Rapid on-site evaluation of fine needle aspiration specimens by cytology scientists: a review of 3032 specimens.

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Running title: ROSE of FNA specimens by scientists

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Abstract

Objectives: To determine (i) the accuracy of cytology scientists at assessing specimen adequacy by rapid on-site evaluation (ROSE) at fine needle aspiration (FNA) cytology collections and (ii) whether thyroid FNA with ROSE had lower inadequacy rates than non-attended FNAs.

Methods: The ROSE of adequacy for 3032 specimens from 17 anatomical sites collected over a 20-month period was compared with the final report assessment of adequacy. ROSE was performed by 19 cytology scientists. The report profile for 1545 thyroid nodules with ROSE was compared with that for 1536 consecutive non-ROSE thyroid FNAs reported by the same cytopathologists during the study period.

Results: ROSE was adequate in 75% (2276/3032), inadequate in 12% (366/3032) and in 13% (390/3032) no opinion was rendered. Of the 2276 cases assessed adequate by ROSE, 99.6% were reported as adequate for assessment; eight specimens had adequacy downgraded on the final report. Forty two per cent of cases with a ROSE assessment of inadequate were reported as adequate (154/366), while 93% (363/390) with no opinion rendered were reported adequate. The overall final report adequacy rate for the 3032 specimens was 94% (2843/3032). Confirmation of a ROSE of adequacy at reporting was uniformly high amongst the 19 scientists, ranging from 98.3 to 100%. The inadequacy rate for thyroid FNAs with ROSE (6%) was significantly ($P<0.0001$) lower than non-ROSE thyroid FNAs (17%). A significantly ($P=0.02$) higher proportion of adequate ROSE thyroid specimens were reported with abnormalities, compared with non-ROSE thyroid collections.

Conclusions: Cytology scientists are highly accurate at determining specimen adequacy at ROSE for a wide range of body sites. ROSE of thyroid FNAs can significantly reduce inadequate reports.

Keywords: Fine needle aspiration; FNA; rapid on-site evaluation; ROSE; cytology; thyroid

INTRODUCTION

DeMay describes fine needle aspiration (FNA) cytology as SAFE (simple, accurate, fast, economic - and safe).[1] In many settings FNA is the most cost-effective investigation for obtaining a morphological diagnosis.[1,2] A definitive diagnosis is not always possible, but FNA results are often of value in determining further patient management. In some settings, particularly when combined with ancillary techniques, a precise definitive diagnosis may be possible. The accuracy of the technique is, however, dependent on representative sampling and optimal preparation of the specimen. False negative results may occur due to non-representative sampling or misinterpretation of poorly sampled or prepared material.[3-5] In addition to technique, the experience of the collector and the nature of some lesions may contribute to inadequate samples.[6] Obtaining representative samples from desmoplastic, cystic or necrotic lesions or very small or heterogenous masses may be problematic.

One of the more commonly sampled sites is the thyroid, where FNA is considered the test of choice for the investigation of nodules. Obtaining adequate well-preserved material from thyroid may be particularly challenging due to the highly vascular nature of the organ and the frequency of cystic change in nodules. Even in experienced hands approximately 10-15% of thyroid aspirates are non-diagnostic.[1,5]

Attendance at the FNA procedure by a cytologist (cytopathologist or cytology scientist) is used in many centres as a means of improving diagnostic accuracy and adequacy rates by ensuring optimal preparation of the specimen. Rapid on-site evaluation (ROSE) of the specimen allows an assessment of the representativeness of the sample and triage of

additional material for ancillary studies. Several studies have found higher adequacy rates with ROSE, [7,8] although a few have found either negligible[9,10] or no improvement.[11] Any benefits also need to be balanced against the demands on cytologists' time and additional procedure time.[7,11] The use of cytology scientists, or cytotechnologists as they are termed in the USA, has been suggested as a cost-effective means of providing ROSE. Demand for this service in our practice, particularly for FNA of thyroid nodules, has grown steadily in recent years. However we are unaware of any studies that have examined the efficacy of ROSE in the Australian setting. This study reviews our service with a view to determining how well cytology scientists performed at assessing adequacy in a range of body sites and if ROSE of thyroid FNA was effective at improving adequacy rates.

