROSE process. The device can also be used to stain other samples such as forceps biopsy. It does not stamp the biopsy tissue on the slide and this needs to be completed manually before insertion of the slide for staining. The device requires low maintenance with a once daily priming protocol that cleans the machine of retained reagents from the day before (that can be done prior to the procedure) and refilling reagents after 20–30 specimens.

Objective

The purpose of this study was to evaluate the feasibility and specimen quality of lymph node biopsies prepared through a novel automated system for automated fixation, drying and staining ("instrument" slide) compared to standard slide preparation method ("SOC" slide). We hypothesize a diagnostic equivalency of greater than 90% between the instrument and conventional preparation method will occur. We present this article in accordance with the SPIRIT reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-81/rc).

Highlight box

Key findings

- Rapid on-site evaluation (ROSE) slides prepared by the automated system are of quality for adequacy assessment with diagnostic concordance when compared to slides prepared by conventional methods for endobronchial ultrasound lung specimens.

What is known and what is new?

- ROSE has been shown to avert additional sampling without reducing diagnostic yield which likely leads to reduced procedural risk and improved patient care. Challenges of ROSE include high variability in sample quality, cumbersome process in the procedure room, and slow specimen preparation leading to extended procedure time.
- Automatic slide preparation may improve workflow of sample preparation and assessment for bronchoscopy procedures.

What is the implication, and what should change now?

- Automatic slide preparation may improve procedural efficiency at institutions with limited resources.

Methods

Study cohort

This was a prospective, single-center pilot study to evaluate the clinical utility and early performance of a novel automated sample preparation system from ASP Health for patients undergoing EBUS sampling of thoracic lymph nodes at an academic institution. The primary outcome was diagnostic equivalency while the secondary outcome was procedural time. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The

Figure 1 ASP device.

study was approved by the institutional ethics board of Mayo Clinic (No. 21-010367, approved 11/16/2021) and informed consent was taken from all individual participants. Patients were enrolled between January 31, 2022, and May 11, 2022. Consecutive patients who met the inclusion and exclusion criteria and provided consent were enrolled in this study.

Study data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at Mayo Clinic (9,10). REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (I) an intuitive interface for validated data capture; (II) audit trails for tracking data manipulation and export procedures; (III) automated export procedures for seamless data downloads to common statistical packages; and (IV) procedures for data integration and interoperability with external sources.

Procedural description

At our institution, EBUS-FNA is performed with a combination of general and local anesthesia using propofol and lidocaine in a dedicated bronchoscopy suite. The Olympus BF-UC180F Bronchoscope (Olympus America, Center Valley, PA, USA) is utilized for EBUS. After bronchoscopic airway inspection, the EBUS bronchoscope is inserted and the mediastinal and hilar lymph nodes are examined visually, dimensions recorded, and sampled if indicated. A 22G ViziShot needle (Olympus America) is used for lymph node sampling via the slow-pull capillary technique for specimen collection. For this study, one to three lymph node per patient were selected to undergo each sample preparation pathway. Additional preparation of lymph node stations via the automated system after the first specimen was at the discretion of the proceduralist. Completion of the procedure in the usual manner was continued after the study sample was obtained.

Standard slide preparation method

Slide preparation and ROSE are performed in the bronchoscopy suite. The material collected from EBUS-FNA is deposited on a glass slide with the remaining tissue flushed into a container for additional assessment post-procedure in the pathology lab. The slide is then smeared with a second slide to create two direct smears. One slide is fixed rapidly with 95% alcohol for permanent cytological assessment using Papanicolaou stain. The second slide is stained promptly with modified Giemsa stain and evaluated by a cytotechnologist in the bronchoscopy suite. The cytotechnologists rotate through the bronchoscopy suite according to a predetermined schedule and availability at the time of the procedure.

Study slides

Stage I

After the determination of cellular adequacy by a cytotechnologist through conventional methods described above, the study slide was then obtained. The next pass taken from the patient was split into conventional standard of care (SOC) slide preparation (described above) and preparation using the device (instrument). An alteration of sample preparation was pursued after our pathology team found discordant material between the two groups (first 24 patients, representing 28 samples). This was not stipulated in the initial protocol but rather the protocol properly revised with this finding.

Stage II

After realizing that the two preparation methods (SOC and instrument) were resulting in two different qualities of cellular material, a step of homogenizing the entire sample in the Eppendorf tube (Eppendorf, Hamburg, Germany) with the buffering media using a pipette was added to the protocol, prior to splitting the sample. This ensured that the material was equally distributed between the standard slide preparation and the preparation using the instrument (*Figure 2*). The IRB was updated regarding the deviation from the original protocol.

