Diagnostic evaluation including:
- Role of diagnostic thyroid US to guide FNA decision-making
- FNA: US versus palpation
Therapeutic recommendations based upon cytology using the Bethesda System

Diagnostic Evaluation of Patients with Thyroid Nodules

HISTORY
Male gender (OR ~ 2.0)
Age of patient (<20 or >65-70)

Boelaert J Clin Endocrinol Metab 2006, Haymart J Clin Endocrinol Metabl 2008

Diagnostic Evaluation of Patients with Thyroid Nodules

HISTORY
- Hx of thyroid cancer in 1st degree relative
- Hx of external beam radiation as a child
- Ionizing radiation in childhood/adolescence
- Prior hemithyroidectomy with thyroid cancer
- 18FDG avidity on PET scanning (incidentally found nodule)
- MEN2/FMTC associated RET mutation

American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer, 2009

Diagnostic Evaluation of Patients with Thyroid Nodules

HISTORY
- Local symptoms?? (rapid growth, hoarseness)
- Duration
- History of coexistent benign thyroid disease (hyperthyroidism, hypothyroidism, other nodules)
SAME cancer rates for patients with solitary and multiple thyroid nodules

<table>
<thead>
<tr>
<th></th>
<th>Definition of nodularity</th>
<th>FNA technique</th>
<th>Cancer in nodule</th>
<th>MNG</th>
</tr>
</thead>
<tbody>
<tr>
<td>McCall/USA</td>
<td>scan/histology palpation</td>
<td>17%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Belfiore/Italy</td>
<td>scan/palpation</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Cochand/France</td>
<td>scan/US US</td>
<td>13%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Sachmechi/USA</td>
<td>scan/palpation</td>
<td>8%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Marqusee/USA</td>
<td>US US</td>
<td>7%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Papini/Italy</td>
<td>US US</td>
<td>9%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Deandrea/Italy</td>
<td>US US</td>
<td>6%</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Cappelli/Italy</td>
<td>US US</td>
<td>9%</td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Frates/USA</td>
<td>US US</td>
<td>15%</td>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>


Diagnostic Evaluation of Patients with Thyroid Nodules

**PHYSICAL EXAM**
- Fixation to adjacent structures
- Adenopathy
- Firm nodule consistency

**FIRST STEP**
- TSH
- Not Indicated
- Radionuclide scans
- CT or MRI
- Antithyroid antibodies
- T4, free T4, T3

**Diagnostic Evaluation of Patients with Thyroid Nodules**

**A low TSH is a way to screen for hyperfunctioning nodules**

TSH 0.03
These “hot” nodules do not need to be aspirated

**BUT, 95% of nodules are hypofunctioning or “cold”**

TSH 1.1mU/L
7-9% of “cold” nodules are malignant

History and physical examination lack the sensitivity and specificity sufficient for diagnosing thyroid cancer
**Serum TSH as a Risk for Thyroid Cancer**

- **Adjusted Odds Ratio for Thyroid Cancer**

  - `<0.4`
  - `0.4-0.9`
  - `1.0-1.7`
  - `1.8-5.5`
  - `>5.5`

  *Adjusted for age and male gender*

**Why should a higher TSH be associated with thyroid cancer?**

- Low TSH may be associated with functioning nodules, very unlikely to be malignant
- TSH has a trophic effect on thyroid cancer growth mediated by TSH receptors on tumor cells
- TSH suppression is an independent predictor of relapse-free survival in differentiated thyroid cancer
- Recently, higher TSH associated with more advanced Stage of thyroid cancer

**Why is a diagnostic US necessary?**

- Confirmation of a sonographically identifiable nodule corresponding to the palpable abnormality
  - 16% no corresponding nodule on US
- Detection of additional nonpalpable nodules for which FNA may be indicated
  - 15% additional nonpalpable nodule >1 cm
- Identification of the sonographic characteristics of the thyroid nodule(s)
- Determination of accuracy of palpation FNA

