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Molecular Diagnosis Using Residual Liquid-Based Cytology Materials for Patients with Nondiagnostic or Indeterminate Thyroid Nodules

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Background: Molecular analysis for common somatic mutations in thyroid cancer can improve diagnostic accuracy of fine-needle aspiration cytology (FNAC) in the nondiagnostic or indeterminate category of thyroid nodules. In this study, we evaluated the feasibility of molecular diagnosis from residual liquid-based cytology (LBC) material after cytological diagnosis.
Methods: This prospective study enrolled 53 patients with thyroid nodules diagnosed as nondiagnostic, atypia of undetermined significance (AUS), or follicular lesion of undetermined significance (FLUS) after FNAC. DNAs and RNAs were isolated from residual LBC materials. *BRAF*^{V600E} and *RAS* point mutations, *PAX8*/peroxisome proliferator-activated receptor γ (*PPARγ*), *RET/PTC1*, and *RET/PTC3* rearrangements were evaluated by real-time polymerase chain reaction and pyrosequencing.
Results: All DNAs from 53 residual LBC samples could be analysed and point mutations were detected in 10 samples (19%). In 17 AUS nodules, seven samples (41%) had point mutations including *BRAF* (n=4), *NRAS* (n=2), and *KRAS* (n=1). In 20 FLUS nodules, three samples (15%) had *NRAS* point mutations. RNA from only one FLUS nodule could be analysed for rearrangements and there was no abnormality.
Conclusion: Molecular analysis for *BRAF* and *RAS* mutations was feasible in residual LBC materials and might be useful for diagnosis of indeterminate thyroid nodules.

Keywords: Molecular diagnostic techniques; Biopsy, fine-needle; Thyroid aspiration; Thyroid nodule; Thyroid neoplasms

INTRODUCTION

Fine-needle aspiration cytology (FNAC) is a reliable, cost-effective, and safe diagnostic method for the evaluation of thyroid nodules [1]. The prevalence of thyroid nodules has been estimated to be about 4% to 10% in adults on physical palpation of the thyroid gland [2,3]. It is much higher on thyroid ultrasonography (US), as 20% to 70% [2,4]. However, the over-all malignancy risk of thyroid nodules is only 5% to 10% [5,6]. By means of FNAC, 15% to 30% samples were classified as inde-

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terminate category, including atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), follicular neoplasm (FN) or suspicious for follicular neoplasm (SFN) [1,6]. The risk of malignancy for AUS/FLUS thyroid nodules was 5% to 15% and repeated fine-needle aspiration (FNA) was recommended [7]. Molecular testing for common somatic mutations of thyroid cancer provides the opportunity to improve the diagnostic accuracy of FNAC in the indeterminate category of thyroid nodules [5-9].

However, routine use of molecular analysis for all FNAC samples remains controversial and is not cost-effective [10]. Usually, molecular diagnosis can be applicable for thyroid nodules which are diagnosed as indeterminate in the first FNAC results. Since currently available molecular tests need fresh FNAC material obtained by standard FNA procedures, an additional FNA is required for molecular analysis. A recent study suggested the feasibility of air-dried FNAC samples for molecular analysis including common point mutations and rearrangements in thyroid cancer [11]. This study suggested that additional molecular analysis using air-dried FNAC samples could improve the sensitivity of FNAC from 67% to 75% [11].

Recently, liquid-based cytology (LBC) is widely used for the preparation of cytological specimens instead of routine air-dried slides. One of the most common U.S. Food and Drug Administration-approved methods for the LBC samples is to use an ethanol-based fixative solution (CytoRich, TriPath Imaging, Burlington, NC, USA). The FNAC samples are collected in this solution, centrifuged twice and slowly transferred on to a single slide by a computer-assisted device and finally stained with a hematoxylin-eosin stain (SurePath, TriPath Imaging) [12]. After the centrifugation, the pellet is used for diagnosis but the supernatant is discarded. If the residual supernatant could be used for molecular diagnosis, repeated FNAs could be avoided for indeterminate thyroid nodules. A recent study demonstrated that residual ThinPrep (Hologic Inc., Bedford, MA, USA) material from thyroid FNAC was adequate for molecular analysis in most of the cases [13]. The majority of *BRAF* mutations were found in cases classified cytologically as malignant or suspicious for malignancy [13]. However, the usefulness of *BRAF* testing was limited by the low rate of *BRAF*-positive cases in other categories such as AUS/FLUS or FN/SFN [13].

