



# Rapid on-site evaluation (ROSE) for fine needle aspiration of thyroid: benefits, challenges and innovative solutions

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**Abstract:** Rapid on-site evaluation/adequacy assessment (ROSE) is considered an essential component of thyroid fine needle aspiration (FNA) and many reported that it significantly decreases the nondiagnostic (ND) rate. The average reported ND rate without ROSE is about 20% and is improved by 12% when ROSE is implemented. However, the data also suggest that the improvement in ND rate after implementation of ROSE is directly related to the ND rate prior to ROSE and that it is mostly beneficial to aspirators with less experience. Several studies have also raised concerns regarding the impact of ROSE as it prolongs the procedure time, requires additional resources and increases the cost incurred by the additional fees. This resulted in a wide variation in the methodology applied to acquire the sample and implement ROSE across the globe with variation in the number of passes performed, stain utilized and the personnel reviewing the slides, e.g., cytotechnologists versus pathologist. This review summarized the literature reporting the impact of ROSE including its pros and cons, its accuracy and reproducibility, concordance between cytotechnologists and pathologists based on final diagnosis and highlights the different ways laboratories attempted to circumvent the challenges. In particular, the review highlights a unique approach practiced in Ito Hospital, Tokyo, Japan.

**Keywords:** Rapid on-site evaluation (ROSE); thyroid; fine needle aspiration (FNA); adequacy assessment

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## Introduction

Fine needle aspiration (FNA) biopsy is currently recommended as the primary method for the evaluation of a suspected thyroid nodule. While aspiration of palpable nodules by clinicians was a common practice in the past, ultrasound (US)-guided aspiration became the leading method in the last two decades especially since the guidelines recommend aspiration of all suspected thyroid nodules measuring  $\geq 10$  mm (1). The procedure is predominantly performed by radiologists although

endocrinologists, surgeons and pathologists can also perform the procedure if properly trained and certified. As a result, the number of thyroid FNA exponentially increased and led to heightened demand on rapid on-site evaluation/adequacy assessment (ROSE), resulting in increased demand on the cytopathology laboratories' time, staff and resources. The following report reviews the current literature evaluating the impact of ROSE including both the benefits and challenges. The report also describes the various modalities laboratories developed to deliver the service.

## How ROSE is performed

While the technique of specimen preparation and how and who provides adequacy assessment may vary by institutions or geographic locations, the basic principal is the same. A staff is available onsite that handles the aspirated sample in real time. The most common practice across the globe is to deposit a drop of the sample on a slide and smear it by a second slide. A variety of quick stains are utilized depending on the preference of the individual laboratory such as Diff Quik (2-9), toluidine blue (10) and rapid Papanicolaou or hematoxylin and eosin (4,11) stain. One slide is stained with a fast stain for immediate review while the other is fixed with alcohol. The needle is rinsed in a collecting media and later processed along with the fixed slide according to laboratory protocols.

## Impact of ROSE

### Sample adequacy

The reported rate of nondiagnostic (ND) FNA is as high as 20% (5,12-15). Several studies concluded that ROSE resulted in a significant decrease in the number of ND cases. In a Chinese study by Jiang *et al.* (10), the authors evaluated 1,103 FNA performed on suspicious thyroid nodules by two trained radiologists with at least 5 years of experience. Of those, 513 were performed with ROSE (WR) attended by a cytotechnologist and a pathologist who evaluated smears stained by Toluidine Blue. The remaining 590 cases were performed without Rose (WOR), in which the radiologists assessed the adequacy based on gross examination of the sample and the imaging findings. They concluded that while ROSE had no significant effect on the overall ND rate (NDR) (6.6% WR *vs.* 9.5% WOR), it resulted in significant reduction in NDR in certain nodules (sub-centimeter, mixed solid-cystic, macro-calcified and hyper vascular nodules). The NDR for other types of nodules was not significantly different. There was no significant difference in the incidence of complications. The mean number of passes was significantly reduced from  $2.8 \pm 0.5$  in the WOR group to  $1.7 \pm 0.6$  in the WR group ( $P < 0.001$ ).