METHODS

Sullivan Nicolaides Pathology operates a busy FNA cytology service, reporting over 14,000 specimens per year, collected throughout the states of Queensland and New South Wales. This includes an FNA ROSE service with cytology scientists attending collections in over 30 locations within the Brisbane metropolitan area, where the reporting laboratory is situated. Most collections occur under ultrasound guidance in radiology practices, with some deeper aspirates performed under computed tomography (CT) guidance or endoscopic ultrasound (EUS) guidance in operating theatres. The laboratory has operated a ROSE service for many years, but due to increased demand a formal training programme in ROSE for cytology scientists was implemented in 2007. All participants have postgraduate cytology certification and a minimum of three years diagnostic experience, including routine involvement in the examination of FNA specimens from a wide range of sites. The ROSE training includes instruction in specimen preparation, adequacy assessment and triaging of material into additional cytology preparations, cell block, flow cytometry or microbiology. The course runs for several weeks and includes accompanying experienced attenders to a variety of collection sites and a final assessment.

Scientists performing ROSE complete an attendance form with each collection to ensure capture of relevant information and communication with the reporting pathologist. This form accompanies the request through processing and reporting and details the number of slides made, any other specimen collected, relevant clinical information in addition to that stated on the request form, macroscopic features of the specimen, any other useful information relating to the procedure and the final ROSE interpretation of adequacy. Criteria for adequacy during the study period were the presence of a diagnostic

sample of lesional cells or representative cells of the lesion being sampled, e.g. lymphoid cells in the case of lymph node lesions. For thyroid nodules a minimum of six groups of ten or more follicular cells, preferably in one pass, was required. Exceptions to this were when the radiological findings were consistent with a colloid nodule and abundant colloid but scant follicular cells were collected or in the case of cystic lesions. For the latter the attending scientist verbally reported that cystic material only was present and that that was adequate providing no solid component was present and the cyst was completely drained.

Scientists usually prepared two slides from each needle pass. One was wet fixed for Papanicolaou staining in the laboratory and the other air-dried and stained on-site with a rapid Romanowsky stain (Rapid Differential Stain, POCD, NSW). The latter was used to assess adequacy and was examined wet without a coverslip. In most cases additional passes were collected until either the scientist indicated adequate material for a definitive diagnosis was obtained or the radiologist decided to terminate the procedure. After the preparation of slides the needle was usually rinsed into an FNA washout fluid (HAMS). In some cases additional material was requested so that a dedicated pass could be rinsed into FNA washout fluid for laboratory processing as a cell block for immunocytochemical staining or cell surface marker analysis by flow cytometry.

We reviewed results from over 3000 consecutive FNA collections that were attended with ROSE by cytology scientists over a 20-month period to March 2013. The specimens were collected from a wide range of sites, with thyroid FNAs constituting about half. The ROSE of adequacy was compared with the final reported diagnosis. The ROSE opinion communicated to the collector was recorded on the attendance form as adequate; inadequate or adequacy not assessed. The latter were cases where the procedure was

terminated due to concern for the patient or if the radiologist was satisfied that the lesion was sampled, without the attending scientist confirming adequacy. Cytologists only viewed some of the material collected. Therefore a proportion of cases evaluated as inadequate on-site would be expected to be reported adequate after evaluation of all the material in the laboratory. The accuracy of adequacy assessment was therefore calculated as the proportion of cases evaluated as adequate at ROSE that were reported adequate. This was calculated for the total cases and for the 19 individual scientists that provided the service during the study period. Eight cytopathologists reported specimens throughout the study period.