The aim of this study was to evaluate the feasibility of molecular diagnosis using residual supernatant of SurePath samples after cytological diagnosis. We evaluated *BRAF*, *NRAS*, *HRAS*, and *KRAS* point mutations and rearrangements of *PAX8*/ peroxisome proliferator-activated receptor γ (*PPARγ*), *RET/PTC1*, and *RET/PTC3* using residual SurePath samples of cytological nondiagnostic or indeterminate thyroid nodules such as AUS/FLUS or FN/SFN categories.

METHODS

Patients and samples

This prospective study enrolled 53 thyroid nodules from 53 patients, which were diagnosed nondiagnostic or indeterminate thyroid nodules after thyroid FNAC from July 2014 to September 2014 in Asan Medical Center. The thyroid nodules were classified as non-diagnostic ($n=16$), AUS ($n=17$), and FLUS ($n=20$). The supernatant of LBC material after the centrifugation for collecting pellets to make slides for cytological diagnosis was collected. The samples were blinded to the medical record number, age, gender, and other personal information with the exception of FNAC results. The study was approved by the Institutional Review Board of the Asan Medical Center, Seoul, Korea. Informed consents were obtained from all patients.

US guided FNAC

As we previously described, all US examinations were performed using one of two US systems; an iU22 unit or an HDI-5000 (Philips Healthcare, Bothell, WA, USA) equipped with a linear, high-frequency probe (4 to 15 MHz) [14]. The scanning protocol included both transverse and longitudinal real-time imaging of the thyroid. FNA was performed under US guidance using a 23-gauge needle connected to a 10-mL syringe. All US examinations and FNAs were performed by experienced radiologists or endocrinologists.

Cytological diagnosis

Cytological diagnoses were made by experienced endocrine cytopathologist (D.E.S). FNAC diagnoses were categorized into six categories using the Bethesda System as follows: nondiagnostic or unsatisfactory, benign, AUS or FLUS, FN or SFN, suspicious for malignancy, and malignant [1].

DNA and RNA extraction from residual SurePath material

Centrifugation of residual SurePath material was performed at 20,000 rpm for 2 minutes and the pellet was used. DNAs and RNAs were extracted from the samples using the AllPrep DNA/RNA FFPE Kit (Qiagen, Hilden, Germany). RNA was extracted from the upper phase of the initial extraction step, and DNA was extracted from the lower phase as previously reported [11,13].

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cDNA synthesis

According to the manufacturer's protocol, cDNA was synthesized from RNA using the miScript Reverse Transcription Kit (Qiagen) [11]. A 7.5 μL template RNA was added to a master mix consisting of 2 μL miScript RT buffer and 0.5 μL miScript Reverse Transcriptase Mix. The mixture was incubated for 60 minutes at 37°C and 5 minutes at 95°C.

Detection of point mutations and rearrangements by high-resolution melting peak analysis

BRAF (codon 600), *NRAS* (codon 61), *KRAS* (codon 12/13), and *HRAS* (codon 61) point mutations were detected by real-time polymerase chain reaction (RT-PCR) and high-resolution melting (HRM), using the LightCycler 480 High Resolution Melting Master chemistry (Roche, Mannheim, Germany) on a LightCycler 480 (Roche) as previously reported [11,13,15].

PAX8/PPARγ, *RET/PTC1*, and *RET/PTC3* rearrangements were also evaluated after cDNA synthesis from RNAs. PCRs were performed as previously reported [11].

Detection of point mutations and rearrangements by pyrosequencing

BRAF (codon 600), *NRAS* (codon 61), *KRAS* (codon 12/13), and *HRAS* (codon 61) point mutations were detected by pyrosequencing on a PyroMark Q24 (Qiagen) according to the manufacturer's instructions. Pyrosequencing was applied if the samples were considered as mutation positive or showed ambiguous results by HRM peak analysis. When the samples were determined wild-type by HRM peak analysis, some samples of them were analyzed by pyrosequencing as previously reported [11].