In Brazil where nearly all procedures were performed by radiology residents, Pastorello *et al.* (4) evaluated a total of 4,649 FNA (3,469 WR and 1,180 WOR). The authors found that ROSE resulted in a significant difference with an adequacy rate of 93.4% for those WR and 69.4% for those WOR ( $P < 0.0001$ ). The NDR WR was 6.6%. While the operator routinely performed 3 passes when ROSE was not

performed, the mean number of passes was reduced in the WR group to  $1.48 \pm 0.71$ . No significant difference in the rate of malignancy among the two groups was noted.

In Colombia, Medina Chamorro *et al.* (2) evaluated 522 FNA and found that the NDR was 40% among the 197/522 WOR and only 9.5% in the 325/522 WR leading to a 30.5% decrease in the NDR.

Similarly in the USA, Zhu *et al.* (8) evaluated 833 cases and reported a significant reduction ( $P < 0.001$ ) in NDR from 31.8% (140/440) WOR to 5.9% (26/443) WR. Of interest, the authors found that follow up either by repeat FNA or resection of ND cases did not reveal any missed malignancy in the WR group while 7 carcinomas were found in the follow up of the WOR group. A meta-analysis by Witt *et al.* (16,17) concluded that ROSE significantly improves specimen adequacy with an average adequacy of 92% in the WR group *vs.* 83% in the WOR group. However, the authors also suggested that based on gross inspection of the data, the improvement in adequacy due to ROSE may be related to the adequacy rate WOR. According to the meta-analysis by Schmidt *et al.* (17), studies with high adequacy rates WOR showed low improvement after ROSE was implemented and therefore had little opportunity for improvement.

On the other hand, others have questioned the value of ROSE. Ghofrani *et al.* (18) assessed a total of 981 US-guided FNA and 521 thyroid FNA performed by palpation alone. The NDR for the entire group was 5.3% WR and 10.5% WR. However the NDR for the US-guided group alone was 4.5% WR *vs.* 7.1% WOR. The authors concluded that although ROSE significantly reduced the NDR among the entire group ( $P < 0.01$ ) as well as among FNA performed by palpation ( $P < 0.025$ ), the 2.6% reduction in NDR noted among US-guided cases was not significant.

### Time and cost

In an effort to evaluate the cost effectiveness of ROSE, Eedes *et al.* (3) retrospectively reviewed 331 thyroid FNA. In their institution, such FNA could be performed by endocrinologist, surgeons or radiologist with and without ROSE. A thyroid clinic was also conducted twice a week to evaluate thyroid nodule referrals and staffed by an endocrinologist, a surgeon and a radiologist. They examined the NDR and calculated the time spent by cytology to provide ROSE. The NDR decreased from 14.9% (32/215 WOR) to 9.5% (11/116 WR). Their estimated time spent for ROSE performed based on Diff Quik stain in the

Thyroid Clinic was 35 and 57 minutes outside the Thyroid Clinic. The authors concluded that ROSE resulted in 23 additional adequate specimens in the WR group at a relatively high “cost” of 220 minutes of additional cytologist time per specimen and recommended the judicious use of ROSE. The authors also noted that ROSE was provided only within the hospital vicinity and that all the operators for these aspirates were highly experienced. Accordingly, if their results could be attributed to the operators, the bias would have been in favor of ROSE and US guidance. Selection bias could have also been a factor since ROSE tend to be requested for repeat FNA and those that are probably difficult to aspirate. Similarly, Tambouret *et al.* (19) reported that cytotechnologists spent about 70 minutes in performing ROSE and that radiologists with least experience had the highest benefit from ROSE. O’Malley *et al.* (11) raised a significant concern regarding the benefit of thyroid ROSE. The authors reviewed 50 retrospective and 21 prospective thyroid FNAs WR and compared them with 50 prospective FNA performed WOR. ROSE was performed by a cytopathologist based on examining on-site H&E stained slides. When they evaluated the 71 prospective FNA in which the total procedure time has been recorded, they found that ROSE prolonged the average procedure time from 12.5 to 44.4 minutes, without any significant decrease in the percentage of NDR (24% *vs.* 20%). About 50% of those WR and WOR were either limited or ND.