The reporting profile and the number of direct smear slides made for thyroid FNA nodules with ROSE were compared with reports on 1536 consecutive thyroid FNA nodules from 1432 patients reported during the same period that were not attended with a ROSE. Specimens with ROSE were collected by 109 radiologists. Non-ROSE specimens were collected by 141 referrers, almost all by radiologists (1459/1536; 95%), with the remainder collected by surgeons (68/1536; 4.4%) and a few by rural general practitioners (9/1536; 0.6%). A small number of radiologists (14) had both ROSE and non-ROSE specimens included in the study. The number of cases sampled by individual aspirators varied widely in both groups (ROSE range = 1 to 107; non-ROSE range = 1 to 65). Cases collected without ROSE were generally performed outside either the hours of operation or the geographic scope of our attendance service or by collectors who routinely chose not use the service.

All ROSE and non-ROSE cases were reported by the same eight cytopathologists. In Australia national standardised reporting terminology for thyroid FNA is still under development. Cases reported in the study were classified into the following categories: (i)

inadequate; (ii) benign; (iii) cyst; (iv) atypical cells; (v) atypical follicular pattern or suspicious for follicular neoplasm; (vi) suspicious for malignancy; (vii) malignant , corresponding to the British classifications Thy1, Thy2, Thy2C, Thy3A, Thy3F, Thy4 and Thy5, respectively. The inadequate rate for the ROSE and non-ROSE thyroid FNAs was compared using Fisher's exact test.

RESULTS

During the study period 19 scientists attended the FNA collection of 3032 specimens from 17 anatomic sites (Table 1). The ROSE was adequate in 75.1% (2276/3032), inadequate in 12.1% (366/3032) and in ~~13~~12.8% (390/3032) no opinion was rendered. Of cases assessed adequate by ROSE, ~~97~~99.6% (2268/2276) were reported as adequate for assessment, with only eight cases being reported as insufficient for diagnosis in the final report. Forty two per cent of cases with a ROSE assessment of inadequate were reported as adequate (154/366), while 93.1% (363/390) with no opinion rendered were reported adequate.

The overall final report adequacy rate for the 3032 specimens was ~~94~~93.8% (2843/3032). The FNAs were collected from a wide range of body sites, with thyroid making up just over half. Inadequacy rates varied from 0-21% for the various sites, although some sites had low numbers of cases. FNA axilla specimens showed the highest inadequacy rates (10%) of sites with more than 50 cases. A high proportion of inadequate specimens from leg/lower extremity were noted to be unsatisfactory (21.4%; 6/28), probably reflecting the nature of lesions sampled in these sites, with many ill-defined or soft tissue lesions. Of the three non-thyroid aspirates with ROSE adequate but report inadequate, one was a breast lesion that had a concurrent biopsy showing fibrocystic change, one was a parotid mass with no further results and one a lymph node which was thought to be non-suspicious on ultrasound. The lymph node had an extra pass dedicated to the washout but it contained insufficient cells for statistically viable cell surface marker analysis. A repeat FNA was reported as B cell lymphoma.

Nineteen scientists provided ROSE in the study period and varied considerably in experience with ROSE (<1 to >20yrs) and in the number of specimen collections attended in

the period (between 4 and 701 specimens each (median=109). Figure 3 summarises the performance of scientists carrying out ROSE. One scientist attended only four cases, all of which were assessed adequate and confirmed on the final report. Excluding these data, the percentage of specimens with a ROSE of adequate by the 18 scientists varied from 62.5-88% (mean 75.2, sd= 6.7; median = 75.6). Confirmation of a ROSE of adequacy at reporting was uniformly high, ranging from 98.3 to 100% (median= 98.5%; mean= 99.75, sd = 0.51). The eight cases with an incorrect ROSE of adequate were assessed by four different scientists.

A total of 1593 thyroid FNA specimens were collected with ROSE in the study period. Overall 94.2% were reported adequate (1501/1593) giving an inadequate rate of 5.8% for attended thyroid FNAs. A ROSE evaluation of adequacy was made in 77.7% (1239/1593) of cases and this was confirmed in 99.6% (1234/1239). Five thyroid cases were assessed adequate on-site but reported as inadequate. These included two specimens collected post-hemithyroidectomy, a patient under treatment for lymphoma with a complex nodule with calcification, a solid and cystic lesion, and one with a dominant nodule in the isthmus. One of these patients had a repeat FNA with our laboratory, which was reported benign.

