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RAPID COMMUNICATION

Potential role of mobile rapid on-site evaluation® in thyroid fine-needle aspiration cytology to reduce delayed repeated aspiration

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Abstract. Rapid on-site evaluation of fine-needle aspiration cytology is time-consuming and requires specialized cytopathology staff. Mobile Rose® is a newly developed device for rapid on-site evaluation of fine-needle aspiration cytology. This study aimed to investigate the potential role of Mobile Rose® in reducing delayed repeated aspiration of the thyroid. A total of 120 cytological samples were collected and observed using Mobile Rose® after fine-needle aspiration cytology between September and October 2020, with immediate assessment of minimal or no cell clusters after conventional smear preparation. After qualifying and scoring, needle washout materials were prepared using the BD CytoRich™ method and correlated with cytology results. The average turn-around time of Mobile Rose® was found to be 1.5 minutes. Sensitivity, specificity, positive predictive value, and negative predictive value were 94.4%, 100%, 100%, and 57.1%, respectively. False-negative results were attributed to small aggregates of cells that were difficult to distinguish from the background and artifacts. Mobile Rose® may represent an important innovation for rapid on-site evaluation that is fast, has high diagnostic performance, does not require the presence of specialized cytology staff, and can reduce delayed repeated aspiration of the thyroid gland. However, further minor improvements and confirmation are required.

Key words: Mobile rapid on-site evaluation, Fine needle aspiration cytology, Thyroid, Adequacy, Delayed repeated aspiration

FINE-NEEDLE ASPIRATION CYTOLOGY (FNAC) has emerged as a reference diagnostic tool for the evaluation of thyroid nodules and has proven to be a rapid, cost-effective, and reliable method. Major problems of FNAC of thyroid nodules arise due to non-diagnostic results and delayed repeated aspirations caused by inadequately obtained FNA specimens [1]. Cytological diagnosis is often hampered by reasons such as blood contamination, fixation artifacts, or scanty cells. At the same time, the increase in the number of passes and slides submitted was correlated with additional burden and health care costs for medical staff and patients [2]. The most effective way to increase the rate of accurate diagnosis in cytology is to ensure proper sampling and optimize specimen handling [1].

In this context, the rapid on-site evaluation (ROSE) method has recently been gaining attention [1, 3]. ROSE is performed by a trained cytotechnologist or cytopathologist with an immediate microscopic analysis of the material after aspiration to improve the overall specimen adequacy with a combined decrease in the number of needle passes necessary to optimize the material for diagnosis [1]. However, there are some limitations, as using ROSE significantly prolongs the procedure time and requires additional human resources, including professional cytopathology staff. It also increases the costs incurred [3, 4]. To the best of our knowledge, no simple and quick method for ROSE of FNAC materials is available to date.

Mobile Rose® (MR®; Yamachu Co., Ltd, Medical Equipment Research and Development Corporation, Chiba, Japan, https://yamachutech.com/) is a newly developed multifunctional phase-contrast method for evaluating the adequacy of FNAC aspirates.

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This study aimed to investigate the potential role of MR® in reducing delayed repeated aspiration of thyroid FNAC, correlating the cytological results.

Materials and Methods

Case selection

The study protocol was reviewed and approved by the Institutional Review Board of Kuma Hospital (20200910-1) and was in accordance with the 1964 Helsinki declaration and its amendments or comparable ethical standards.

In the previous pilot study, a large number of cell clusters were identified by MR® in cases that were determined to have sufficient material by the naked eye. Only cytological samples with inadequate visual assessment, defined as minimal or no cell collection after conventional smear preparation, were considered for analysis by MR® assessment. This study included 120 cytological samples selected from 500 patients who underwent thyroid FNAC for 648 cytological samples performed by a cytopathologist (T.H.) at Kuma Hospital between September and October 2020. The remaining cytological samples were considered adequate by visual assessment and were excluded from this study.

US-guided FNA procedure

All FNACs were performed using a 22-gauge needle with ultrasound guidance in the cytological room of the outpatient department of Kuma Hospital using a GE ultrasound system (LOGIQ P6 Expert, General Electric Company, WI, USA) with a linear IIL-D probe. All FNAC procedures and slide smears for selected cases were performed by a certified cytopathologist (T.H.). Cytological slides were prepared by expressing the aspirated materials from the needle onto glass slides and compressing them with a second slide, which was immediately fixed using Cytorop (Alfresa Pharma Co., Osaka, Japan), a cytological fixative for further Papanicolaou staining as described previously [5].

