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Journal of the American Society of Cytopathology (2022) **11**, 375–384

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

Liquid-based rapid onsite evaluation of endobronchial ultrasound cytologies

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Received 3 June 2022; received in revised form 15 June 2022; accepted 7 July 2022

KEYWORDS
Liquid-Based ROSE;
EBUS;
Validation;
Performance

Introduction Rapid onsite evaluation (ROSE) generally uses smears made at the site of the procedure ("smear-based ROSE"). It requires considerable time, generally 2 individuals, technical expertise, and it can be difficult to estimate material available for ancillary studies. We developed an alternative ROSE using liquid-based cytology ThinPrep with hematoxylin and eosin (H&E) stain ("liquid-based ROSE") and assessed its advantages.
Materials and methods Clinicians rinse the sample(s) into CytoRich Red and send to Pathology. A defined proportion of the needle rinse is removed for a ThinPrep stained with a rapid H&E. Adequacy and diagnosis were compared to final outcome. Total time was recorded.
Results Among 52 liquid-based ROSE readings, 28 (53.8%) were interpreted as "adequate" with final as adequate; 17 (32.7%) were interpreted as "inadequate" with final as inadequate; 7 (13.5%) were interpreted as "inadequate" with final as adequate. Of 23 readings provided with onsite diagnosis, 15 (65.2%) were interpreted as definitive positive or negative diagnoses; 6 (26%) were interpreted as nondiagnostic; and 2 (8.7%) were interpreted as atypical. All definitive diagnoses were concordant with final diagnoses. The time for liquid ROSE performance ranges from 6 to 22 minutes (mean: 13 minutes) and required only 1 individual.
Conclusions Liquid-based ROSE allows accurate adequacy determination and diagnosis, takes about 15 minutes of cytologist time, and can be performed by just 1 person. The technique produces well-preserved and stained slides, it may allow a better estimation of the total amount of material in the specimen vial and may provide a better platform for telecytology.

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https://doi.org/10.1016/j.jasc.2022.07.004

Introduction

Lung cancer is the most common cancer and the leading cancer-related cause of death in both sexes in the United States.[1] Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is emerging as the safest[2] and most efficient tool for diagnosis and staging. It is a minimally invasive procedure for obtaining a cytology sample, using ultrasound to visualize structures near airways with or without radiofrequency-guided navigation. Rapid onsite evaluation (ROSE) is an important component in cytology, and its usage is highest in EBUS-TBNA among all procedures.[3] The overall goal of ROSE is to help ensure that a patient only needs 1 procedure for a minimally invasive, complete diagnostic evaluation.[4-8] The methodology of ROSE is largely similar among institutions: material from EBUS-TBNA needles or brushes are expressed onto a glass slide for making a direct smear, either air-dried or alcohol-fixed. We therefore refer to this technique as "smear-based" ROSE.

There are problems with ROSE. First, it is time-consuming. In a 2019 American Society of Cytopathology survey on the use of ROSE,[3] 86% of 261 cytotechnologists and 53.9% of 255 cytopathologists reported a typical ROSE procedure requires more than 30 minutes; 40.3% and 13.5%, respectively, reported a typical ROSE procedure requires more than 45 minutes. The smears must be made near the room where the procedure is taking place, precluding the ability to do other work. Second, 2 people may be needed for each procedure[3] since the time to collect a sample can be faster than the ability to prepare, stain, and read the smear. Third, smear-based ROSE can generate more readings than are clinically actionable. This is because material in the needle or brush must immediately either be made into a smear or transferred to a stable rinse solution. There is not a way to postpone the preparation, because material would clot in the needle or dry on a brush. Fourth, the reimbursement is typically below the costs for a cytopathologist,[9] and a professional fee cannot be charged at all if ROSE is performed by a cytopathology fellow or cytotechnologist. Fifth, it is technically challenging to produce a well-preserved and well-stained smear for ROSE. In our experience, cytologists cannot rely on ad hoc technical support for triaging an appropriate amount of material to smear, creating a relatively thin and well-preserved smear devoid of crushing or air-drying, and staining. These are advanced technical skills that require hands-on training. A sixth problem with ROSE is that ancillary tests often need to be conducted on the material that is NOT evaluated in the actual ROSE process—for example, a cellblock. The evaluation of a ROSE smear provides only a very indirect and poorly quantifiable measure of the material that has NOT been smeared.

A long term-goal of many laboratories is to use telecytology to enable more productive use of cytologists' time, but smear-based ROSE techniques still require use of a skilled technician. Telecytology would seem to confound the issue of being able to assess whether sufficient material has been collected, since the interpreting microscopist would be physically separated from the triaging, and smear-based ROSE necessarily creates multiple slides to examine, which can be laborious for telecytology.

To overcome some of these challenges and potentially improve ROSE, it is first essential to understand its value and quantify performance. Together with clinicians, we identified the following 5 indications for ROSE used in EBUS-TBNA:

(1) Clinicians need to know if they have reached a lesion. Especially with navigation bronchoscopy, it is not always obvious if a pulmonary lesion is within reach of the needle. Atelectasis and post-obstructive pneumonia may camouflage lesions.
(2) Clinicians need to know whether a lesion is yielding diagnostic material with FNA or brushing. Some lesions, especially benign ones, do not shed many cells with the available minimally invasive biopsy devices and require larger or more invasive techniques for diagnosis. Newer microbiopsy devices may help in this regard. Other lesions may be nearly entirely necrotic or yield overwhelming amounts of blood, and a modification of technique can be suggested upon consultation with cytology.
(3) An intraoperative decision needs to be made whether ancillary studies will be needed (eg, flow cytometry, microbiology, immunohistochemistry, or molecular studies).
(4) Clinicians need assurance that a sample has been collected for all indicated ancillary tests.
(5) In some instances, clinicians need to know whether a lesion is definitively malignant or benign. For example, if the sampling does not show a neoplastic process, other sites may need to be sampled. Conversely, if a high station lymph node is positive, then sampling of an apparent primary tumor may not be needed.