Comparison of the 1593 ROSE thyroid FNAs with 1536 non-ROSE thyroid FNA reports is summarised in Figure 1. Inadequacy rates were significantly ($P<0.0001$) lower for cases with a ROSE (92/1593; 5.8%) compared with non-ROSE cases (262/1536; 17.1%). Figure 2 displays the report profile for adequate ROSE and non-ROSE specimens. A significantly ($P=0.0371$) higher proportion of specimens with ROSE (13.5%; 203/1501) were reported with a cellular abnormality (atypical + atypical follicular pattern + suspicious for malignancy + malignant: Thy3,4, or 5) than non-ROSE (10.9%; 139/1274). Overall, similar numbers of

direct smear slides per specimen were made for ROSE (mean = 5.75; median = 6) and non-ROSE thyroid (mean = 5.263 ; median =6) specimens.

Discussion

The efficacy of FNA as a means of providing a rapid reliable morphological diagnosis is well established. However the accuracy of the test is reliant on adequate sampling, preparation and appropriate handling of the specimen for adjunctive testing. Rapid on-site evaluation of specimens by a cytologist has been recommended as a means of obviating some of these limitations. Reported advantages of ROSE include improved specimen preparation, lower inadequate rates,[12] greater accuracy with a reduction in indeterminate diagnoses,[13] fewer passes and hence less complications,[14] improved adequacy of histology specimens collected at the same procedure, improved specimen handling for additional tests[8] and educational benefits by providing immediate feedback for specimen collectors.[9,15] Considerable cost-savings to the health system may also be achieved by reducing repeat procedures .[7,15-17] These benefits have been recognised in Australia by the provision of items in the Medicare schedule for ROSE by both medical and non-medical cytologists. However not all studies have found ROSE to be either effective or economical and there has been debate over who should perform the evaluation.

Some studies have failed to find a significant improvement in adequacy rates or accuracy with ROSE, including ultrasound-guided thyroid samples[11] and CT-guided thoracic and abdominal FNAs.[9,10] However a majority of studies, particularly those with larger sample sizes, have found ROSE significantly improves adequacy rates. Schmidt et al[18] recently published a meta-analysis designed to determine the influence of ROSE on adequacy rates for FNA of various body sites. Twenty-five studies examining 12,407 cases that compared cohorts with ROSE FNA samples and non-ROSE FNAs reported by the same laboratory were included and showed an overall improvement of 12% in adequacy rates with ROSE. They found considerable variation across studies and highlighted that much of the variability in comparative adequacy rates with and without ROSE reflected variations in non-ROSE adequacy rates amongst laboratories. There was more variation in non-ROSE adequacy across centres for each of the nine body sites examined than there was between body sites. This

may in part reflect variation in adequacy criteria for some body sites. As might be expected, institutions reporting high adequacy rates with non-ROSE FNAs showed less improvement with ROSE. However the improvement in adequacy rates with ROSE was statistically significant for all body sites after adjusting for the non-ROSE adequacy rate.