Preparation of LBC specimen and MR® observation

The LBC samples included in the present study were obtained by washing the needles after expressing the aspirated materials onto the slides. The needles were rinsed gently within the MR® View Cap with 0.5 mL CytoRich™ RED collection fluid (Becton Dickinson and Company, Franklin Lakes, NJ) with hemolytic and proteolytic abilities.

MR® is a compact, lightweight device measuring 229 × 265 × 224 mm (width × depth × height) that can be used in a 25 cm^2 space and incorporates a finite phase contrast lens with 20× magnification and white LED lighting. An external smartphone was mounted on the MR® and achieved up to 300× magnification (Fig. 1). The aspirated material can be observed real-time on the smartphone, and images and videos can be saved for comparison with the final cytology or histology (https://yamachutech.com/assets/pdf/catalog_MR20201.pdf). After washout of the needle, the MR® View Cap containing the washout material was connected to the test tube and inserted into the insertion slot of MR® with the MR® View Cap down and observed (Fig. 2; https://yamachutech.com/assets/video/mobile-rose_official.mp4) before submitting the material for processing. In addition, because the entire content of the MR® View Cap cannot be observed at once, the MR® View Cap must be manually moved to observe the entirety.

Fig. 1 Mobile Rose® and its sample container (MR® View Cap, inset). A wide range of Apple iPhones and Android smartphone models can be applied to MR® by using the adjustment pad (white arrow).

The sum of cell clusters from a batch of four field images projected onto the screen of the smartphone connected to MR® was recorded and scored into two tiers as follows: (a) MR®0, absence of cell clusters and (b) MR®+, presence of cell clusters. Based on their shape, the cell clusters were categorized into small, cloud-like, stick-shaped, or large clusters (Fig. 3). Liquid-based preparation (LBC) specimens prepared by the BD CytoRich™ method were then evaluated according to the Bethesda System for Reporting Thyroid Cytopathology [6]. The turn-around time for MR® was defined as the interval for observation using MR® to define the adequacy of aspirated material, measured from the time of immediate gross visual assessment of conventional smears to the time at which the LBC material was ready to be submitted to the pathology department for further processing.

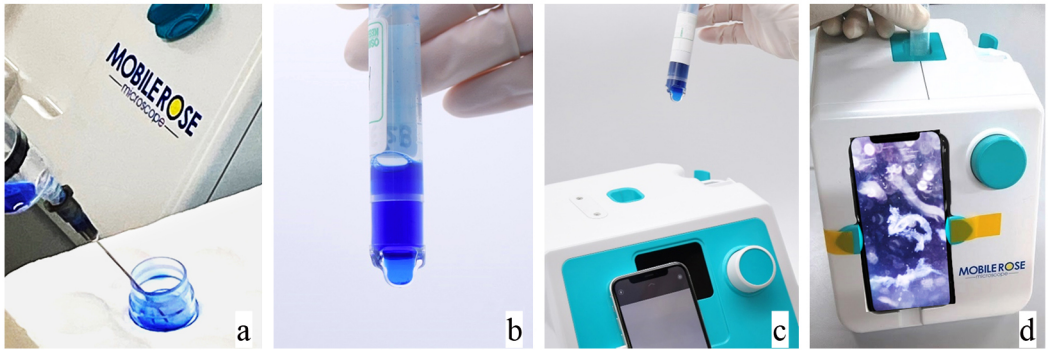

Fig. 2 Mobile Rose® (MR®) observation procedure: (a) Step 1: washing out the needle with a fixative in the MR® View cap; (b) Step 2: Connection of the MR® View Cap and test tube; (c) Step 3: Insertion of the MR® View Cap containing the washout material for observation; (d) Step 4: Observation of aspirate on the equipped smartphone screen before submitting the material for LBC processing.