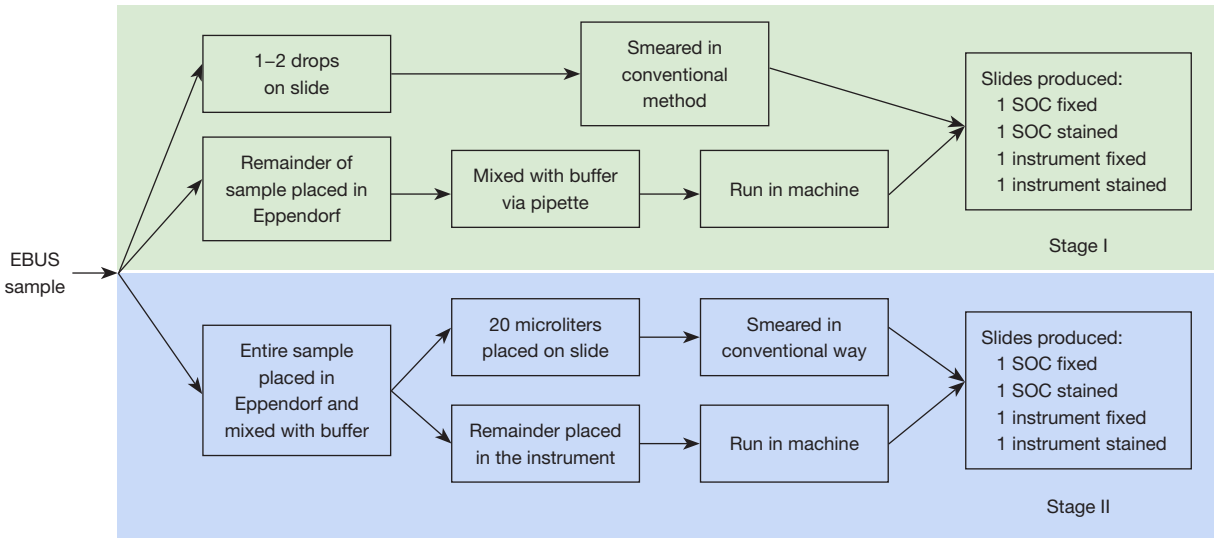

Figure 2 Workflow of sample preparation. EBUS, endobronchial ultrasound; SOC, standard of care.

Time measurement

Time of sample preparation using both methods was recorded. The SOC time included the time from sample deposition on the slide until first evaluation by the cytotechnologist (not including the evaluation time). The study instrument time included the time from collection into the Eppendorf tube to the completion slide creation by the machine.

Pathology assessment

Two pathologists compared the SOC slides to the slides prepared by the automated system and assessed the following metrics independently: nuclear and cytoplasmic quality, presence of debris/artifact, staining quality, creation of a monolayer, and ease of adequacy/diagnosis assessment. A score between 1 (lowest quality) and 3 (highest quality) was assigned to the above metrics. Nondiagnostic samples were excluded from analysis in this study. One pathologist reviewed each pair of SOC and instrument slides to account for potential differences in evaluation between pathologists.

Consideration of the data from stage I and stage II for analysis

During the study period, the protocol was revised to account for the discordant material that we discovered on the SOC and instrument slides. We pursed establishing a protocol with homogenizing the sample prior to splitting the sample to ensure reproducibility of future results. However, we recognize that this change did not contribute to the alteration of certain study measurements including individual slide scoring of those slides deemed adequate for complete assessment and the variable of time. Therefore, we report the results of both stages of the protocol for the slide assessment and time but only assess diagnostic equivalency for those slides produced after the protocol was revised.

Statistical analysis

Categorical variables were summarized as frequency (%) and compared using chi squared test. Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student's *t*-test or paired Student's *t*-test when appropriate. A P value <0.05 was considered statistically significant. All statistical analysis was performed using BlueSky Statistics software (BlueSky Statistics LLC, Chicago, IL, USA) and R Studio Integrated Development Environment (Boston, MA, USA).

Results

Sixty patients were recruited. One to three lymph nodes were sampled for each patient for study purposes for a total of 72 paired samples collected. All samples were sent to the pathologist for slide grading and determination of diagnostic equivalency.