**Diagnostic thyroid ultrasound**

**4 recently published guidelines/consensus statements for selection of thyroid nodules for FNA**

- **Size**
  - American Thyroid Association 2006
  - Size and sonographic features
  - Society of Radiologists in Ultrasound 2005
  - Size, sonographic features and clinical history
  - American Association of Clinical Endocrinologists 2006
  - European consensus 2006
  - American Thyroid Association 2009

---

**US characteristics of thyroid nodules**

1. **Echogenicity** (hypo-, hyper-, iso-)
2. **Calcifications** (micro-, dense)
3. **Margins** (infiltrative*, well-defined regular)
4. **Vascularity** (intranodular*, peripheral, absent)
5. **Shape** (taller than wide*)

*associated with thyroid cancer

**HIGH RISK**

- Hypoechoic, solid
- Infiltrative margin
- MicroCa
- Intranodular vascularity
- Papillary carcinoma

**LOW RISK: “Spongiform” without hypervascularity**

- Aggregation of multiple microcystic components in more than 50% of the volume of the nodule
- “Honeycomb of internal cystic spaces”

**“Spongiform” nodules**

- Moon Radiology 2008; 247: 762–70
- Bonavita AJR 2009; 193:207–13
- Ronavita AJR 2009; 193:207–13

**INTERMEDIATE RISK:**

66% of benign nodules have at least one positive US predictor of papillary thyroid cancer¹

66% of papillary cancers have at least one nonsuspicious US feature²,³

Most other nodules!

¹Wienke J Ultrasound Med 2003; ²Chan, J Ultrasound Med 2003; ³Yuan, Clin Imaging 2006
American Thyroid Association 2009

<table>
<thead>
<tr>
<th>Nodule Sonographic / Clinical Features</th>
<th>Recommended nodule threshold size for FNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk history</td>
<td>Recommendation A</td>
</tr>
<tr>
<td>Nodule WITH suspicious sonographic features**</td>
<td>&gt;5 mm Recommendation I</td>
</tr>
<tr>
<td>Nodule WITHOUT suspicious sonographic features**</td>
<td>&gt;5 mm</td>
</tr>
<tr>
<td>Abnormal cervical lymph nodes</td>
<td>Recommendation A</td>
</tr>
<tr>
<td>Microcalcifications present in Nodule</td>
<td>Recommendation B</td>
</tr>
<tr>
<td>Solid nodule AND hypoechoic AND iso- or hyperechoic</td>
<td>≥1cm Recommendation B</td>
</tr>
<tr>
<td>Mixed cystic/solid nodule WITH any suspicious ultrasound feature**</td>
<td>≥1.5cm – 2cm Recommendation B</td>
</tr>
<tr>
<td>Spongiform nodule</td>
<td>≥2cm Recommendation C</td>
</tr>
<tr>
<td>Purely cystic nodule</td>
<td>FNA not indicated Recommendation B</td>
</tr>
</tbody>
</table>

**suspicious features: microcalcifications; hypoechoic; increased nodular vascularity; infiltrative margins; taller than wide on transverse view.

---

Palpation versus US-guided FNA

If what you feel is what you see on US (and it’s predominantly solid) → Palpation FNA

---

US-guided FNA for nodules

ATA Guidelines 2009

1. Nonpalpable and difficult to palpate nodules (posterior, low lying), "incidentalomas"
2. Predominantly cystic nodules
3. Nodules with a nondiagnostic cytology from palpation or US FNA
   - nondiagnostic rate 50% lower than palpation FNA
4. Nodules with a prior benign cytology that have grown
   - false negative rate lower than palpation FNA (decreased sampling error)


---

Therapeutic recommendations based upon cytology results
Nondiagnostic cytology
(< 6 groups of 10 cells each)

- 5-13% of nodules have nondiagnostic cytology after US-FNA\(^1,2\).
- Cystic content is the only significant predictor of nondiagnostic cytology\(^2\).
- Repeat US-FNA yields a diagnostic cytology in ~65% of patients\(^2,3\).