Thyroid is now one of the most commonly sampled sites for FNA. The cost-effectiveness of thyroid FNA is largely reliant on the rates of inadequate and indeterminate diagnoses.[16,19] Inadequate rates as high as 27% or more have been reported,[19,20] although a rate of less than 10-15% for individual collectors is considered desirable.[21,22] The importance of specimen adequacy in thyroid FNA is further underlined by an analysis of errors in thyroid FNA reporting that determined the root cause of most errors was poor specimen quality resulting in either over- or underinterpretation.[5] The use of ultrasound-guided collection,[23,24] increasing experience[6] and ROSE have been demonstrated to be effective at reducing inadequacy rates. Our study demonstrated a highly significant improvement in specimen adequacy for thyroid FNA with ROSE (94%) compared with non-ROSE thyroid specimens (83%). The inadequate rate for non-ROSE thyroid FNA in our practice was quite high at 17%, even though almost all were collected by radiologists, however this reduced to less than 6% with ROSE. Training and aspirator experience with FNA technique are known to influence adequacy rates.[6,25] Unfortunately, in the current study we were unable to quantify this for the ROSE and non-ROSE aspirators. It is therefore possible that the aspirators utilising ROSE in our study were generally more experienced and more interested in the FNA procedure and this may have contributed to the higher adequacy rate observed in the ROSE specimens. However the sample was large, some aspirators were common to both groups and some of those with non-ROSE collections are known to be very experienced, so we believe this alone is unlikely to have ~~contiributed~~ accounted for~~to~~ the magnitude of difference in adequacy rates between the two groups. The improvement in thyroid FNA adequacy rate of 11% was similar to the overall improvement of 12% reported in the metaanalysis by Schmidt et al.[18]

Some authors have highlighted the demands on pathologists' time, the inadequate reimbursement for cytopathologist ROSE in the USA[26] and also noted significantly increased procedure times when ROSE is implemented. Eades et al[7] reviewed 331 thyroid FNA ROSE and found improved adequacy rates, but they felt the additional commitment in pathologist time rendered pathologist ROSE impractical. They suggested a better strategy might be to routinely collect three passes and use ROSE for special circumstances, such as re-collections. However, ROSE generally requires fewer passes than fixed sample size policies and achieves higher adequacy rates.[14]

The use of non-medical staff to perform ROSE has therefore been examined as a means of providing a more economical on-site evaluation of the material collected. Savoy et al[27] compared the ability of endosonographers, who had received some training in cytology, at performing ROSE for EUS FNA specimens with that of a cytotechnologist, using a cytopathologist interpretation as the standard. They found the former to be variable and inferior to a cytotechnologist in terms of both determination of adequacy and accuracy. Renshaw[28] has also commented that ROSE involves more than just counting cells. Therefore personnel, such as endocrinologists, who are not skilled in microscopy and not subject to the same quality controls and scrutiny as diagnostic laboratories, are inappropriate to perform the evaluation.

Some authors[17] believe that only experienced cytopathologists should perform ROSE. A study of 552 thyroid FNAs with ROSE performed by cytopathologists (324) and cytotechnologists (228) found an assessment of adequacy to be correct significantly more frequently when rendered by a cytopathologist (97% vs 93% respectively). [29] However cytology scientists have been increasingly used in this role and more recently, several large studies have demonstrated that cytology scientists are highly accurate in determining adequacy.[15,30] Olson et al[13] found no difference in adequacy downgrade rates for medical versus non-medical cytologists performing ROSE in a sample of 2,261 thyroid FNAs. Wotruba and colleagues[15] reported concordance of adequacy assessment by

cytotechnologists with a preliminary diagnosis by pathologists in 98.8% of cases and a recent large study of 5241, mainly non-thyroid, FNAs found cytotechnologists to be 95% accurate at determining adequacy.[30] The current study confirmed a high level of accuracy by cytology scientists in predicting adequacy with 99.6% (2268/2276) of total FNAs and the same proportion (1234/1239) of thyroid FNAs with a ROSE of adequate confirmed on the final report. A disadvantage in using scientists rather than pathologists in this role is that a preliminary diagnosis is not issued, only an assessment of adequacy. Although a preliminary report at collection may be of value in some settings by guiding further immediate patient management, it is not an issue in most cases when a timely final report is available. If well trained, and provided with cytopathologist support, we believe scientists are capable of determining adequacy across a range of body sites and appropriately requesting further material.