Table 1 Scoring of sample preparation using SOC and instrument methods for all diagnostic samples

Category	Instrument (N=41), n (%)	SOC (N=51), n (%)	P value
Nuclear detail and quality			0.707
Bad	4 (9.8)	4 (7.8)	
Intermediate	15 (36.6)	23 (45.1)	
Good	22 (53.7)	24 (47.1)	
Cytoplasmic detail and quality			0.908
Bad	4 (9.8)	4 (7.8)	
Intermediate	13 (31.7)	18 (35.3)	
Good	24 (58.5)	29 (56.9)	
Amount of debris/artifact			0.134
Bad	3 (7.3)	0 (0.0)	
Intermediate	9 (22.0)	14 (27.5)	
Good	29 (70.7)	37 (72.5)	
Monolayer			0.025
Bad	1 (2.4)	6 (11.8)	
Intermediate	13 (31.7)	25 (49.0)	
Good	27 (65.9)	20 (39.2)	
Staining			0.917
Bad	1 (2.4)	2 (3.9)	
Intermediate	24 (58.5)	30 (58.8)	
Good	16 (39.0)	19 (37.3)	
Ease of adequacy assessment			0.923
Bad	1 (2.4)	2 (3.9)	
Intermediate	5 (12.2)	6 (11.8)	
Good	35 (85.4)	43 (84.3)	
Ease of diagnosis			0.766
Bad	1 (2.4)	2 (3.9)	
Intermediate	4 (9.8)	7 (13.7)	
Good	36 (87.8)	42 (82.4)	

SOC, standard of care.

In stage I of the study, discrepancy seen in the material on the study slide versus conventional preparation sample were seen which led to a change in the method of slide preparation. In stage I, 27 paired samples were collected from 24 patients. Of these samples, 10 instrument samples were nondiagnostic, 14 samples (7 pairs) were nondiagnostic for both SOC and instrument, and 1 sample was nondiagnostic for the SOC but not the instrument. Nine paired samples remained for assessment of diagnostic equivalency after removal of the 18 nondiagnostic pairs.

In stage II, 45 paired samples were collected from 36 patients. One sample was nondiagnostic for the instrument slide but not the SOC. Thirteen pairs of samples were both nondiagnostic in the SOC and instrument slide. The 14 paired nondiagnostic samples were removed, and the remaining 31 pairs were analyzed for diagnostic equivalency. Of the 31 paired diagnostic samples, 21 were definitively benign.

Table 1 shows the scores of each assessment category for the entire dataset (from both stages I and II) after removal of nondiagnostic samples. Scores for both SOC and instrument samples were analyzed for how often each numerical score was graded. Statistical significance was only reached in the category of monolayer creation in which the instrument sample had 65.9% of the samples graded as "good" versus 39.2% of the SOC samples graded similarly. Otherwise, the instrument and SOC samples were similarly graded in nuclear and cytoplasmic quality, presence of debris/artifact, staining quality, and ease of adequacy/diagnosis assessment.

Analysis of only benign samples for both stage I and II showed consistency with the larger dataset of the monolayer performing statistically higher in the instrument samples (P=0.025). Malignant/atypical/suspicious samples from both stage I and II showed similar monolayer between the two groups but improved staining quality of the instrument slide (66.7% instrument slides rated "Good" *vs.* 7.7% of SOC slides, P=0.012).

All 72 samples were sent to the pathologist for slide grading and determination of diagnostic equivalency. However, after the review described above, only 9 paired samples from stage I of the study could be used to assess diagnostic equivalency. Given this same size and the alteration of the technique, this data was not felt to not be strong enough for meaningful analysis.

After the protocol was revised for stage II with sample homogenization prior to splitting the material, the remaining samples were congruent in quality. From stage II, 31 paired samples were generated for assessment of diagnostic equivalency. The diagnostic equivalency was 96.8%, with the nonequivalent pairing being one which was reported to be "suspicious" on the instrument sample

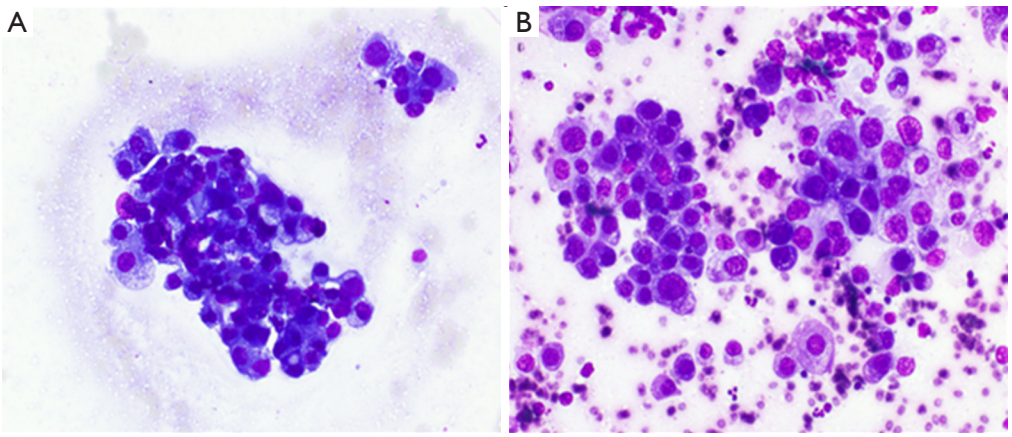

Figure 3 Standard of care slide (A) (×40, modified Giemsa stain), instrument slide (B) (×40, modified Giemsa stain). Both slides diagnosed as adenocarcinoma. Both slides scored with good ease of ROSE interpretation and good ease of final diagnosis. The instrument slide judged by pathologist to have better cellular detail and staining. ROSE, rapid on-site evaluation.

and "atypical" on the SOC. Examples of each staining preparation style are provided in *Figure 3*.