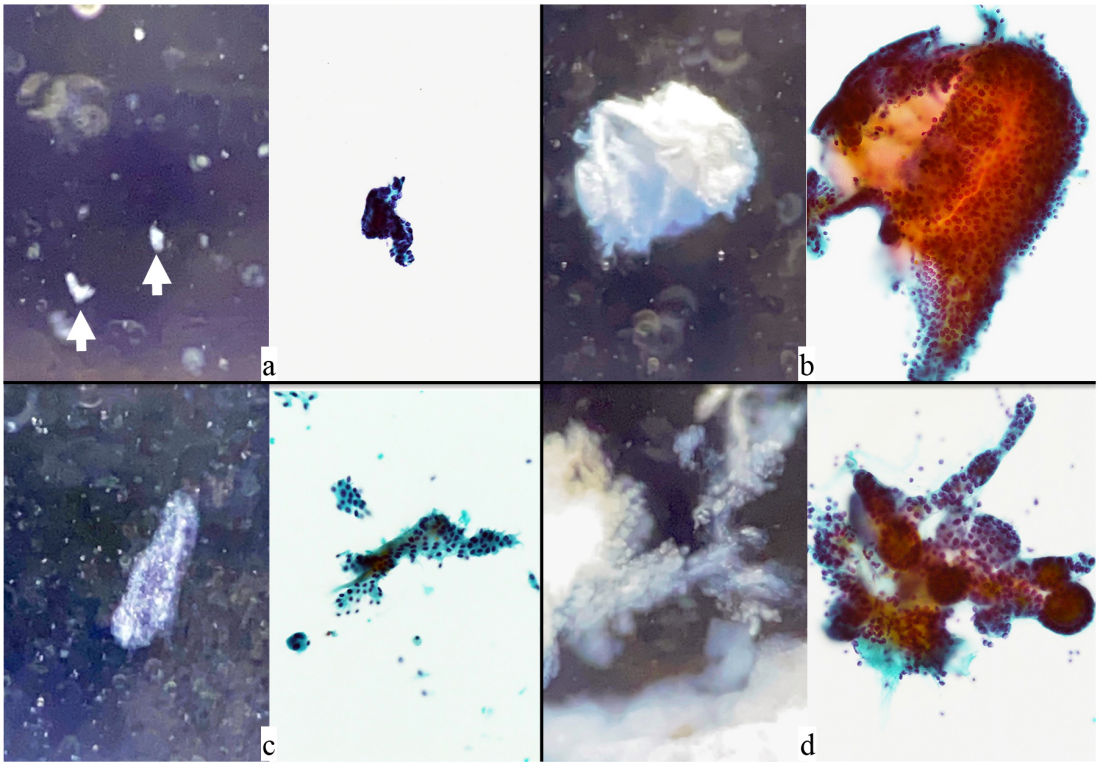

Fig. 3 Proposed morphological classification based on the shape of the cell clusters with cytological correlation: (a) Left: small cluster (white arrow, MR® ×200), Right: cytological correlation of small cluster (LBC, Papanicolaou stain ×200); (b) Left: cloud-like shape cluster; (MR® ×200), Right: cytological correlation of cloud-like cluster (LBC, Papanicolaou stain ×200); (c) Left: stick-like cluster (MR® ×200), Right: cytological correlation of stick-like cluster (LBC, Papanicolaou stain ×200); (d) Left: large cluster; (MR® ×200), Right: cytological correlation of large cluster (LBC, Papanicolaou stain ×200).

To clarify the cause of misinterpretation of false-negative and false-positive cases in MR® findings, cases with contradictory MR® findings on the recording images and cytology were reviewed and correlated with cytology.

Results

MR® findings

The MR® findings were as follows: MR®0 (n = 14; 11.7%) and MR®+ (n = 106; 88.3%). The shapes of cell clusters were as follows: small cluster in 96 (90.1%), cloud-like clusters in 4% (3.8%), stick-shaped clusters in 5 (4.7%), and large clusters in 1 (0.9%) (Fig. 3).

Table 1 Correlation of mobile rapid-onsite evaluation® (MR®) finding and the cytological result of our 120 cytological samples

	Inadequate 6.7% (8)		Adequate 93.3% (112)									TOTAL 100% (120)
	BEI		BEII			BEIII	BEIV	BEV	BEVI			
	UNS	CFO	AN	BENIGN	CT	AUS/FLUS	FN	SFM (HTT)	PTC	MALT	MTC	
MR®0	6	2	2	1	0	0	0	1	2	0	0	11.7% (14)
MR®1	0	0	56	13	4	1	7	0	22	2	1	88.3% (106)

MR®, mobile rapid-onsite evaluation. BE, Bethesda system for reporting thyroid cytopathology; CFO, cystic fluid only; AN, adenomatous nodule; CT, chronic thyroiditis; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN, follicular neoplasm; SFM, suspicious for malignancy; HTT, hyalinizing trabecular tumor; PTC, papillary thyroid carcinoma; MALT, mucosa-associated lymphoid tissue lymphoma; MTC, medullary thyroid carcinoma

Turn-around times

A total of 120 cytological samples were evaluated with MR® with turn-around times ranging from 60 to 110 seconds with an average of 90 seconds (1.5 minutes).