Statistical analysis

Statistical analysis was conducted by using SPSS version 21.0 (IBM Co., Armonk, NY, USA). Graphs were produced using Prism version 5.01 (GraphPad Software Inc., La Jolla, CA, USA). Continuous variables such as age and primary nodule size were presented as mean±standard deviation.

RESULTS

Evaluation for *BRAF* or *RAS* point mutations

All DNAs from 53 residual LBC samples could be analysed for point mutations by RT-PCR or pyrosequencing. Point mutations were detected in 10 of 53 samples (19%) (Table 1, Fig. 1). There

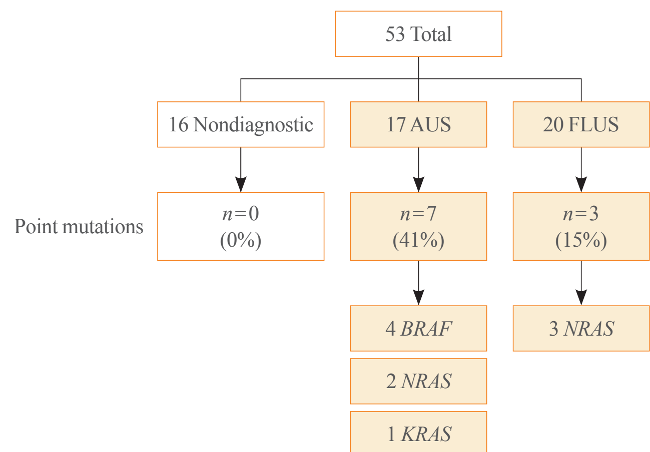

Fig. 1. All DNAs from 53 residual liquid-based cytology samples could be analysed for point mutations by real-time polymerase chain reaction or pyrosequencing. AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance.

Table 1. Polymerase Chain Reaction and Pyrosequencing Results for Point Mutations of Atypia of Undetermined Significance and Follicular Lesion of Undetermined Significance Samples

Cytology	*BRAF*	*NRAS*	*KRAS*	*HRAS*
A2	*V600E* (10%)	Wild type	Wild type	Wild type
A5	Wild type	*Q61R* (27%)	Wild type	Wild type
A6	*V600E* (12%)	Wild type	Wild type	Wild type
A9	*V600E* (19%)	Wild type	Wild type	Wild type
A14	Wild type	*Q61R* (35%)	Wild type	Wild type
A15	Wild type	Wild type	*G12V* (16%)	Wild type
A16	*V600E* (14%)	Wild type	Wild type	Wild type
F2	Wild type	*Q61R* (32%)	Wild type	Wild type
F8	Wild type	*Q61K* (35%)	Wild type	Wild type
F18	Wild type	*Q61R* (20%)	Wild type	Wild type

In pyrosequencing, samples showing a mutation level greater than 10% were considered as positive mutation.

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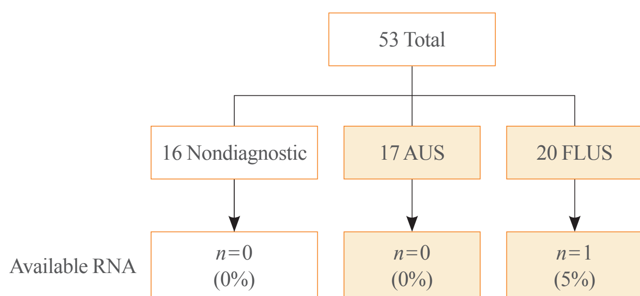

Fig. 2. Only one RNA sample from a follicular lesion of undetermined significance (FLUS) thyroid nodule could be analysed for rearrangements. AUS, atypia of undetermined significance.

were no *BRAF* or *RAS* point mutations in 16 nondiagnostic thyroid nodules. In 17 AUS thyroid nodules, seven samples (41%) had point mutations including *BRAF* ($n=4$), *NRAS* ($n=2$), and *KRAS* ($n=1$). In 20 FLUS thyroid nodules, three samples (15%) had *NRAS* ($n=3$) point mutations.