Cytotechnologists are generally able to provide an adequacy assessment for the first 4 of these indications. For this paper, we define "Accurate Adequacy" of ROSE as a correct answer to these first 4 clinical questions, based on the final clinical diagnosis and initial treatment plan. For medical legal and scope of practice reasons, cytotechnologists do not provide actionable diagnoses (the fifth indication for ROSE) without cytopathologist backup. The fifth indication for ROSE (diagnosis) is quantified in this paper as "Accurate Diagnosis". Accurate diagnosis is determined based on comparison to the final clinical diagnosis. Samples can theoretically be diagnostic, but not adequate.

In this paper, we develop and validate "liquid-based" ROSE in which all the material from one site is collected into a cytology rinse solution, a controlled amount of material is removed in Pathology and made into a single monolayer preparation that is then interpreted intraoperatively. We chose to develop this method because of the anticipation that the technical aspects of preparing the smear would be streamlined such that no special training would be needed, and a uniform presentation of well-preserved cells would be created. We also anticipated that the technique would allow the pathologist reading the slide to better predict if a sample was adequate for ancillary studies, since a defined proportion of the needle rinse suspension can be removed for preparation of the liquid-based ROSE slide. Finally, we anticipated that a uniformly stained monolayer preparation located in a defined area on the slide would ultimately make liquid-based ROSE more useful than smear-based ROSE for telecytology. We report our validation of liquid-based ROSE and report its performance in the first 52 EBUS-TNBA readings for cytopathologists, cytopathology fellows, and cytotechnologists. Our experience with telecytology on liquid-based ROSE is anticipated in a subsequent publication.

Methods

Institutional review board approval was obtained for this retrospective study (Docket 10,396). The Ion (Robotic-assisted Bronchoscopy) platform (Intuitive, Sunnyvale, CA) was used for all cases in this study. Clinicians performed EBUS-TBNA with the 21- or 23-gauge Flexision needle (Intuitive). Transbronchial brushings (along the fine-needle aspiration [FNA] needle tract) were performed with a Celebrity cytology brush (Boston Scientific, Boston, MA), rinsing the brush directly into fixative. Brushes were rinsed in saline to remove fixative before collecting any additional samples from the same site. The clinicians sample an area of interest and express all the material into one vial of rinse solution. Our laboratory uses 10 mL vials of CytoRich Red (ThermoFisher Scientific, Cincinnati, OH), a fixative that lyses and solubilizes red blood cells and serum proteins, exposing granular whitish particles that generally represent tissue fragments, or turbidity that usually represents a sample of fine particles. The clinicians use visual inspection of the vial together with their judgment about the likelihood that a sample should be adequate based on imaging and other clinical findings. For some clinical scenarios, a defined minimal number of passes is performed. For the cases in this study, clinicians performed a minimum of 3 needle passes and 2 brushing samples and a maximum of 8 FNA passes and 4 brushings. When the clinician has the impression that a sample should be informative, it is sent immediately to Pathology like a frozen section.

Within the Pathology laboratory, a cytotechnologist, the cytopathology fellow, or a cytopathologist inspects the sample vial. If turbid or many fine particles are present, only about 1 mL (10%) is removed by pipette from the vial for ROSE. If the sample appears clear with few particles, 2 mL is removed by pipette from the vial. In either case, large particles and clots are avoided in the sample for ROSE. The 1-2 mL of sample is added to a PreservCyt vial (Hologic Corp., Marlborough, MA) from which 1 to 2 mL of PreservCyt has been removed. A ThinPrep slide (Hologic Corp.) is then immediately prepared using the FNA setting (#2) T2000 instructions (available at: https://www.hologic.com/sites/default/files/package-insert/MAN-02585-001_006_02.pdf). The ThinPrep slide, recovered from 95% alcohol in the processor, is then stained with a rapid hematoxylin and eosin (H&E) staining procedure in disposable jars. The stain setup includes the following 12 stations: Distilled water, Epredia hematoxylin 7211 (ThermoFisher Scientific), Distilled water, tap water, Epredia Bluing 7301 (ThermoFisher Scientific), 95% alcohol, Eosin Y solution (intensified, ThermoFisher Scientific) diluted to 10% with 95% alcohol, 95% alcohol, 100% alcohol (2 stations), Xylene (2 stations), followed by coverslipping (Tissue-tek Glas; Sakura, Torrance, CA). For each staining station, 15 dips are used in and out through the meniscus. For the H&E stains, 1 dip per second is used, whereas the other stations can be faster such that the total staining and coverslipping time is less than 3 minutes.

The slide is then evaluated outside the operating room in Pathology, generally by the individual preparing the ThinPrep slide. Cytotechnologists assess only sample "adequacy". Cytopathologists and the cytopathology fellow (with the immediate availability of a supervising board-certified cytopathologist) provide both adequacy evaluation and a diagnosis. The adequacy and diagnosis are recorded along with the time between receipt of the sample in Pathology and the call back to the clinician. We also recorded the amount removed from the specimen vial and whether the ThinPrep for the liquid ROSE ran "dilute". The specimen is deemed to be "adequate" if the existing sample at the time of ROSE appears sufficient to perform all needed ancillary tests. The adequacy was compared to the final diagnoses. We define an "Accurate Adequacy" of ROSE as a correct answer to questions including whether the clinicians are acquiring a sample, whether ancillary testing is needed, and whether the sample is suitable for all indicated ancillary tests. Not every "adequate" sample was necessarily used for ancillary testing. For the positive for malignancy cases, an adequacy reading was deemed accurate when the sample was sufficient for all subsequent necessary ancillary testing, including >200 tumor cells and >10% tumor cells per cell block section in cases potentially requiring next generation sequencing. We defined "Accurate Diagnosis" of ROSE based on the comparison to the final diagnosis.