### R6a
US guidance should be used when repeating the FNA procedure for a nodule with an initial nondiagnostic cytology result.

**Recommendation A**

---

### Malignant Cytology
Papillary, medullary or poorly differentiated (suspicious for papillary cancer cytology)

- **R26** SURGERY—near total thyroidectomy, unless unifocal intrathyroidal <1cm tumor

**Recommendation A**

---

### Follicular neoplasm cytology

**Do all patients require thyroid surgery?** Follicular neoplasm, NOT Hurthle cell neoplasm

**R9** If the cytology reading reports a FOLLICULAR neoplasm, an I-123 thyroid scan may be considered if the serum TSH is in the low-normal range. If a concordant autonomously functioning nodule is NOT seen, lobectomy or total thyroidectomy should be considered. **Recommendation C**

****

---

**Follicular neoplasm cytology**

### Extent of Surgery

**R25a** Because of an increased risk for malignancy, near-total thyroidectomy is indicated for:
- large tumors (>4 cm)
- patients with a family history of thyroid carcinoma
- patients with a history of radiation exposure

Recommendation A

**R25b** Near-total thyroidectomy is also recommended for:
- patients with bilateral nodular disease
- patients who prefer to undergo bilateral thyroidectomy to avoid the possibility of requiring future surgery on the contralateral lobe (already hypothyroid, medical comorbidities)

Recommendation C

---

### What is new?

**R8a** The use of molecular markers (BRAF, Ras, RET/PTC, Pax8-PPARγ or galectin-3) may be considered for patients with indeterminate cytology on FNA to help guide management. Recommendation C

---

### Benign cytology

**To give L-T4 or not to give L-T4?**

**Hypothesis:**
- TSH stimulates nodule growth, therefore TSH suppressive doses of L-T4 cause benign nodules to shrink

---

### Benign cytology

**R16.** Routine levothyroxine suppression therapy of benign thyroid nodules in iodine sufficient populations is not recommended. Recommendation F

---

### Benign cytology

**R14a,b.** US to re evaluate nodule size in 6-18 months
- Growth assessed as either 50\% increase in volume or >20\% increase in at least 2 nodule dimensions with a minimal increase of 2mm
- same size or smaller--continue to follow at longer interval, i.e. 3-5 years
- larger--repeat FNA with US guidance

Recommendation C

---

### Atypia/follicular lesion of undetermined significance

- Bethesda classification noted, but AUS/FLUS not included in ATA 2009 guidelines
- “the heterogeneity of this category precludes outlining all scenarios” Cibas 2009
- Clinician’s concern--

This could represent an cellular adenomatoid nodule, a follicular adenoma, a follicular carcinoma, or the follicular variant of papillary thyroid carcinoma. Clinical correlation suggested

---

ATA Guidelines 2009
Atypia/follicular lesion of undetermined significance

The value of on site assessment

- **PRO**
  - Reduction in nondiagnostic rate from 14-20% to <5%\(^1,2\)
- **CON**
  - Prolonged procedure time\(^3,4\)
- **Benefit of preliminary diagnosis for change in management**
  - FNA of additional nodules in other lobe
  - Lateral cervical LN assessment if suspicious or positive for malignancy


Patient with a thyroid nodule >1-1.5cm

Triage of Nodule Management by Cytology

- Nondiagnostic → Repeat FNA with US
- Malignant/ susp for PTC → SURGERY
- Follicular neoplasm → SCAN if low NL TSH
  - Hyperfunctioning compared to surrounding thyroid → observation
  - Hypofunctioning → surgery
- Hurthle cell neoplasm → surgery
- Benign → follow with US
- AUS/FLUS → repeat FNA

ALL PATIENTS SHOULD HAVE AN ULTRASOUND BEFORE FNA
MOLECULAR “REFLEX TESTING” OF THYROID FNA

Yuri E. Nikiforov, M.D., Ph.D.
Division of Molecular Anatomic Pathology
University of Pittsburgh School of Medicine