In AUS thyroid nodules, the *BRAF V600E* point mutation was detected in sample A2, A6, A9, and A16 by pyrosequencing (mutation percentage 10%, 12%, 19%, and 14%, respectively). *NRAS* point mutations were detected in sample A5 (*Q61R*, mutation percentage 27%) and A14 (*Q61R*, mutation percentage 35%). There was only one *KRAS G12V* point mutation in sample A15 (mutation percentage 16%). There were no *HRAS* mutations in AUS thyroid nodules.

In FLUS thyroid nodules, *NRAS* point mutations were detected in sample F2 (*Q61R*, mutation percentage 32%), F8 (*Q61K*, mutation percentage 35%), and F18 (*Q61R*, mutation percentage 20%). There was no *BRAF*, *KRAS*, and *HRAS* mutations in FLUS thyroid nodules.

Evaluation for rearrangements

Only one RNA sample from a FLUS thyroid nodule could be analysed for rearrangements (Fig. 2). There were no *PAX8/PPARγ*, *RET/PTC1*, or *RET/PTC3* rearrangements in this sample. The other RNA samples were not amplified by PCR.

DISCUSSION

In this study, molecular analysis for *BRAF* and *RAS* point mutations was feasible using residual supernatant of LBC SurePath samples after FNA. We found *BRAF* or *RAS* point mutations in 10 of 37 AUS/FLUS nodules (27%). However, we did not find point mutations in nondiagnostic thyroid nodules, and RNAs from residual SurePath material were not applicable for molecular analysis of gene rearrangements.

FNA is the procedure of choice in the evaluation of thyroid nodules [6]. Once the needle is withdrawn from the lesion, the material is extruded onto glass slides for a conventional smear which is fixed with 95% ethyl alcohol for the Papanicolau stain. Otherwise, the smear may be air-dried and then stained with May-Grunwald Giemsa. The thin-layer or LBC technique has been used popularly as an alternative technique for conventional smear due to cost-effectiveness, time-sparing, and simple application with a semi-automated device [10,12]. It is originally developed for application in gynecologic cervical smears. In gynecologic area, the diagnostic utility of cell blocks prepared from residual LBC material was applied for detection of human papilloma virus by immunohistochemistry or *in situ* hybridization method [16].

A single mutational marker or the molecular testing panel were useful and provided a significant increase in the diagnostic accuracy of FNAC of up to 95% [17-21]. Especially, PTC may carry *BRAF*, *NRAS*, or *RET/PTC* mutations in a total of 70% of cases [9,17,18,20-24]. The revised guidelines of the American Thyroid Association suggested that molecular analysis, such as *BRAF*, *RAS*, *PAX8/PPARγ*, and *RET/PTC*, may be considered in patients with indeterminate FNAC to help guide clinical management and improve the final diagnostic accuracy [6,24].

A repeated FNAC is required for molecular testing, because it is usually applied with fresh FNAC material before fixation. Molecular analysis using residual LBC material takes advantage to avoid repeated FNA or needle biopsy. In this study, we used the supernatant of LBC samples after isolation of the cell pellet which was used for cytological diagnosis. We could apply molecular testing only in nondiagnostic or AUS/FLUS nodules which were diagnosed by cytopathological examination. This approach could enhance cost-effectiveness. The nucleic acids are stable in the preservative solution for up to 6 months after sampling [12,25]. Isolated DNAs from residual LBC SurePath samples were stable and useful for mutational analysis in the present study. The previous study using residual ThinPrep material reported that adequate results were obtained in 402 of 597 cases (67%) [13]. In this study, all DNAs from 53 residual LBC SurePath samples could be analysed for point mutations by RT-PCR or pyrosequencing.