After ROSE is complete, the residual material is prepared in the main Cytopathology laboratory. Cases called "inadequate" were generally prepared with a second ThinPrep slide and the laboratory's standard Papanicolaou stain; if the ThinPrep did not run "dilute", the residual material was then prepared into a Cellient cellblock (Hologic Corp). For cases that were called "adequate", generally the entire sample was prepared into a Cellient cellblock.

Results

Validation steps

To enable ROSE, a rapid stain is needed, and the stain needs to ultimately be compatible with telecytology. We first tested use of the Diff-Quik blue (RAL Diagnostic, Martillac, France) on monolayer preparations from residual material in ThinPrep vials following diagnosis. We found the Diff-Quik stain to have variability and generally lack cytoplasmic-to-nuclear contrast. We also experimented with a rapid 2-step Papanicolaou stain, but it was slow. Neither the Diff-Quik or the rapid Papanicolaou stains had optimal contrast for the Motic Easy Scan (San Antonio, TX) telecytology system (both stains have blue cytoplasmic staining that decreases ability to resolve shades of blue in the digital images of chromatin). We developed a rapid H&E stain that provided contrast between cytoplasm and nuclei and improved the appearance of Motic telecytology images (not shown). It was necessary to dilute the eosin to 10% of the original strength (see Methods). To validate the rapid H&E stain, 20 ThinPrep vials with residual material from FNA samples were prepared into a monolayer slide and stained with H&E. All cases demonstrated comparable chromatin preservation and display with roughly stoichiometric staining of chromatin compared to DNA content (not shown), transparency of groups, and cellularity. The diagnoses on the 20 cases by H&E matched the (blinded) diagnosis of the original Papanicolaou-stained slide from the original cases in all 20 cases. We next validated that a 10%-20% aliquot is sufficient for assessing adequacy of a sample and for establishing a diagnosis by using ThinPrep vials in which material from an FNA remained within the vial after a case had been finalized. We deliberately chose cases that had been either diagnosed as "negative" or "positive". Based on the turbidity of the sample, 10% to 20% of the remaining sample was removed for a monolayer preparation. Twenty samples were assessed, and the diagnosis of the 10%-20% sample compared to the final diagnosis for the case. We reasoned that if a 10%-20% sample was deemed not diagnosable, or was falsely negative, then the 10%-20% sampling technique was not valid. Scoring and diagnosis were done blindly but with the specimen source given. The results showed 100% concordance between the 10% and 20% sampling method and the final diagnosis.

Liquid-based ROSE performance

The first total 52 liquid-based ROSE readings from 30 patients used in EBUS-TBNA are presented in this study (Table 1). Of all 52 readings, 28 readings (53.8%) were interpreted as "adequate" in ROSE with final assessment as adequate sample; 17 cases (32.7%) were interpreted as "inadequate" in ROSE with final assessment as inadequate sample. There were no false positive adequacy readings (reading interpreted as "adequate" but not actually adequate for ancillary testing). There were 7 false negative adequacy readings (13.5%), interpreted as "inadequate" in ROSE in which the final assessment was actually adequate for a complete diagnosis (Table 1). Four of the 7 readings from 2 patients were benign inflammatory necrotizing processes on final diagnosis (readings 7-9 [patient 4], Fig. 2B, C; and reading 25 [patient 13]) and 3 cases were positive for malignant cells on final diagnosis. In only 2 samples was molecular testing ultimately requested (readings 34 and 50). Both cases had sufficient quantity and quality of DNA in the cellblocks for complete next-generation sequencing using the Ion-Torrent system (Thermo Fisher Scientific).

Twenty-three readings were provided with a diagnosis by a cytopathologist or cytopathology fellow (Fig. 1). As shown graphically in Table 2, 13 (56.5%) were interpreted as positive for malignant cells; 2 cases (8.7%) were interpreted as negative for malignant cells; 6 cases (26%) were interpreted as nondiagnostic; and 2 cases (8.7%) were interpreted as atypical. At a threshold of definitive benign and definitive malignant on liquid-based ROSE, the sensitivity and specificity are both 100%. For the 2 cases with liquid ROSE diagnosis of "atypical", 1 was positive for malignant cells (reading 3, predominantly lepidic pattern broncholavaeolar adenocarcinoma, Fig. 2A) and 1 was negative for malignant cells on final diagnosis (reading #8, atypia was due to reserve cell hyperplasia in the setting of a benign necrotizing process, Fig. 2C). In our growing experience, we believe low-grade lepidic pattern broncholavaeolar adenocarcinoma, though diagnostically challenging, will be easier to diagnose on liquid-based preps compared to smears. If "atypical" is considered a false negative, the sensitivity of liquid-based ROSE for the diagnosis of malignancy drops to 93%. If atypical is considered a false positive, then the specificity drops to 80% in this sample with only 4 true negatives. Among 6 cases with "nondiagnostic" immediate interpretations, 5 remained as nondiagnostic on final diagnosis. One case (reading 7 [patient 4]) was eventually diagnosed as a benign inflammatory process on final diagnosis. All cytologic final diagnoses are concordance with clinical or surgical final diagnosis.

Delivery of the sample from the operation room to Pathology lab took an average of 3 minutes. The time from receipt of the sample in the lab to a reported ROSE ranged from 6 to 22 minutes (mean: 13 minutes; median: 12.5 minutes; mode: 12.5 minutes).