To assess the cost effectiveness of the additional cost and resources incurred by ROSE against the overall health care cost of managing a patient with a thyroid nodule, Nasuti *et al.* (5) reviewed 5,688 FNA performed in various organs WR. The authors compared the very low NDR (0.98%) at their institution when ROSE is provided to the reported NDR of 20% for FNA WOR according to the literature. The authors concluded that despite the additional fees charged by cytology for ROSE, it was responsible for an estimated cost savings of >\$400,000 per year as a result of avoiding repeated aspirates (based on the assumption that all ND aspirates will be repeated). The authors also emphasized that their calculations may be underestimated because they did not take into account indirect cost to the patient such as additional time off work and longer hospital stays due to repeated biopsy.

### *Number of passes*

When ROSE is not requested, 3–4 passes are routinely

performed and grossly assessed by the operator. However, when ROSE is also performed, the number of passes vary since the operator continues to perform new passes until an adequate verdict is issued. Several studies attempted to assess the optimal number of passes. Eedes *et al.* (3) reported that 66% of their cases were deemed adequate with 2 passes and 98% with 3 or fewer passes and that additional passes were not useful. Additional studies have also demonstrated that the significant increase in diagnostic yield was after the second pass and that additional passes gave little further improvement (20,21). Zhu and Michael (8) correlated the number of passes with adequacy in their cases. They found that the number of ND cases decreased from 25% to 11.0% ( $P < 0.001$ ) in the WR procedures when 4 passes were performed. Their data demonstrated a continuous reduction of ND cases with the increase in number of FNA passes up to 6 passes. Additional passes did not significantly improve adequacy.

### **Concordance between ROSE and final diagnosis**

In the report by Eedes *et al.* (3), the authors noted that criteria for adequacy varied somewhat among their cytologists. Generally, they considered a specimen adequate when several groups of at least a dozen follicular cells were present and that they required less cellularity when abundant colloid was identified. Of the cases deemed adequate by ROSE in their study, 20% had suboptimal cellularity upon final review. The authors explained that there seems to be a tendency by pathologists to consider borderline samples as adequate assuming that additional material will be seen on the TP, however such expectation may not always materialize. Meanwhile, Jing *et al.* (7) retrospectively evaluated their concordance rate after their institution self- implemented a standardized requirement of at least 6 clusters of follicular cells, 10 cells each on the examined Diff-Quik slides. There was total agreement on specimen adequacy in 93% of cases between adequacy by ROSE and final assessment. Adequacy was converted in 70/178 samples from ND to diagnostic while only 2/853 specimens were reversed to ND on final diagnosis. Their cytohistologic concordance rate was 93.9% for non-neoplastic nodules and 82.3% for neoplastic ones.

To assess concordance rate for adequacy provided by cytotechnologists on-site versus pathologists, Olson (6) retrospectively reviewed 2,261 cases, 64.7% ( $n=1,462$ ) attended by cytotechnologists and 35.3% ( $n=799$ ) attended by cytopathologists. The authors found no difference in the

adequacy downgrade rate for cytotechnologists compared with that for cytopathologists (4.1% *vs.* 5%).

## Discussion

FNA is an important triage tool for assessing thyroid nodules and evaluate for malignancy. The majority of the literature favors the value of utilizing ROSE during the procedure and confirms that it results in lower NDR. The Bethesda Reporting System for Thyroid Aspirates recommends the use of ROSE when possible and particularly in repeat aspirates (22,23). However, several studies as described above, have either doubted the value of ROSE or raised concerns primarily regarding the related cost, prolonged procedure time and the burden imposed on the cytology laboratories to provide this service.

The variation among studies may be a result of multiple factors. For example, the nodule size requirement set by different institutions may affect the NDR since the smaller the size, the higher the expected NDR (24). The stain protocol used by pathology can impact the time required for ROSE. Some studies performed ROSE on fixed and H&E stained slides (11). This technique requires at least 2 minutes and necessitate the use of mounting media and coverslip. Others described using air dried Diff-Quik stained (3,8) or Toluidine Blue stained slides (10), both are done within 30–40 seconds and require no mounting media. The latter, would save at least one minute per pass. Also, the reported operator experience varied among studies ranging from residents to well experienced operators. In fact, according to the meta-analysis by Schmidt *et al.* (17), while on average, ROSE improved the adequacy rate by 12%, there was considerable variability across studies. Based on the data, 65% of the variability in the adequacy rate WR was found to occur because of differences in the adequacy rate WOR. Based, on these studies, the value of ROSE seems to be highest when the operators are less experienced.