FNA cytology is the most reliable diagnostic test for thyroid nodules and it establishes the definitive diagnosis of a benign or malignant lesion in the majority of cases. However, 10-40% of all thyroid FNA samples are diagnosed as indeterminate for malignancy [1-3]. The general category of indeterminate cytology encompasses several subcategories, i.e. follicular lesion of indeterminate significance (FLUS), follicular neoplasm/Hürthle cell neoplasm (FN/HCN), and suspicious for malignancy, which correlate with the estimated risk of malignancy of 5-10%, 20-30%, and 50-75%, respectively [4]. Due to the lack of definitive diagnosis, most patients with indeterminate cytology undergo surgery, although only 8-17% of surgically removed thyroid nodules are malignant [5,6]. Patients with indeterminate FNA cytology and malignant tumors are not adequately treated as well, since most of them initially undergo thyroid lobectomy and later have another surgery to complete thyroidectomy.

In the recent years, our understanding of molecular genetics of thyroid cancer has expanded dramatically. This knowledge has started to be translated into clinical practice, offering significant improvement in the preoperative FNA diagnosis of thyroid cancer and better tumor prognostication [7]. Four mutation types constitute the majority of known mutations occurring in papillary and follicular cancers and carry the highest impact on tumor diagnosis and prognostication. They are \textit{BRAF} and \textit{RAS} point mutations and \textit{RET/PTC} and \textit{PAX8/PPARY} rearrangements.

\textbf{BRAF point mutations}

Activating mutations of the \textit{BRAF} gene is the most common genetic alteration in papillary carcinoma and found in \textasciitilde45\% of these tumors [8-10]. About 95\% of all mutations involve nucleotide 1799 and result in a substitution of valine to glutamate at residue 600 (V600E). This point mutation leads to constitutive activation of BRAF kinase and chronic stimulation of the MAPK pathway, and is tumorigenic for thyroid cells [11,12]. Other and rare mechanisms of BRAF activation in thyroid papillary cancer include K601E point mutation, small in-frame insertions or deletions surrounding codon 600 (reviewed in [13]), and \textit{AKAP9/BRAF} rearrangement, which is more common in papillary carcinomas associated with radiation exposure [14]. \textit{BRAF} V600E mutation is typically found in papillary carcinomas with classical histology and in the tall cell variant, and is less common in the follicular variant of papillary carcinoma [8,15]. This mutation can also be seen in anaplastic and poorly differentiated thyroid carcinomas arising from papillary carcinoma [16-18]. However, \textit{BRAF} V600E is not found in follicular carcinomas and benign thyroid nodules, and therefore among primary thyroid lesions it represents a specific marker of papillary carcinoma and related tumor types.

\textbf{RET/PTC rearrangements}
Chromosomal rearrangement known as RET/PTC rearrangement is another genetic alteration found in papillary carcinomas [19]. It is formed by the fusion between the 3’ portion of the RET receptor tyrosine kinase gene and the 5’ portion of various unrelated genes. Two most common rearrangement types, RET/PTC1 and RET/PTC3, are paracentric inversions since both RET and its respective fusion partner, H4 or NCOA4 (ELE1, RFG), reside on the long arm of chromosome 10 [20-22]. RET/PTC2 and nine more recently identified types of RET/PTC are all interchromosomal translocations (reviewed in [23]). RET/PTC is found in approximately 20% of adult sporadic papillary carcinomas, although its prevalence is highly variable between various observations, largely due to the difference in sensitivity of the detection methods and also due to some geographic variability [24,25]. RET/PTC occur with higher incidence in patients with the history of radiation exposure (50-80%) and in papillary carcinomas from children and young adults (40-70%) [26-29]. The distribution of RET/PTC rearrangement within the tumor may be quite heterogeneous, varying from involving almost all neoplastic cells (clonal RET/PTC) to being detected only in a small fraction of tumor cells (non-clonal RET/PTC) [30,31]. Although RET/PTC has been found in several studies in adenomas and other benign thyroid lesions, it can be assumed that clonal RET/PTC (i.e. rearrangement that is found in most cells within the tumor) is reasonably specific for papillary thyroid carcinoma [24,30]. Proper techniques should be used for the clinically-relevant detection of RET/PTC are discussed below. Among the different types of rearrangement, RET/PTC1 is typically the most common and comprises up to 60-70% of positive cases, whereas RET/PTC3 accounts for 20-30% and RET/PTC2 and other novel rearrangement types for less than 5% [32,33]. RET/PTC-positive papillary carcinomas typically present at younger age, reveal classic papillary architecture, and have a high rate of lymph node metastases [15].