In this study, we found the *BRAF V600E* mutation in four AUS nodules. There was no *BRAF V600E* mutation in a FLUS nodule. The AUS nodules had several atypical nuclear features including nuclear grooves, prominent nucleoli, elongated nuclei and cytoplasm, and/or intranuclear cytoplasmic inclusions. The FLUS nodules had a prominent population of microfollicles or

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Hurthle cells but did not otherwise fulfill the criteria for FN/SFN. The *BRAF V600E* mutation is known to be found in 29% to 69% of classical PTC and in 80% of tall cell variant of PTC, but in only 10% to 25% of follicular variant of PTC and very rarely in follicular thyroid carcinoma [17,20,21,26,27]. When a *BRAF V600E* mutation was detected in FNAC specimens, the possibility of PTC was approximately 99% [23,26,28-30].

In this study, RNAs isolated from the supernatant of LBC samples were not useful for molecular diagnosis. RNA from only one sample could be analyzed for rearrangements. The other samples were not amplified in RT-PCR. Since we used Surepath which includes a small amount of formalin for preservation [31]. Limited availability of RNAs from residual LBC samples is most likely due to a chemical linkage between proteins and RNAs generated by formalin [32,33].

This prospective study has several limitations. First, we could not evaluate sensitivity and specificity of molecular analysis in residual LBC materials because our prospective study protocol was approved by the Institutional Review Board in condition of blind for other medical records including follow-up information. We could be accessible only for the cytological results and residual LBC samples of the study patients. Therefore, we could not obtain final pathological results. Second, our study included only a limited number of patients to indicate general application of molecular testing from residual LBC material. However, this study provides a basis for future progression in the development of a larger prospective study using residual LBC samples for a DNA based molecular analysis of point mutations.

In conclusions, molecular analysis for *BRAF* and *RAS* mutations was feasible in residual LBC materials and might be useful for the diagnosis of indeterminate thyroid nodules. This approach might be expected to reduce repeated FNA, needle biopsy, or diagnostic surgery for indeterminate thyroid nodules.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Cibas ES, Ali SZ. The bethesda system for reporting thyroid cytopathology. Thyroid 2009;19:1159-65.
2. Dean DS, Gharib H. Epidemiology of thyroid nodules. Best Pract Res Clin Endocrinol Metab 2008;22:901-11.
3. Vander JB, Gaston EA, Dawber TR. The significance of nontoxic thyroid nodules: final report of a 15-year study of the incidence of thyroid malignancy. Ann Intern Med 1968;69:537-40.
4. Guth S, Theune U, Aberle J, Galach A, Bamberger CM. Very high prevalence of thyroid nodules detected by high frequency (13 MHz) ultrasound examination. Eur J Clin Invest 2009;39:699-706.
5. Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013;381:1058-69.
6. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009;19:1167-214.
7. Crippa S, Mazzucchelli L, Cibas ES, Ali SZ. The Bethesda System for reporting thyroid fine-needle aspiration specimens. Am J Clin Pathol 2010;134:343-4.
8. Hsiao SJ, Nikiforov YE. Molecular approaches to thyroid cancer diagnosis. Endocr Relat Cancer 2014;21:T301-13.
9. Song YS, Lim JA, Park YJ. Mutation profile of well-differentiated thyroid cancer in Asians. Endocrinol Metab (Seoul) 2015;30:252-62.
10. Rossi ED, Martini M, Capodimonti S, Straccia P, Cenci T, Lombardi CP, et al. Diagnostic and prognostic value of immunocytochemistry and BRAF mutation analysis on liquid-