Cytological results and diagnostic accuracy

The cytological results were as follows: unsatisfactory: 8 (inadequate: 6; cystic fluid only: 2), benign: 76 (adenomatous nodule: 58; benign: 14; chronic thyroiditis: 4), atypia of undetermined significance/ follicular lesion of undetermined significance: 1, follicular neoplasm: 7, suspicious for malignancy: 1 (hyalinizing trabecular tumor: 1), malignant: 27 (papillary thyroid carcinoma: 24; mucosa-associated lymphoid tissue/ MALT lymphoma: 2, medullary thyroid carcinoma: 1) (Table 1).

The sensitivity, specificity, positive predictive value, and negative predictive value were 94.4%, 100%, 100%, and 57.1%, respectively. Moreover, eight false-negative cases (false-negative fraction, 6.7%), but no false-positive cases were found (false positive fraction, 0%). Among the MR®0 group, there were eight true negative cases (57.1%) and six false-negative cases (42.9%) (Table 1). Smears of the two false-negative cases showed cystic fluid only with macrophages. The smears of the remaining six false-negative cases showed the presence of tiny cell aggregates that were difficult to distinguish from scratches, bubbles, or dirt present in the MR® View Cap (n = 6, Fig. 4).

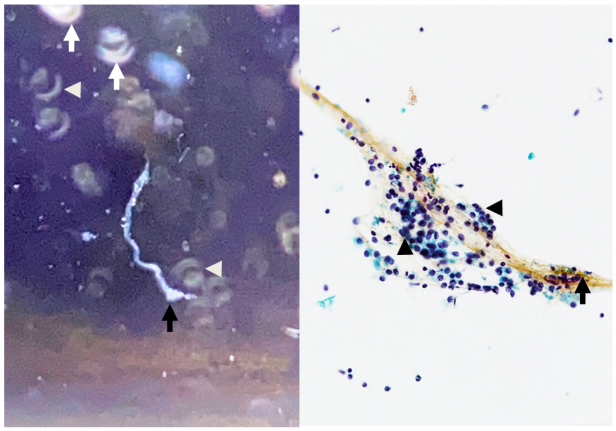

Fig. 4 Left: Presence of scratches (white arrowheads), air bubbles (white arrows), or dirt (black arrows) present in the MR® View cap that interferes with MR® observation (MR® ×200), Right: cytological correlation of a case where small aggregates of tiny cells (black arrowheads) are attached to the dirt obscuring the MR® observation (LBC, Papanicolaou stain ×200).

Discussion

Mobile Rose® (MR®) is a recently developed device that allows observation of unstained cells of FNAC aspirated material in a sample container. However, its usefulness and accuracy have not yet been evaluated. This pilot study demonstrated that the average time spent for MR® was 1.5 min and was feasible by a single aspirator. Our study also showed that the sensitivity, specificity, and positive predictive value of MR® were high, indicating exemplary diagnostic performance. However, the high number of false negatives in the MR®0 group (6 cases, 42.9%) is a concern. In this study, MR® was used to observe cases for which visual assessment was inadequate (120 cases); without MR®, all such cases may have required repeated aspiration. In this sense, it was effective in avoiding unnecessary passes in the MR®+ group (88.3%, 106 cases) and delayed repeated aspiration in the true-negative group (6.7%, 8 cases). Nasuti *et al.* reported that ROSE was essential for an estimated cost savings of >$400.000 per year as a result of avoiding repeated FNAC and additional workload or longer hospital stays due to the delayed procedure. Furthermore, the adequacy of the specimen should be determined before the patient left [7, 8]. Effective use of MR® observation could avoid unnecessary aspirations, including delayed repetition, which could effectively reduce cost, patient anxiety, and time for optimal treatment.