**RAS point mutations**

Mutations of the RAS genes are not restricted to a particular type of thyroid tumors and found in follicular carcinomas, papillary carcinomas, and follicular adenomas. The RAS genes (HRAS, KRAS, and NRAS) encode highly related G-proteins that propagate signals arising from cell membrane receptors along the MAPK and other signaling pathways, such as PI3K/AKT. Point mutations in the specific domains of the RAS gene result in the chronic stimulation of the downstream signaling pathways. In thyroid cancer, mutations involving NRAS codon 61 and HRAS codon 61 are by far the most common, although mutations have been found in different hotspots of all three genes. In papillary carcinomas, RAS mutations occur in 10-15% of tumors [34-39]. Papillary carcinomas harboring RAS mutation almost always have the follicular variant histology; this mutation also correlates with more frequent encapsulation and low rate of lymph node metastases [15,40]. RAS mutations are also found in 40-50% of conventional type follicular carcinomas [37,41-45] and 20-40% of conventional type follicular adenomas [34,41-44]. A lower incidence has been seen in oncocyctic tumors [46,47]. RAS mutations have also been reported in some adenomatous nodules and goiter nodules, although it is likely that these lesions are true neoplasms and therefore could be better designated as follicular adenomas, despite their frequent macrofollicular and colloid-rich histology.

**PAX8/PPARγ rearrangements**
PAX8/PPARγ rearrangement is a result of the translocation t(2;3)(q13;p25), leading to the fusion between the PAX8 gene coding for the thyroid-specific paired domain transcription factor, and the peroxisome proliferator-activated receptor (PPARγ) gene [48]. PAX8/PPARγ rearrangement leads to strong overexpression of the PPARγ protein, although the mechanisms of cell transformation induced by this genetic event are yet to be fully understood. PAX8/PPARγ is found in 30-40% of conventional type follicular carcinomas, and with lower prevalence in oncocyctic carcinomas [49-51]. Tumors harboring PAX8/PPARγ tend to present at a younger age, be smaller in size, have a solid/nested growth pattern, and more frequently reveal vascular invasion [49,50]. This rearrangement can also be found in a small fraction (2-10%) of follicular adenomas and occasionally in the follicular variant of papillary carcinoma [50-53]. Follicular adenomas positive for PAX8/PPARγ typically have a thick capsule and show the immunohistochemical profile characteristic of thyroid cancer, suggesting that they may represent pre-invasive (in situ) follicular carcinomas or malignant tumors where invasion was overlooked during histological examination [50].