Discussion

Among all cytologic sampling procedures, ROSE has the highest reported mean and median usage rate (59.1% and

Table 1 Adequacy and preliminary diagnosis by liquid-based ROSE and correlation with final sign-out.

Reading Number	Patient number	Specimen type	ROSE adequacy	Final adequacy	ROSE diagnosis	Final diagnosis	LB ROSE evaluator
1	1	Nodule FNA	Adequate	Adequate	Positive	Positive	Path
2	1	Nodule brushing	Adequate	Adequate	Positive	Positive	Path
3	2	Nodule FNA	Inadequate	Inadequate	Atypia	Positive	Path
4	2	Nodule brushing	Adequate	Adequate	Positive	Positive	Path
5	3	Nodule FNA	Inadequate	Adequate	Positive	Positive	Path
6	3	Nodule brushing	Adequate	Adequate	Positive	Positive	Path
7	4	LN FNA	Inadequate	Adequate	ND	Negative	Path
8	4	LN FNA	Inadequate	Adequate	Atypia	Negative	Path
9	4	Nodule FNA	Inadequate	Adequate	Negative	Negative	Path
10	5	Nodule FNA	Inadequate	Inadequate	ND	ND	Path
11	5	Nodule brushing	Inadequate	Inadequate	ND	ND	Path
12	5	Nodule FNA	Inadequate	Inadequate	ND	ND	Path
13	6	Nodule FNA	Adequate	Adequate	Positive	Positive	Fellow
14	7	Nodule FNA	Adequate	Adequate	Positive	Positive	Fellow
15	7	LN FNA	Adequate	Adequate	Negative	Negative	Fellow
16	8	Nodule FNA	Inadequate	Inadequate	Positive	Positive	Fellow
17	9	Nodule FNA	Adequate	Adequate	Positive	Positive	Fellow
18	10	Nodule FNA	Adequate	Adequate	Positive	Positive	Fellow
19	10	Nodule brushing	Adequate	Adequate	Positive	Positive	Fellow
20	11	Nodule FNA	Adequate	Adequate	Positive	Positive	Fellow
21	11	Nodule brushing	Adequate	Adequate	Positive	Positive	Fellow
22	12	Nodule FNA	Inadequate	Inadequate	ND	ND	Fellow
23	12	Nodule brushing	Inadequate	Inadequate	ND	ND	Fellow
24	13	Nodule FNA	Inadequate	Inadequate	N/A	ND	CT
25	13	Nodule FNA	Inadequate	Adequate	N/A	Negative	CT
26	14	Nodule FNA	Inadequate	Inadequate	N/A	Positive	CT
27	14	Nodule brushing	Inadequate	Inadequate	N/A	Positive	CT
28	15	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
29	16	LN FNA	Adequate	Adequate	N/A	Positive	CT
30	17	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
31	17	Nodule brushing	Adequate	Adequate	N/A	Positive	CT
32	18	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
33	18	Nodule brushing	Adequate	Adequate	N/A	Positive	CT
34	19	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
35	20	Nodule FNA	Adequate	Adequate	N/A	Negative	CT
36	20	Nodule brushing	Adequate	Adequate	N/A	Negative	CT
37	21	LN FNA	Inadequate	Adequate	N/A	Positive	CT
38	22	LN FNA	Inadequate	Adequate	N/A	Positive	CT
39	22	LN FNA	Adequate	Adequate	N/A	Positive	CT
40	23	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
41	24	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
42	24	Nodule brushing	Adequate	Adequate	N/A	Positive	CT
43	25	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
44	26	Nodule FNA	Inadequate	Inadequate	N/A	Positive	CT
45	26	Nodule brushing	Adequate	Adequate	N/A	Positive	CT
46	27	Nodule FNA	Inadequate	Inadequate	N/A	ND	CT
47	27	Nodule brushing	Inadequate	Inadequate	N/A	ND	CT
48	28	Nodule FNA	Inadequate	Inadequate	N/A	ND	CT
49	28	Nodule brushing	Inadequate	Inadequate	N/A	Suspicious	CT
50	29	Nodule FNA	Adequate	Adequate	N/A	Positive	CT
51	30	Nodule FNA	Inadequate	Inadequate	N/A	ND	CT
52	30	Nodule brushing	Inadequate	Inadequate	N/A	ND	CT

Abbreviations: ND, nondiagnostic; N/A, not applicable; Path, cytopathologist; CT, cytotechnologist; LB, liquid-based; LN, lymph node.