Whether ROSE is still worth the additional cost or not is still controversial. There is no doubt that the exponential increase in the number of thyroid FNA in the last two decades have put tremendous burden on the cytology laboratories. First, not all laboratories have the resources to offer ROSE which requires mobilizing the cytotechnologist or trainee to the FNA site; time spent away from doing other duties in pathology; and in some countries the mandate for a cytopathologist presence as well. Second, the reimbursement fees vary tremendously depending on the third party payers, and regulations imposed by

such payers. For example, some healthcare payers put a cap on procedure fees regardless of whether ROSE or other ancillary tests were performed or not. In the USA, where many third party payers still reimburse for ROSE, the Medicare and Medicaid impose a ruling that for any charged fee, a pathologist has to be present and personally reviews the key portion of the procedure. As a result, even when cytotechnologist or trainee are available and quite capable to perform ROSE, a pathologist is still required to personally review the slides and provides the assessment of adequacy (key portion).

Some may argue that the value of ROSE outweighs its additional cost and that it results in decrease in the overall healthcare cost (5). This conclusion is based on the assumption that ND cases will require repeat aspiration which will inflict additional cost on both the health care system and the patient. Others caution against the routine use of ROSE and recommend judicious use (3). Layfield *et al.* (25) analyzed the compensation for ROSE which varied by healthcare providers and concluded that ROSE is insufficiently compensated by current Medicare.

## ROSE: to do it or not to do it?

The decision whether to offer ROSE or not lies in each institution individually depending on its own resources, patient population and fee coverage. As a result, institutions have responded to the need for ROSE in various ways. Some institutions situated pathologists attending ROSE to perform their routine duties in a location adjacent to the ultrasonography/procedure rooms thus minimizing the time spent by the cytopathologist (4). Many institutions adopted selective use of ROSE only for suspicious nodules or for repeat FNA. Who prepares the smears also vary by institution, some will still send their cytotechnologists or trainee while others elected to train the radiology staff to prepare and stain the smears. Others instituted telecytology systems and reported great results (9,26), where cytotechnologists prepare, stain and screen the slides then connect with the cytopathologist's working station using a secure server to transmit in time the reviewed slides and project the concerning areas. This procedure saves the cytopathologist time, yet still requires cytotechnologist attendance. However, such success relies heavily on well trained and capable cytotechnologists since they lead the pathologist through the transmitted images.

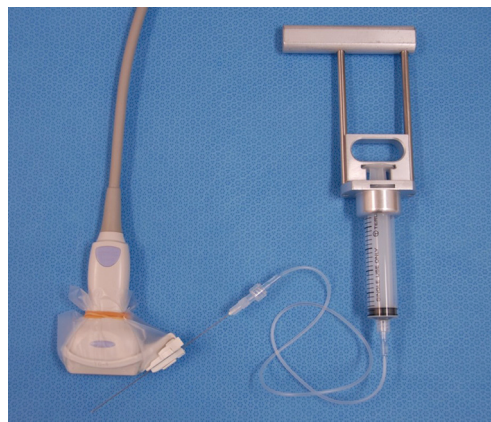
The first author had the pleasure of visiting Ito Hospital in Tokyo Japan where she observed their different approach



**Figure 1** Patient lies in a supine position and asked to look up. The nodule and safest path for FNA is located using the US probe. FNA, fine needle aspiration; US, ultrasound.

to thyroid FNA and ROSE. Ito Hospital deals exclusively with the evaluation and management of thyroid nodules. Consequently they perform a large number of US-guided FNA on a daily basis but a cytopathologist attends the hospital three times a week only to review the cases. While in the USA and many other countries including Japan, the free hand technique is the preferred method for US-guided aspirations, Ito Hospital utilizes a slightly different technique as described below (27).