**Testing for BRAF Mutations in Thyroid FNA Samples**

A number of studies have shown that molecular testing of FNA samples significantly improves the accuracy of cytologic diagnosis of thyroid nodules. Most studies have explored the diagnostic role of BRAF mutation. To date, the results of BRAF testing in 2,766 samples have been reported, including 9 prospective FNA studies [54-62], 7 retrospective FNA studies [63-69] (study in ref. 87 was also partially prospective), and 2 studies of research FNA performed on surgically removed thyroid glands [70,71]. All 450 BRAF-positive clinical FNA samples studied prospectively and retrospectively were papillary carcinomas, and only one reported BRAF-positive sample, obtained by research aspiration of the nodule in a surgically removed thyroid gland, appeared to be benign [71]. This reportedly benign nodule, pathologically diagnosed as “atypical nodular hyperplasia”, has not be worked up using modern immunohistochemical techniques helpful in the diagnosis of thyroid malignancy in difficult cases [72], and the nature of atypical changes has not been described or illustrated in the study published [71]. Even if this case is accepted as false positive, it appears that 580 our of 581 BRAF-positive nodules tested in various types of FNA samples, are papillary carcinomas, with the false-positive rate of 0.2%. Importantly, a significant proportion (15-39%) of BRAF-positive FNA samples in many of these studies were indeterminate or non-diagnostic by cytology, demonstrating that testing for BRAF is helpful in establishing the definitive diagnosis of cancer in nodules with indeterminate cytology [54,58,61,64,65,69]. In addition, occasional FNA samples with benign cytology were also tested positive for BRAF, and those were papillary carcinomas after surgery [54,62].

**Testing for a Panel of Mutations in Thyroid FNA Samples**

The biggest diagnostic impact can be achieved by testing FNA samples for a panel of mutations rather then for a single mutation. A recent study explored the diagnostic utility of molecular testing for a panel of mutations consisting of BRAF, RAS, RET/PTC and PAX8-PPARγ [54]. The study employed 470 consecutive FNA samples from thyroid nodules that were prospectively tested and yielded 32 mutations, including 18 BRAF, 8 RAS, 5 RET/PTC, and 1 PAX8-PPARγ. The presence of any mutation was a strong predictor of cancer, as 31 (97%) of mutation-positive nodules had a malignant diagnosis after surgery and one case (3%) was a follicular adenoma. This study showed that testing for a panel of mutations is particularly useful in nodules with
indeterminate cytology. Among those, the probability of malignancy was 40% based on cytologic evaluation, whereas the addition of molecular testing separated this category into mutation-positive samples with 100% probability of malignancy, and mutation-negative samples with a 14% probability of malignant outcome. The molecular testing was especially helpful in the lowest risk subgroup of indeterminate cytology, i.e. FLUS. In this subgroup, the positive mutational status had a 100% accuracy in predicting the risk of a malignancy, whereas mutation-negative nodules were all benign. In addition, this study showed that molecular testing decreased the false-negative rate of cytology from 2.1% to 0.9 [7]. However, routine testing of all samples with benign cytology is unlikely to be cost effective. Therefore, further studies are needed to identify a set of clinical and possibly imaging criteria that would determine which nodules with benign cytology should be re-screened using molecular testing.

Regarding specific mutations that constituted the panel, BRAF, RET/PTC and PAX8/PPARγ mutations had all a 100% positive predictive value for cancer [54]. Patients with these mutations would be candidates for total thyroidectomy irrespective of the cytologic diagnosis. This would eliminate the need for intraoperative pathology consultation and subsequent second surgery to complete thyroidectomy, reducing costs and additional morbidity. Detection of RAS mutation, which was the second most common mutation after BRAF, also appeared to be of high diagnostic value in FNA samples, as it conferred a 87.5% probability of malignancy. Importantly, RAS mutations were identified in tumors which are difficult to diagnose by cytology alone, i.e. follicular variant of papillary carcinoma and follicular carcinoma. One RAS-positive nodule was diagnosed as a benign follicular adenoma, corresponding to a false positive rate of 12.5% [54]. However, it is conceivable that RAS-positive follicular adenomas serve as precursor lesions for RAS-positive follicular carcinomas [50]. Therefore, surgical removal of follicular adenomas that carry this oncogenic mutation by lobectomy may be considered as justifiable to prevent this putative progression.

The accumulation of knowledge on diagnostic use of molecular markers has been reflected in the Revised Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer, recently released by the American Thyroid Association [73]. The Guidelines state that the use of molecular markers, such as BRAF, RAS, RET/PTC and PAX8/PPARγ, may be considered for patients with indeterminate FNA cytology to help guide management.