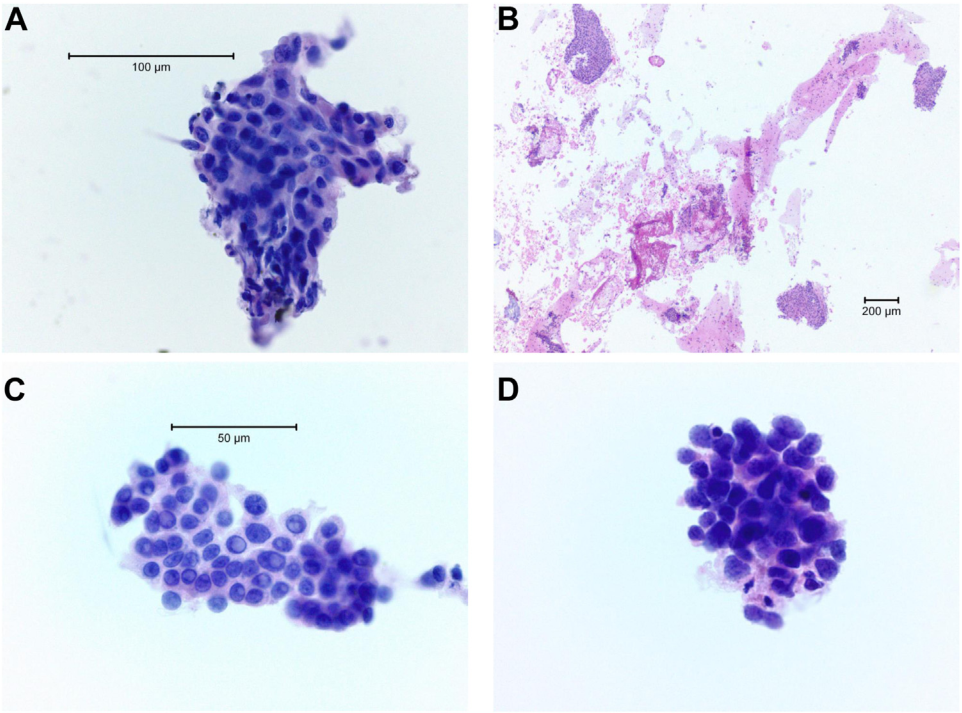

Figure 1 A, Squamous cell carcinoma (reading 41 [patient 24]) on liquid-based ROSE showed 3-dimensional tumor cells with waxy cytoplasm and cell bridges (hematoxylin and eosin, 400×). B, Squamous cell carcinoma in cell block (same case as A, reading 41 [patient 24]), showing abundant tumor cells as anticipated based on the amount of material in the 10% of the sample used for liquid-based ROSE (hematoxylin and eosin, 40×). C, Adenocarcinoma (reading 43 [patient 25]) on liquid-based ROSE showing mostly 2-dimensional tumor cells with intranuclear cytoplasmic inclusions (hematoxylin and eosin, 600×). D, Small cell carcinoma (reading 18 [patient 10]) on liquid-based ROSE showing nuclear molding, highly variable amount and abnormal chromatin, and single cell necrosis (hematoxylin and eosin, 600×).

70%, respectively) in EBUS-TBNA.[3] College of American Pathology (CAP) guidelines currently recommend ROSE to be utilized when performing EBUS-TBNA if available. Together with our clinicians, we established the key measurable values of ROSE in determining adequacy and an actionable diagnosis. Adequacy encompasses knowing whether a lesion has been reached, whether a lesion is yielding cells, whether ancillary studies are needed, and whether material is sufficient for performing all necessary ancillary studies. Adequacy can be performed by cytotechnologists. An actionable diagnosis is performed by a cytopathologist.

As detailed in the Introduction, the existing smear-based method for performing ROSE has several limitations that include poorly reimbursed use of technical and professional time, often using 2 people, generation of more readings than are actionable, challenges to making a good quality smear, and difficulty judging the material that is NOT smeared but needed for ancillary testing. This is the first study using a liquid-based cytology method for ROSE procedure in which a controlled and measured amount of material is prepared into a ThinPrep slide, stained with a rapid H&E, and interpreted. Previous studies[10-12] have demonstrated a comparable or higher diagnostic yield of liquid-based cytology compared to the conventional smear method in samples obtained by EBUS-TBNA. Our goal in developing liquid-based ROSE was to achieve the values of smear-based ROSE and to overcome some of its limitations.

Since 2000, our laboratory has used CytoRich Red (Thermo Scientific) as the collection media for all FNAs and non-gynecologic brushings, due to its ability to remove all red cells and prevent precipitation of serum proteins. Our immunohistochemistry and molecular procedures have all been validated on this platform (rarely with a post-fixation in formalin for PD-L1 and a few other IHC stains). To be able to use ThinPrep processing in routine samples, our laboratory centrifuges material out of CytoRich Red and resuspends the sample in PreservCyt (Hologic does not support the use of Cytorich Red in ThinPrep processors). Once resuspended in PreservCyt, most routine FNA and brushing samples have a ThinPrep prepared, and if the sample is not "dilute" or if the sample has 5 visible particles in an otherwise empty container, a Cellient (Hologic Corp.) cellblock is prepared. We adopted the monolayer ROSE procedure to allow it to be used on CytoRich Red needle rinses, making the treatment of the sample identical to all of our existing protocols and thereby allowing all our validated protocols to continue to be used. In order to make the procedure as fast as possible, we developed an off-label use of the T2000 processor by adding 1-2 mL of CytoRich Red