We found ROSE performed by scientists to be accurate in determining adequacy, even with a large group of staff examining specimens from a wide variety of body sites. We believe this highly reliable performance is due in large part to the thorough training provided, the clear criteria for adequacy and the scientist's familiarity with FNA interpretation gained through involvement in routine reporting. As noted by others[13] there is a tendency for scientists to undercall adequacy. Olson et al[13] found cytotechnologists to be significantly more likely to undercall thyroid FNA adequacy at ROSE than pathologists. Around 40% of the 366 cases assessed inadequate at ROSE in the current study were reported adequate. This probably reflects the emphasis during training that is placed on not overcalling adequacy, as well as the limited material examined during ROSE. In practice this is not a problem providing the patient does not undergo unnecessary passes. All FNA cases in our laboratory have a report issued within 24hrs of collection, ensuring unnecessary repeat collections do not occur.

Interestingly, most (93%) of the 390 cases where the radiologist terminated the procedure without a ROSE opinion being rendered were reported adequate. This may in part reflect better

preparation and specimen handling and the additional clinical information obtained by attending the collection. Collins et al [31] recently reported that ROSE of endobronchial ultrasound guided FNAs led to sampling of significantly fewer sites and to fewer slides per case. We found no difference in the number of direct smear slides made for thyroid FNA with or without ROSE. However we believe a significant benefit of ROSE is ensuring the collection of additional material for ancillary studies. A recent study from our laboratory[32] examined cell block adequacy and found the preparations were almost 20% more likely to contain sufficient diagnostic material when a scientist attended the FNA collection compared with non-attended FNAs. This has significant implications for patient management and the cost-effectiveness of the procedure, given the improvements in diagnostic precision that can often be achieved when adequate cell block material is available.

This study did not address the cost-effectiveness of ROSE but the potential reduction in inadequate, particularly thyroid, FNA collections is significant if applied on a large scale. From a private practice perspective we also believe that a scientist-based service can be cost-effective if well managed. In summary we have demonstrated ROSE of adequacy of FNA specimens by cytology scientists to be highly accurate and reproducible across a range of body sites. ROSE of thyroid FNA by cytology scientists may allow significant reduction in inadequate thyroid FNA reports.

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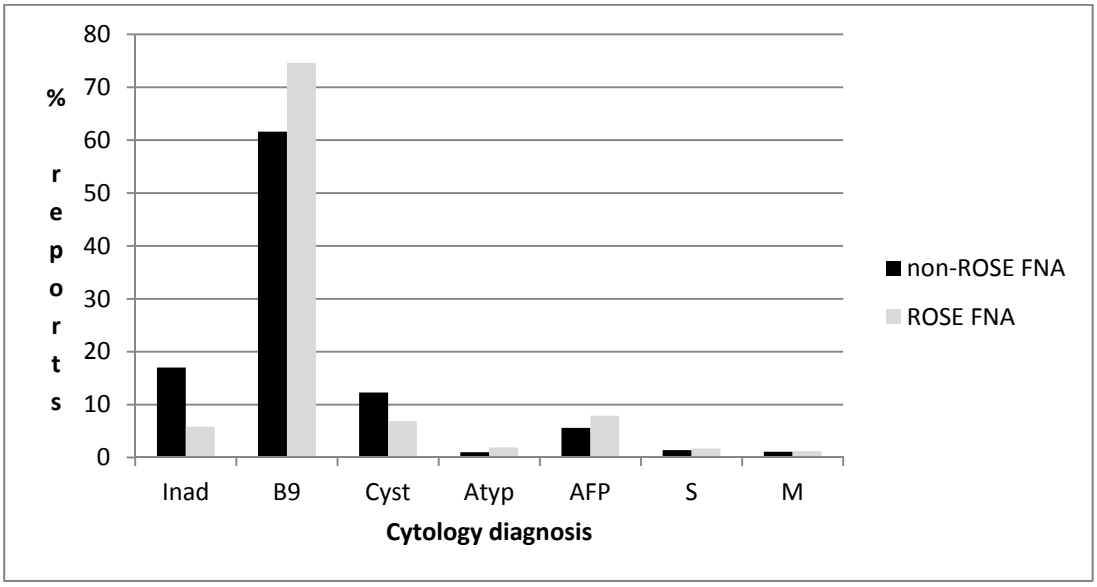

Figure 1. Reporting profile for 1593 Thyroid FNA cases with rapid on-site evaluation (ROSE) and 1536 collected without ROSE. (Inad=inadequate (Thy1); B9=Benign (Thy2); Atyp =Atypical (Thy3a); AFP= atypical follicular pattern or suspicious for follicular lesion (Thy3f); S=suspicious for malignancy (Thy4); M=malignant (Thy5).