**Technical Issues in Molecular Testing of Thyroid Samples**

Although various scientific methods are used to study genetic alterations in thyroid cancer, the detection of mutations and other diagnostic markers for clinical use must be limited to those laboratory techniques that provide highly accurate, reproducible, and clinically relevant information. The choice of most appropriate technique/s is determined by the mutation type and samples type available for the analysis.

Testing for point mutations, such as those of BRAF and RAS, is relatively straightforward and can be achieved by a variety of molecular techniques including conventional PCR and Sanger sequencing, pyrosequencing, real-time PCR amplification and post-PCR melting curve analysis, allele specific PCR, and others [9,17,54,63,69,70,74,75]. Some of these techniques are illustrated in Figure 1. These techniques typically provide a reliable and sensitive detection of BRAF
mutations in various types of thyroid samples. One study that compared four different approaches, i.e. probe-specific real-time PCR, real-time allele specific PCR, direct sequencing and colorimetric assay, found all the techniques showing similarly high sensitivity in the detection \textit{BRAF} mutation in fixed FNA samples [63]. However, some caution has to be exercised in setting up the sensitivity of a diagnostic assay in order to avoid ultrasensitive detection of point mutations, which may increase probability of false-positive results and decrease diagnostic specificity.

The choice of a method for the detection of chromosomal rearrangements, such as \textit{RET/PTC} and \textit{PAX8/PPAR\gamma}, is dictated largely by the type of sample available. When freshly collected or snap-frozen FNA or tumor tissue samples are available, the testing can be reliably performed by reverse-transcriptase PCR (RT-PCR) in a conventional or real-time mode (Fig. 2). The sensitivity of detection should generally be not higher than 1\% of tumor cells (i.e. should detect 1\% or more tumors cells in the background of normal cells) to avoid detecting non-clonal rearrangements, which have no diagnostic implications. When only formalin-fixed and paraffin-embedded tissue is available for testing, fluorescence in-situ hybridization (FISH) is the assay of choice. The appropriate cut-off levels have to be established, which generally should be no less than 8-12\% of cells with the rearrangement pattern of signals, as this assure the reliable detection and avoid detecting non-clonal rearrangements [76]. Usage of RT-PCR for clinical detection of \textit{RET/PTC} and \textit{PAX8/PPAR\gamma} rearrangements in formalin-fixed and paraffin-embedded tissues should be avoided due to severe RNA degradation, which can not be compensated by choosing ultrasensitive conditions. The latter results in the loss of diagnostic specificity of the test due to increased detection of non-clonal rearrangement and higher risk of false-positive results. Fixed thyroid FNA samples are generally not suitable for testing for \textit{RET/PTC} and \textit{PAX8/PPAR\gamma} rearrangements in clinical setting.

\textbf{Key words:} thyroid cancer, fine-needle aspiration (FNA), molecular testing, \textit{BRAF}, \textit{RAS}, \textit{RET/PTC}, \textit{PAX8/PPAR\gamma}
References

5. Baloch ZW, Fleisher S, LiVolsi VA, Gupta PK. Diagnosis of "follicular neoplasm": a gray zone in thyroid fine-needle aspiration cytology. Diagnostic cytopathology 2002;26:41-44
7. Nikiforova MN, Nikiforov YE. Molecular Diagnostics and Predictors in Thyroid Cancer. Thyroid 2009
24. Nikiforov YE. RET/PTC Rearrangement in Thyroid Tumors. Endocr Pathol 2002;13:3-16
34. Namba H, Rubin SA, Fagin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol 1990;4:1474-1479


70. Hayashida N, Namba H, Kumagai A, et al. A rapid and simple detection method for the BRAF(T1796A) mutation in fine-needle aspirated thyroid carcinoma cells. Thyroid 2004;14:910-915