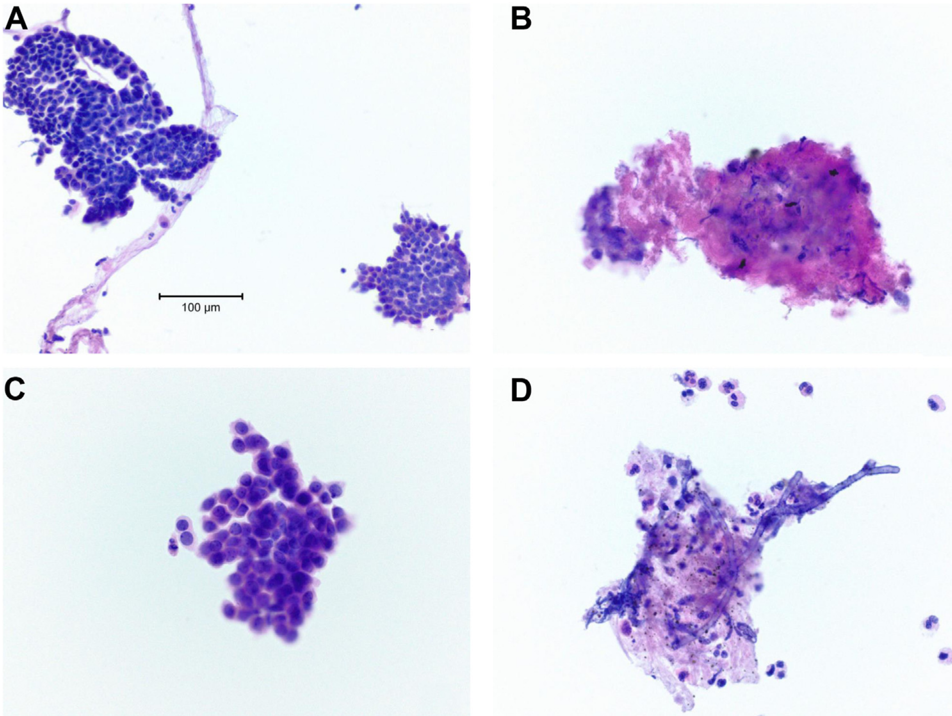

Figure 2 A, Adenocarcinoma (reading 3 [patient 2]). Liquid-based ROSE was interpreted as "atypical". Note the distinct but subtle hyperchromasia of the malignant cell group on the left in comparison to the benign bronchial cells on the right (hematoxylin and eosin, 200×). B, Benign necro-inflammatory process (reading 7 [patient 4]). Liquid-based ROSE was interpreted as "non-diagnostic" (hematoxylin and eosin, 400×). C, Benign necro-inflammatory process (same [patient as B, reading 8 [patient 4]) showing reserve cell hyperplasia, which was interpreted as "atypical" on liquid-based ROSE (hematoxylin and eosin, 400×). D, Fungal hyphae (reading 35 [patient 20]) on liquid-based ROSE (hematoxylin and eosin, 400×).

to the PreservCyt vial and immediately running the ThinPrep, rather than taking the time to centrifuge the sample and incubate it for 15 minutes per the ThinPrep T2000 protocol. We did not identify any loss of cells or alteration in the appearance of cells based on this modification. However, we do observe some protein precipitation when a clear supernatant of a Cytorich Red−collected specimen is mixed into PreservCyt solution. The precipitation is slow and progressive, appearing to start after a few minutes and reaching a maximum in about an hour. Thus, we note it is important to make the ThinPrep immediately after adding the CytoRich Red to the PreservCyt vial. This minor off-label modification saves about 20 minutes. In this study, we validated a rapid H&E stain used in liquid-based ROSE. We found that H&E was faster than the rapid Papanicolaou stain without decreasing diagnostic accuracy. H&E stain also seemed more suited for use with the MOTIC system (data not shown).We identified several advantages of liquid-based ROSE over smear-based ROSE. A first advantage is substantial time saving to the Pathology lab. Total time spent on each liquid-based ROSE ranges from 9 to 22 minutes, with a mean 13 minutes, median 12.5 minutes, and mode 12.5 minutes. The time savings is mostly because only 1 reading per site was necessary. Unnecessary ROSE interpretations are avoided by providing a mechanism for clinicians to pool several needle or brushing passes in a specimen container. Clinicians then decide when a ROSE is needed, in part by looking at the specimen container

Table 2 Liquid-based ROSE preliminary diagnosis and correlation with final diagnosis.

	ROSE Non-diagnostic	ROSE Negative for malignant cells	ROSE Atypical	ROSE Positive for malignant cells
FINAL Positive for malignant cells			1	13
FINAL Negative for malignant cells	1	2	1	
FINAL Non-diagnostic	5			

(thereby also gaining feedback about their sampling technique), and then they send the needle rinse to the laboratory like a frozen section. Although each pass of a conventional ROSE procedure takes about 5 minutes, the whole procedure takes more than 30 minutes.[3] The increased time per reading for liquid-based ROSE is largely due to the increased staining and transport time. Liquid-based ROSE uses laboratory personnel efficiently. Cytotechnologists, cytopathologists or cytology fellows do not need to be physically in the operating room, thereby allowing them to remain productive in the Pathology laboratory near the operating room.

A second advantage may relate to the number of individuals involved with ROSE. In a previous survey of ROSE practices,[3] most labs reported that 2 individuals were used: a cytotechnologist with a cytopathologist interpretation. We found that liquid-based ROSE procedure requires only 1 individual. A third advantage is that liquid-based preparations require only minimal training and provide a uniform presentation of cells, free from blood and air-drying artifact in a defined area of the slide.[13]

Compared with conventional ROSE, the billing and supply costs to the laboratory are less favorable. Only 1 reading per site was needed for all of our cases, equating to one 88172 CPT code. Clinicians found the reduction in the number of readings to one per site to be satisfactory for all of the patients in this study. For most conventional ROSE cases, multiple units of 88177 are also applied for each subsequent reading of the same site. The liquid-based preparations also require purchase of ThinPrep materials including the processor and disposable filters. Laboratories would have to evaluate the cost−benefit balance of liquid-based ROSE. For our lab, the decrease in personnel costs more than offset the decreased reimbursement and increased ThinPrep costs.

One limitation in this study is that we did not compare the anesthesia time of smear-based ROSE EBUS procedure with that of liquid-based ROSE EBUS procedures, but the impression of the clinicians is that 15 to 20 minutes was not problematic. Flow cytometry was not indicated in any of our patients. If a liquid-based ROSE reading indicated that flow cytometry was needed, then additional passes would have to be collected in saline transport media. If it were necessary to assess the adequacy of the flow cytometry sample, it would be possible for us to convert a liquid-based ROSE into a conventional ROSE by going to the operating room and making and assessing a smear. It should be possible to adapt a liquid-based ROSE procedure to collect the sample into saline from which a liquid-based preparation could be made after fixation of the cells.