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based biopsies of thyroid neoplasms suspicious for carcinoma. Eur J Endocrinol 2013;168:853-9.
11. Eszlinger M, Krogdahl A, Munz S, Rehfeld C, Precht Jensen EM, Ferraz C, et al. Impact of molecular screening for point mutations and rearrangements in routine air-dried fine-needle aspiration samples of thyroid nodules. Thyroid 2014; 24:305-13.
12. Fadda G, Rossi ED. Liquid-based cytology in fine-needle aspiration biopsies of the thyroid gland. Acta Cytol 2011;55: 389-400.
13. Krane JF, Cibas ES, Alexander EK, Paschke R, Eszlinger M. Molecular analysis of residual ThinPrep material from thyroid FNAs increases diagnostic sensitivity. Cancer Cytopathol 2015;123:356-61.
14. Kwon H, Kim WG, Choi YM, Jang EK, Jeon MJ, Song DE, et al. A cut-off value of basal serum calcitonin for detecting macroscopic medullary thyroid carcinoma. Clin Endocrinol (Oxf) 2015;82:598-603.
15. Rehfeld C, Munz S, Krogdahl A, Jensen EM, Siebolts U, Ferraz C, et al. Impact of different methodologies on the detection of point mutations in routine air-dried fine needle aspiration (FNA) smears. Horm Metab Res 2013;45:513-7.
16. Afify A, Yu C, Hejazi N, Howell L. The diagnostic utility of cell blocks prepared from residual SurePath Pap material for detection of human papilloma virus. Appl Immunohistochem Mol Morphol 2009;17:108-14.
17. Ohori NP, Singhal R, Nikiforova MN, Yip L, Schoedel KE, Coyne C, et al. BRAF mutation detection in indeterminate thyroid cytology specimens: underlying cytologic, molecular, and pathologic characteristics of papillary thyroid carcinoma. Cancer Cytopathol 2013;121:197-205.
18. Musholt TJ, Fottner C, Weber MM, Eichhorn W, Pohlenz J, Musholt PB, et al. Detection of papillary thyroid carcinoma by analysis of BRAF and RET/PTC1 mutations in fine-needle aspiration biopsies of thyroid nodules. World J Surg 2010; 34:2595-603.
19. Eszlinger M, Paschke R. Molecular fine-needle aspiration biopsy diagnosis of thyroid nodules by tumor specific mutations and gene expression patterns. Mol Cell Endocrinol 2010;322:29-37.
20. Nikiforova MN, Nikiforov YE. Molecular diagnostics and predictors in thyroid cancer. Thyroid 2009;19:1351-61.
21. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 2009;94:2092-8.
22. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev 2007;28:742-62.
23. Hong AR, Lim JA, Kim TH, Choi HS, Yoo WS, Min HS, et al. The frequency and clinical implications of the BRAF (V600E) mutation in papillary thyroid cancer patients in Korea over the past two decades. Endocrinol Metab (Seoul) 2014;29:505-13.
24. Kim JG. Molecular pathogenesis and targeted therapies in well-differentiated thyroid carcinoma. Endocrinol Metab (Seoul) 2014;29:211-6.
25. Rossi ED, Zannoni GF, Moncelsi S, Stigliano E, Santeusanio G, Lombardi CP, et al. Application of liquid-based cytology to fine-needle aspiration biopsies of the thyroid gland. Front Endocrinol (Lausanne) 2012;3:57.
26. Yip L. Molecular diagnostic testing and the indeterminate thyroid nodule. Curr Opin Oncol 2014;26:8-13.
27. Cohen Y, Xing M, Mambo E, Guo Z, Wu G, Trink B, et al. BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst 2003;95:625-7.
28. Xing M, Tufano RP, Tufaro AP, Basaria S, Ewertz M, Rosenbaum E, et al. Detection of BRAF mutation on fine needle aspiration biopsy specimens: a new diagnostic tool for papillary thyroid cancer. J Clin Endocrinol Metab 2004; 89:2867-72.
29. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab 2011;96:3390-7.
30. Chung KW, Yang SK, Lee GK, Kim EY, Kwon S, Lee SH, et al. Detection of BRAFV600E mutation on fine needle aspiration specimens of thyroid nodule refines cyto-pathology diagnosis, especially in BRAF600E mutation-prevalent area. Clin Endocrinol (Oxf) 2006;65:660-6.
31. Michael CW, McConnel J, Pecott J, Afify AM, Al-Khafaji B. Comparison of ThinPrep and TriPath PREP liquid-based preparations in nongynecologic specimens: a pilot study. Diagn Cytopathol 2001;25:177-84.
32. Gilbert L, Oates E, Ratnam S. Stability of cervical specimens in SurePath medium for human papillomavirus testing with the Roche cobas 4800 assay. J Clin Microbiol 2013;51: 3412-4.
33. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961-71.

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