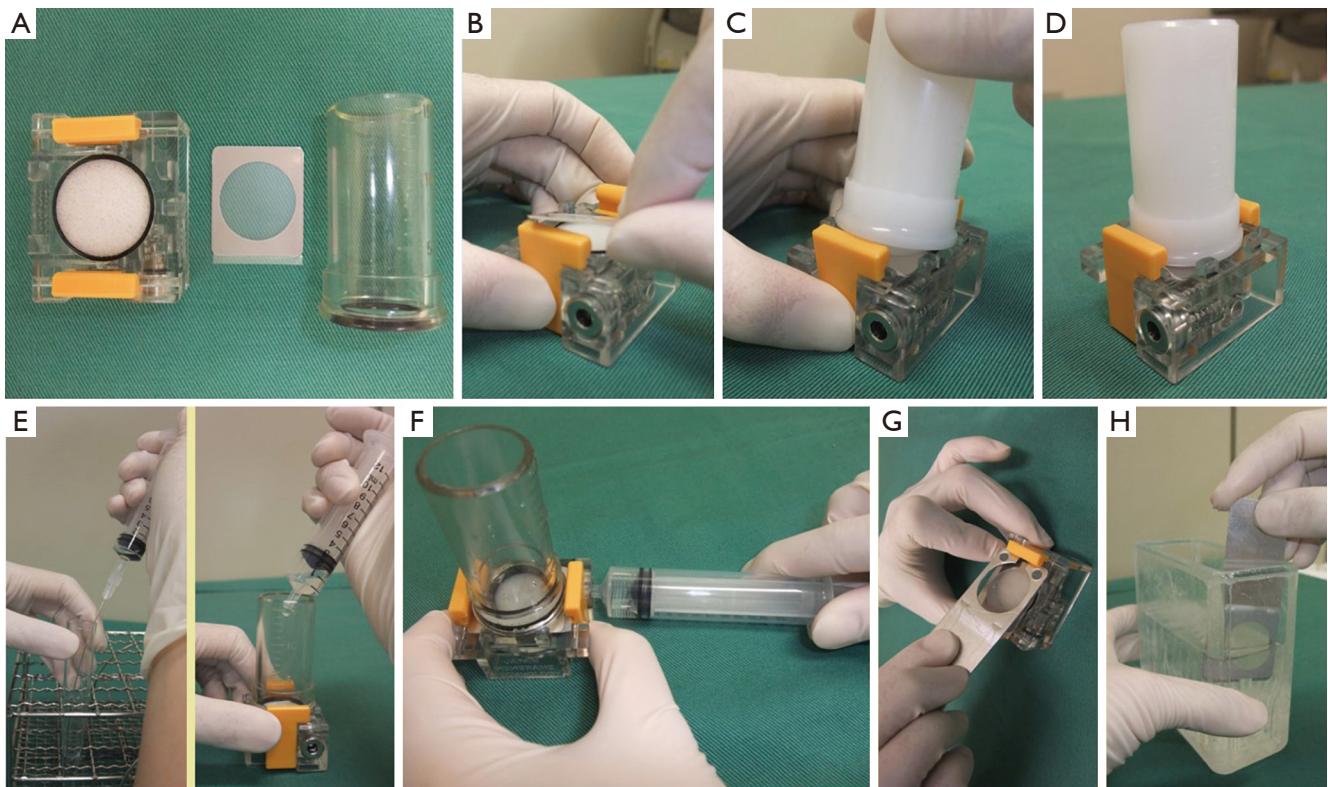
While US-guided FNA technique is beyond the scope of this review, a short description to highlight the different methods is warranted. In general, the patient lies in a supine position and is asked to look up. Once the thyroid nodule is identified using US and the vascularity of the nodule is assessed by Doppler, the safest access to avoid large vessels is mapped. The site is prepped in the regular, sterile fashion and the appropriate sterile coverage is applied to maintain sterility; 1% lidocaine is used as a local anesthetic (some providers use 4% lidocaine topical anesthetic cream or perform the procedure without any local anesthetics). Then a sterile probe cover is applied to maintain site sterility. With US-guidance, using a freehand technique, the nodule is accessed with a 22–27-gauge needle (most commonly 25-gauge). After removing the needle styloid, the needle is moved from one side to another of the nodule without applying any suction (capillary action). Needle motion is continuously monitored during sample acquisition. A minimum of 5 cycles of to-and-fro is recommended (27). In Ito Hospital, the patient is prepped in the same way but no local anesthesia is used. The US probe used has a mounted needle guidance bracket (*Figure 1*). A 22-gauge needle, 120 mm long (TOP Corporation, Tokyo, Japan) is attached