Liquid-based ROSE performed well as a platform for determining the 4 components of adequacy, with some tendency to underestimate adequacy, but no overestimation of adequacy. Of all 52 readings, 28 were interpreted as "adequate" in ROSE with the final sample being adequate. Seventeen cases were interpreted as "inadequate" in ROSE with the final sample truly being inadequate. Seven cases were falsely interpreted as "inadequate" in ROSE but in fact were adequate in the final sample preparations. Four of the 7 falsely inadequate cases were benign inflammatory processes on final diagnosis and 3 cases were positive for malignant cells on final diagnosis. It is safer to err on the side of calling an apparently benign case inadequate. Underestimating adequacy of a malignant case is further discussed below. Two samples (from 2 patients) were requested by clinicians for next-generation sequencing testing, and both were successfully analyzed. The accuracy of a reading of "adequate" in our liquid-based cases was 100% with the final interpretation. Burlingame et al[14] showed a correct adequacy assessment of 94.8% and 94.4% in lung and mediastinal FNAs from all cases interpreted as adequate by cytotechnologists.[14]

We underestimated the adequacy in 3 readings from 3 patients with malignancy (3 of 26 [12%] of patients with malignancy). One case (reading 5 [patient 3]), read by a pathologist, proved more cellular with immunohistochemistry in the cellblock than expected. The other 2 (reading 37 [patient 21] and reading 38 [patient 22]) were read by cytotechnologists, both with final diagnoses of squamous cell carcinoma. Upon review, both were determined to actually have sufficient material based on the liquid ROSE slide, consisting of mostly individual squamous cells with abundant necrosis. All cases eventually had >200 cells and >10% tumor cells per cell block section, our guideline for determining adequacy of cases for molecular testing.

Estimating whether a sample has adequate tumor cells for ancillary testing at ROSE is a major problem for cytology. In a 2013 survey of participants in a CAP interlaboratory comparison program in nongynecologic cytology,[15] the greatest number of respondents used "general impressions" of the samples to assess adequacy in pulmonary cytology. Smears are often made until a diagnosis is rendered, then extra needle passes are still collected (blindly) for a cellblock, apparently to help offset any imprecision in the estimate of adequacy.[16] This common practice of collecting more sample after ROSE is finalized confounds analysis of the value of ROSE and can help explain some of the findings in the literature: In a randomized trial of EBUS-TBNA with and without ROSE for lung cancer genotyping,[8] ROSE was associated with only an insignificant 10% increase in the success rate for optimal lung cancer genotyping.

Our goal differs sharply from previous studies: Our goal was to facilitate estimation of the adequacy at the time of ROSE. For our study, sample collection stopped when a sample was deemed adequate. Accurate estimation requires knowledge of which tests will be needed and the requirements for the tests, knowing which platform will provide material for the tests (whether smears or cellblocks), and the amount of material and proportion of tumor cells in the platform that will used for the test. The most demanding ancillary test in our experience is Foundation Medicine, Inc. (Cambridge, MA), requiring a minimum of 20% tumor nuclei, and a minimum 25 mm^2 of tissue area in 10 unstained slides to provide 1 mm^3 of tissue. (https://assets.ctfassets.net/w98cd481qyp0/6qYLg8jUuEYEvUytoBz8p6/f7764c8e3fcadda9ec1374fe26bb999e/F1CDx_Specimen_Instructions.pdf). When smears are used for the ancillary test, a direct count can be performed at the time of smear-based ROSE. Our laboratory, like the majority of laboratories,[17,18] performs ancillary testing on cellblocks. When cellblocks are used, then it is the material not smeared at ROSE that will be used for testing. To overcome the problem of assessing what will be available in the cellblock, there are only a few options. It would be ideal to be able to image the sample in the specimen container, and never actually use the cells or tissue fragments that are imaged. Though this is completely feasible[19] (and it appears feasible to perform this analysis on unfixed cells such that the material can be triaged for any ancillary test including flow cytometry), there is no industry support for laboratories to acquire such a fluorescence-based device. Liquid-based ROSE provides another option: Remove a defined proportion of the sample, so the overall cellularity of the sample can be more accurately quantified. If the ThinPrep slide exhausts all the material for the liquid-based ROSE, the amount delivered to the cellblock can be estimated by counting tumor cells or particles on the liquid-based ROSE slide and then multiplying by 4 or 9 (if 20% or 10% was removed for the liquid-based ROSE, respectively). Our method errs on the side of underestimation: We avoid taking larger fragments for the liquid-based ROSE slide since large particles are harder to recover and see in liquid-based preparations. Our unpublished experience with Cellient is that the chance of recovering a fragment in a cellblock histologic section is roughly 80%.

The other key component of ROSE is to provide an accurate intraoperative diagnosis. Liquid-based ROSE diagnostic accuracy appears similar to previous studies. At a threshold of definitive benign and definitive malignant on liquid ROSE, the sensitivity and specificity are both 100%. The sensitivity and specificity drop if atypical is considered a false negative or false positive, respectively. Geisler et al[20] reported 99.1% of concordance rate with final diagnosis by selecting cases with only definitive ROSE diagnosis in all anatomic sites, and found the concordance to be similar to intraoperative frozen section. Nakajima et al[21] showed smear-based ROSE can achieve concordance rate of 94.3% with final diagnosis in EBUS-TBNA nodal staging of lung cancer.

Our institution has begun to perform the Robotic One Anesthetic Diagnosis and Treatment program (ROADAT program) for patients with a high clinical probability of low-stage lung cancer, to improve the timeliness of diagnosis and treatment of lung cancer. ROADAT bases the decision to perform a robotic lobectomy on the ROSE diagnosis, so accuracy becomes crucial. Based on the data from this study, we have successfully started to use liquid-based ROSE for ROADAT.

Other advantages of liquid-based ROSE are likely to be for telecytology: fewer slides per site, with material in a defined area in a relatively thin preparation, and with an optimal H&E stain for the imager. Studies are underway to test the hypothesis telecytology using this platform will lead to time savings with comparable diagnostic accuracy.