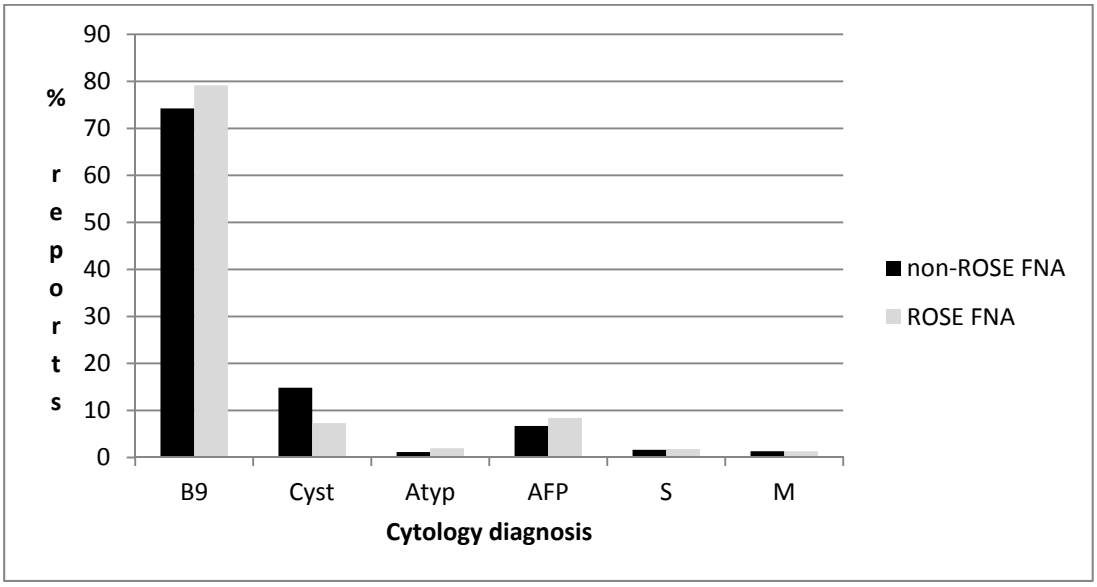

Figure 2. Reporting profile of adequate reports for 1501 Thyroid FNA cases with rapid on-site evaluation (ROSE) and 1274 collected without ROSE. (B9=Benign (Thy2); Atyp =Atypical (Thy3a); AFP= atypical follicular pattern or suspicious for follicular lesion (Thy3f); S=suspicious for malignancy (Thy4); M=malignant (Thy5).