The Bethesda Reporting System for Thyroid Cytology

Table 2 Comparison of applications of mobile rapid-onsite evaluation (Mobile rose®) and rapid-onsite evaluation (ROSE) in the management of thyroid fine-needle aspiration cytology

	Mobile rose®	ROSE
Turn-around times (average)	1.5 minutes	12.5 to 44.4 minutes
Specialized cytopathological staff	Not required	Required

Mobile rose®, Mobile rapid-onsite evaluation; ROSE, Rapid-onsite evaluation; FNAC, Fine-needle aspiration cytology

recommended the use of ROSE, particularly in repeat aspirates [6]. Nevertheless, ROSE was currently limited by the availability of professional cytopathology staff and the extension of the procedure time. It can be difficult or nearly impossible to provide an on-site cytopathologist for every thyroid FNAC. In the comparison of turn-around times and manpower, potential use to reduce the delayed repeated aspiration of MR®, and ROSE, we used the data published by O'Malley *et al.* [9]. They reported that ROSE prolonged the average FNAC procedure time from 12.5 to 44.4 min and the requirement of specialized cytological staff. The comparison of the above parameters in the management of thyroid FNAC showed the eminent superiority of MR® over ROSE (Table 2). After washing the aspirated material with CytoRich™ RED solution, MR® has the same application procedure as CytoRich™, and all stains applicable to specimens prepared using CytoRich™ RED (*e.g.* Papanicolaou, Hematolyxin & Eosin, special stains, *etc*.) can be applied. In combination with the LBC method, it can be used for immunohistochemistry, cell block preparation, molecular studies, and other ancillary studies to improve diagnostic accuracy. Alternatively, stains that are not applicable to CytoRich™ RED (*e.g.*, Giemsa) can be used in conventional smears.

In routine practice, repeated aspirations are performed relying on gross visual assessment due to the difficulty in implementing ROSE universally in all institutions. Nonetheless, the accuracy is not sufficient, and intending to compensate for this issue, we conducted this study to investigate the role and potential usefulness of MR®. Since our hospital specializes in thyroid care, all procedures for this study were performed by a single cytopathologist skilled in FNAC procedures, and then MR® analysis was performed only on cytology specimens with insufficient visual assessment. In our opinion, MR® should be introduced in institutions where ROSE is not available, for aspirators who have difficulty making empirical judgments with the gross visual assessment, and for specimens for which it is difficult to confirm cells (*e.g.*, specimens with abundant blood contamination).

We proposed an algorithm for thyroid FNAC using MR® and considered repeated aspiration only when MR® findings were negative.

MR® can be used with Apple iPhone X or later models (*e.g.* iPhone 11 and 12) and Android smartphones with a camera function of 10× digital zoom or higher (*e.g.* Samsung Galaxy S20, HUAWEI P40, AQUOS sense4, *etc*). A wide range of smartphones of various sizes and camera positions can be accommodated by using the adjustment pad. From an operator's perspective, the application of MR® provided real-time feedback and valuable information for repeated FNAC on-site. Real-time feedback to the operator was important when sampling necrotic or hypocellular thyroid nodules, which may have prompted the operator to sample a different, less necrotic region or areas of different echogenicity of the nodule to obtain viable material for diagnosis or ancillary studies [5, 7]. In addition, the captured images of MR® could be observed and recorded in photos or videos, which could also provide valuable feedback and teaching materials with the connected smartphone. This was considered useful for enhancing the skill development of operators, especially less experienced physicians and trainees.

We also demonstrated a good correlation between MR® observation and cytological results. However, there was a false negative fraction of MR® of 5% (6/120, Table 1) due to the difficulty in recognizing small cell aggregates from background artifacts, scratches, air bubbles, and stains on the test tube (Fig. 4). Minor improvements were needed, including the development of next-generation equipment such as equipped smartphones with high-resolution cameras, autofocus adjustment, new MR® View Caps, and fixatives. Interestingly, smartphone-equipped MR® can produce images comparable to cytology at up to ×300 magnification, providing important clues to material adequacy and cell morphology. The potential correlation between MR®, cell morphology, and final histological diagnosis may require further study and confirmation. Our observations may serve as a starting point for future studies.

The limitation of this study was that it was a single-center study with a relatively small sample size. Nevertheless, all procedures performed by a single interventional cytopathologist could minimize the possibility of the operator's variation and inconsistency in the interpretation of results. A large-scale multicenter study

is needed to confirm the usefulness of MR® for FNAC of the thyroid.

In conclusion, MR® was rapid, had high diagnostic performance, and did not require the presence of cytology staff. This device could represent an important innovation for the ROSE of thyroid FNAC to reduce delayed repeated aspiration. However, it had a high false-negative rate and requires minor improvements and further confirmation.

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Disclosure

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the ethics committee of Kuma Hospital (reference number, 20200910-1, 10th September 2020).

Informed consent statement

Written informed consent for fine-needle aspiration cytology was obtained from all subjects involved in the study before the procedures.

Conflicts of interest

The authors declare that there was no conflict of interest.

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