In summary, liquid-based ROSE achieves adequacy and diagnostic accuracy comparable to smear-based ROSE, but it saves substantial time for the Pathology lab and cuts personnel requirements from 2 to 1. The aliquot examined from the total needle pass sample is likely to be more representative of the targeted lesion and provide a more quantifiable measure of the total sample. Uniform quality of the preparation on a defined area offers advantages for telecytology. This study established essential metrics of ROSE quality that should be useful for future studies.

Funding Sources

This work was supported soley by educational funds from the Department of Pathology, UMass Chan Medical School.

Conflict of interest disclosure

Dr. Andrew Fischer is an inventor of Cellient cell block technology and receives royalties for sales of the technology. Other authors made no disclosures.

References

1. Siegel RL, Miller KD, Fuchs HE, et al. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72:7−33.
2. Folch EE, Pritchett MA, Nead MA, et al. Electromagnetic navigation bronchoscopy for peripheral pulmonary lesions: one-year results of the prospective, multicenter NAVIGATE study. *J Thorac Oncol*. 2019;14:445−458.
3. VanderLaan PA, Chen Y, Alex D, et al. Results from the 2019 American Society of Cytopathology survey on rapid on-site evaluation—part 1: objective practice patterns. *J Am Soc Cytopathol*. 2019;8:333−341.
4. Eapen GA, Shah AM, Lei X, et al. Complications, consequences, and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration: results of the AQuIRE registry. *Chest*. 2013;143:1044−1053.
5. Jain D, Allen TC, Aisner DL, et al. Rapid on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspirations for the diagnosis of lung cancer: a perspective from members of the Pulmonary Pathology Society. *Arch Pathol Lab Med*. 2018;142:253−262.
6. Stevenson T, Powari M, Bowles C. Evolution of a rapid onsite evaluation (ROSE) service for endobronchial ultrasound guided (EBUS) fine needle aspiration (FNA) cytology in a UK Hospital: a 7 year audit. *Diagn Cytopathol*. 2018;46:656−662.
7. Stoy SP, Segal JP, Mueller J, et al. Feasibility of endobronchial ultrasound-guided transbronchial needle aspiration cytology specimens for next generation sequencing in non-small-cell lung cancer. *Clin Lung Cancer*. 2018;19:230−238.e2.

8. Trisolini R, Cancellieri A, Tinelli C, et al. Randomized trial of endobronchial ultrasound-guided transbronchial needle aspiration with and without rapid on-site evaluation for lung cancer genotyping. *Chest*. 2015;148:1430–1437.
9. Dhillon I, Pitman MB, Demay RM, et al. Compensation crisis related to the onsite adequacy evaluation during FNA procedures-Urgent proactive input from cytopathology community is critical to establish appropriate reimbursement for CPT code 88172 (or its new counterpart if introduced in the future). *Cytojournal*. 2010;7:23.
10. Hou G, Yin Y, Wang W, et al. Clinical impact of liquid-based cytology test on diagnostic yields from transbronchial needle aspiration. *Respirology*. 2012;17:1225–1228.
11. Nalwa A, Walia R, Singh V, et al. Comparison of conventional smear and liquid-based cytology preparation in diagnosis of lung cancer by bronchial wash and transbronchial needle aspiration. *J Cytol*. 2018;35:94–98.
12. Qiu T, Zhu H, Cai M, et al. Liquid-based cytology preparation can improve cytological assessment of endobronchial ultrasound-guided transbronchial needle aspiration. *Acta Cytol*. 2015;59:139–143.
13. Wallace WA, Monaghan HM, Salter DM, et al. Endobronchial ultrasound-guided fine-needle aspiration and liquid-based thin-layer cytology. *J Clin Pathol*. 2007;60:388–391.
14. Burlingame OO, Kesse KO, Silverman SG, et al. On-site adequacy evaluations performed by cytotechnologists: correlation with final interpretations of 5241 image-guided fine-needle aspiration biopsies. *Cancer Cytopathol*. 2012;120:177–184.
15. Sturgis CD, Marshall CB, Barkan GA, et al. Respiratory cytology—current trends including endobronchial ultrasound-guided biopsy and electromagnetic navigational bronchoscopy: analysis of data from a 2013 supplemental survey of participants in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. *Arch Pathol Lab Med*. 2016;140:22–28.
16. Tang W, Plank C, Kiepura C, et al. Rapid onsite evaluation uses a very small proportion of total material aspirated at endobronchial or endoscopic ultrasound in the investigation of suspected thoracic malignancy. *Cytopathology*. 2021;32:416–427.
17. Roy-Chowdhuri S, Aisner DL, Allen TC, et al. Biomarker testing in lung carcinoma cytology specimens: a perspective from members of the Pulmonary Pathology Society. *Arch Pathol Lab Med*. 2016;140:1267–1272.
18. Schmitt F, Di Lorito A, Vielh P. Molecular testing on cytology for gene fusion detection. *Front Med (Lausanne)*. 2021;8:643113.
19. Liao H, Sheridan T, Cosar E, et al. Deconvolution microscopy: a platform for rapid on-site evaluation of fine needle aspiration specimens that enables recovery of the sample. *Cytopathology*. 2022;33:312–320.
20. Geisler DL, Nestler RJ, Mosley BL, et al. Accuracy of definitive rapid onsite evaluation cytopathology diagnoses: assessment of potentially critical diagnoses as a quality assurance measure. *J Am Soc Cytopathol*. 2022;11:133–141.
21. Nakajima T, Yasufuku K, Saegusa F, et al. Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for nodal staging in patients with lung cancer. *Ann Thorac Surg*. 2013;95:1695–1699.