**Figure 2** The US probe used has a mounted needle guidance bracket. A 22-gauge needle, 120 mm long (TOP Corporation, Tokyo, Japan) is attached to a 20 mL syringe with an extension tube. US, ultrasound.

to a 20 mL syringe with an extension tube (*Figure 2*). After confirming the needle tip is in the target nodule under US-guidance by the operator, negative pressure is generated by the assisting nurse using a pestle type syringe holder. The operator rotates the needle between their thumb and index finger in a fast motion to collect the sample. Then the negative pressure is released and the needle is removed and handed to a medical technologist for processing and adequacy assessment. Only one pass is performed unless the sample is deemed inadequate. It is worth noting that while the utility of the needle guidance mounted on the probe cover with the plastic attachment is slightly more expensive, it does provide easier and faster access to the nodule. However, the free hand technique provides more flexibility with the approach (not locked to specific angle set by the guide) and consequently the operator can use a perpendicular, parallel or both approaches while obtaining the sample. Rotating the needle during aspiration improves sample adequacy.

In Ito Hospital, once the needle is handed to the medical technologist, a small portion is dropped on a slide and smeared in the conventional method with one slide air dried for Diff-Quik stain and the other slide fixed in ethanol for Papanicolaou stain (both stains performed later in the laboratory). The remaining sample is processed on site using a filter apparatus (Senshin Medical Inc.). In short a filter is installed within the apparatus and a cylinder is installed above the filter. The cylinder is filled with lactate Ringer solution-Saviosol™ adjusted osmotic pressure with



**Figure 3** Use of the filter apparatus. (A) Components consist of the main apparatus, a filter and a cylinder; (B) the filter is installed; (C) the cylinder is installed above the filter; (D) apparatus ready to use; (E) cylinder is filled with Ringer solution and specimen is rinsed in the solution; (F) negative pressure is applied to the cylinder; (G) the filter is grossly examined and (H) filter is put in ethanol fixative.

low molecular weight dextran 40 (Otsuka pharmaceutical Co. Ltd, Tokyo, Japan). The needle is rinsed in the Ringer solution and a negative pressure is applied to pull the solution containing the needle rinse through the filter. The filter is removed and grossly examined for adequacy and then fixed in ethanol for Papanicolaou stain (Figure 3). Their reported NDR for a total of 14,668 FNA from 11,830 patients was 1,066 (7.3%). However, when cyst fluid only cases (732/1,066 or 5%) that are diagnosed as benign per Japan Reporting System are excluded, the NDR is only 2.3% (334 cases) (27).

ROSE plays a much larger role that goes beyond providing adequacy assessment of the sample or a preliminary diagnosis. The immediate feedback provided by pathology can impact the operator in several ways. First, it assists the operator in deciding the best needle path and whether it needs to be differently oriented. Second, it provides on time feedback that enhances the skill development of the operator particularly the less experienced and/or trainee. Third, the presence

of pathology results in direct correlation of the ultrasonographic appearance of the mass with the correlating morphology thus facilitating further decisions, e.g., acquiring a concurrent biopsy, additional passes, etc.

While therapeutic decisions are not made based on the preliminary diagnosis, it is still essential to triage the sample for further work-up and ancillary tests. For example, a suspicion for medullary carcinoma will trigger the collection of additional passes beyond simple adequacy to prepare a cell block for immunochemistry. Similarly, when lymphoma is suspected the sample can be triaged for flow cytometry. As a result, the specimen is appropriately handled and repeat procedures to collect for the ancillary tests can be avoided. With the integration of molecular testing of thyroid FNA for indeterminate diagnoses, ROSE is becoming essential to achieve an adequate sample for both diagnosis and testing.

In conclusion, whether the sample is triaged in the first FNA utilizing ROSE or differed to repeat procedure selectively utilizing ROSE or intraoperative consultation, i.e., frozen section is decided by the individual institutions

as they weigh the value of ROSE against the incurred expenses and available resources.

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