The mean time of sample preparation of the SOC slides was 147.7 (SD 58.2) seconds which included the time from sample acquisition, smearing, and staining prior to the technologist's review. The instrument sample's mean preparation time was 48.3 (SD 21.9) seconds which included the sample collection, preparation, and loading into the machine. The machine automatically distributed the sample on the glass slide, dried the sample, and stained it to create a final sample for evaluation. The total time from sample acquisition to preparation of a slide ready for evaluation was 204.1 (SD 30.7) seconds for the instrument samples compared to 147.7 (58.2) seconds for the SOC method ($P<0.001$).

Discussion

While the use of ROSE for EBUS-FNA has been shown to improve the diagnostic accuracy of the procedure, high variability in sample quality may limit its impact. Our study illustrates the novel, automated system produced adequate quality for adequacy assessment with diagnostic concordance of all samples except for one. In the categories of nuclear and cytoplasmic quality, presence of debris/artifact, staining quality, creation of a monolayer, and ease of adequacy/diagnosis assessment, the instrument samples were rated highly, although not statistically significant, apart from the monolayer creation. The instrument utilizes a unique method of spraying the specimen on the glass slide which creates a thin monolayer on the glass slide. This is different from a traditional smearing process which applies pressure between the two glass slides to make a smear. Hence, this is likely the reason for the samples prepared by the instrument yielding a higher percentage of monolayer cells compared to the traditional smearing process.

A significant finding from this study was that the material collected in the hollow needle from an EBUS procedure is not identical throughout the needle shaft. For the first 24 patients, the first few drops of cellular material (those last collected) were first plated in the SOC method, and the remaining sample material was used to generate the instrument slide. After feedback from our pathology group, we realized that the higher quality material was seen on the SOC slides while blood was mainly seen in the instrument slides. This led to an altered approach and revision of our protocol to homogenize the sample with the buffering material prior to the sample being split into two slides for evaluation. The remaining samples were prepared in this manner.

It is important to note that ROSE may not be available at institutions with limited resources. Dedicated and consistent procedural suites with an established area for sample preparation may not be reliable at all centers leading to a cumbersome process with slow specimen preparation and extended procedure time. The instrument's samples compact silhouette can easily be incorporated in most areas and can be placed on a cart for transport between different procedural suites, if needed, as was done during this study. The milliliters of reagent deposition allow multiple

days of procedures to be completed without the material being replaced. At the time of this publication, the study instrument retails for 15,000 USD. More detailed analysis regarding the cost-benefit of using the device for specimen preparation is needed.

Elzamly *et al.* highlights that performing ROSE can be challenging and stressful to cytotechnologists with limited time between passes to not only prepare and stain the slides but also review the cytology slides (11). While the mean time of sample preparation of the SOC slides was shorter than the mean combined time of preparation and machine run time of the device (147.7 *vs.* 204.7 seconds), the machine removes the work of slide sample deposition and staining, allowing time for the cytotechnologist to review slides without additional, competing responsibilities.

Our study has several notable limitations including that samples were only obtained from lymph nodes. Further evaluation of the system with different tissue types (i.e., pulmonary nodules) would be needed to assess sample quality prior to full use in our bronchoscopy suite. Additionally, our institution has had a robust ROSE practice in place for many years with highly efficient cytotechnologists who perform sample preparation and evaluation. The time for sample in the standard workflow may have been influenced by significant experience compared to the novel preparation of the instrument. For this reason, we performed a noninferiority study.

Conclusions

Our study results suggest that slides prepared by the automated system are of noninferior quality for adequacy assessment with diagnostic concordance when compared to SOC slides. With EBUS-FNA used as a minimally invasive, cost-effective procedure for diagnosis and staging of lymphadenopathy complemented by ROSE, the novel automated system may improve workflow of sample preparation and assessment for bronchoscopy procedures.

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Footnote

Reporting Checklist: The authors have completed the SPIRIT reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-81/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-81/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-81/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-81/coif). AB is an employee of ASP Health. HS is the co-founder and shareholder of ASP Health. JR received an internal grant and equipment loan for this study from ASP Health. The grant was an intramural career development award given to JR to further her research in bronchoscopy. The grant covers internal costs associated with publication fees, statistical costs, and IRB-related costs to facilitate career development for physician scientist investigators. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics board of Mayo Clinic (No. 21-010367, approved 11/16/2021) and informed consent was taken from all individual participants.

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