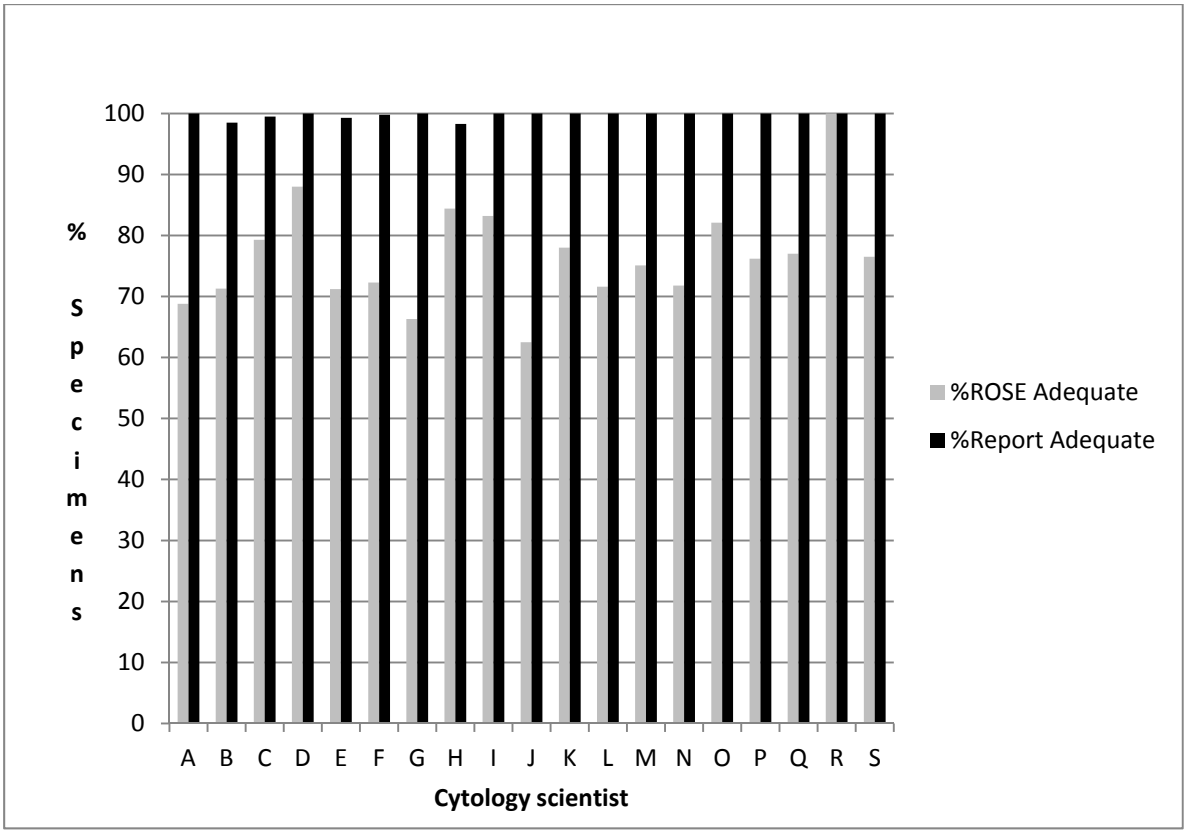

Figure 3. Adequacy rates at ROSE and confirmation of adequacy on final report for 19 scientists performing rapid on-site evaluation of 3032 FNAs. The scientists attended between 4 specimens (S) and 701 (F).

Table 1. Comparison of rapid on-site evaluation (ROSE) of adequacy according to final report by body site

FNA site	ROSE Adequate (75%)		ROSE Inadequate (12%)		ROSE No comment (13%)		Total
FNA site (total specimens)	Total	Final report Adequate	Total	Final report Adequate	Total	Final report Adequate	Final report Inadequate
Abdomen (11)	11	11	0	0	0	0	0
Arm/upper extremity (22)	14	14	6	5	2	2	1 (4.5%)
Axilla (66)	53	53	9	3	4	3	7 (10.6%)
Bone (9)	6	0	2	2	1	1	0
Chest (66)	48	48	14	8	4	4	6 (10%)
Head/face (26)	16	16	7	5	5	5	2 (7.7%)
Leg/lower extremity (28)	10	10	13	7	5	5	6 (21.4%)
Neck (281)	223	222	27	13	31	30	16 (5.7%)
Retroperitoneum (5)	4	4	1	1	0	0	1 (20%)
Back (7)	6	6	1	1	0	0	0
Breast (623)	402	401	75	43	146	138	41 (6.6%)
Groin (50)	44	44	4	1	2	2	3 (6%)
Liver (18)	18	0	0	0	0	0	0
Lung (76)	64	64	11	6	1	1	5 (6.6%)
Pancreas (38)	34	34	3	2	1	1	1 (2.6%)
Salivary gland (113)	84	83	14	7	15	15	8 (7.6%)
Thyroid (1593)	1239	1234	179	109	175	158	92 (5.7%)
TOTAL (3032)	**2276**	**2268 (99.6%)**	**366**	**212 (57.9%)**	**390**	**363 (93.1%)**	**189 (6.2%)**