Template for Reporting Results of Biomarker Testing of Specimens From Patients With Thyroid Carcinoma

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For the Members of the Cancer Biomarker Reporting Committee, College of American Pathologists

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CAP Thyroid Carcinoma Biomarker Template Revision History

**Version Code**
The definition of version control and an explanation of version codes can be found at www.cap.org (search: cancer protocol terms).

**Version:** ThyroidBiomarkers 1.0.0.0

**Summary of Changes**
This is a new template.
Thyroid Carcinoma Biomarker Reporting Template

Template web posting date: August 2015

Completion of the template is the responsibility of the laboratory performing the biomarker testing and/or providing the interpretation. When both testing and interpretation are performed elsewhere (e.g., a reference laboratory), synoptic reporting of the results by the laboratory submitting the tissue for testing is also encouraged to ensure that all information is included in the patient’s medical record and thus readily available to the treating clinical team.

THYROID

Select a single response unless otherwise indicated.

Note: Use of this template is optional.

+ SPECIMEN ADEQUACY

+ Adequacy Assessment of Thyroid Fine-Needle Aspirates (Note A)
  + ___ Adequate
  + ___ Inadequate
  + ___ Suboptimal (specify reason): ______________

+ Adequacy of Resected Specimens or Cell Blocks for Testing (Note A)
  + ___ Adequate
    + Estimated tumor cellularity (area used for testing): _____%
  + ___ Suboptimal (specify reason): ______________

Note: If "Adequate" not selected, please refer to original laboratory report for explanation.

+ RESULTS

+ BRAF Mutational Analysis (Note B)
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.V600E, c.1799T>A
    + ___ p.K601E, c.1801A>G
    + ___ Other BRAF mutation (specify): ______________
      + Indicate mutant allele frequency: _____%
  + ___ Cannot be determined (explain): ______________

+ TERT Mutational Analysis (Note B)
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ c.1-124 (C228T)
    + ___ c.1-146 (C250T)
    + ___ Other TERT mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ Data elements preceded by this symbol are not required.
+ **NRAS Mutational Analysis (Note C)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.Q61R, c.182A>G
    + ___ p.Q61K, c.181C>A
    + ___ Other NRAS mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **HRAS Mutational Analysis (Note C)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.Q61R, c.182A>G
    + ___ p.G12V, c.35G>T
    + ___ Other HRAS mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **KRAS Mutational Analysis (Note C)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.G12D, c.35G>A
    + ___ Other KRAS mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **AKT1 Mutational Analysis (Note D)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.E17K, c.49G>A
    + ___ Other AKT1 mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **TP53 Mutational Analysis (Note D)**
  + ___ No mutation detected
  + ___ Mutation identified (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **PIK3CA Mutational Analysis (Note D)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.H1047R, c.3140A>G
    + ___ Other PIK3CA mutation (specify): ______________
  + ___ Cannot be determined (explain): ______________

+ **CTNNB1 (β-catenin) Mutational Analysis (Note E)**
  + ___ No mutation detected
  + ___ Mutation identified
    + ___ p.S33A, c.97T>G
    + ___ Other CTNNB1 mutation (specify): ______________
  + ___ Cannot be determined
+ RET Mutational Analysis (Note F)
+ ___ No mutation detected
+ ___ Mutation identified
  + ___ p.M918T, c.2753T>C
  + ___ Other RET mutation (specify): __________________
+ ___ Mutation type:
  + ___ Germline (inherited)
  + ___ Somatic (sporadic)
  + ___ Unknown
+ ___ Cannot be determined (explain): __________________________

+ ALK Rearrangement (Note G)
+ ___ No rearrangement detected
+ ___ Rearrangement identified
  + ___ STRN/ALK
  + ___ EML4/ALK
  + ___ Other ALK rearrangement (specify): __________________
+ ___ Cannot be determined (explain): __________________________

+NTRK1 Rearrangement (Note H)
+ ___ No rearrangement detected
+ ___ Rearrangement identified
  + ___ NTRK1/TPM3
  + ___ NTRK1/TFG
  + ___ Other NTRK1 rearrangement (specify): __________________
+ ___ Cannot be determined (explain): __________________________

+NTRK3 Rearrangement (Note H)
+ ___ No rearrangement detected
+ ___ Rearrangement identified
  + ___ NTRK3/ETV6
  + ___ Other NTRK3 rearrangement (specify): __________________
+ ___ Cannot be determined (explain): __________________________

+ RET Rearrangement (Note F)
+ ___ No rearrangement detected
+ ___ Rearrangement identified
  + ___ RET/PTC1
  + ___ RET/PTC3
  + ___ Other RET rearrangement (specify): __________________
+ ___ Cannot be determined (explain): __________________________

+ PPAR gamma Rearrangement (Note I)
+ ___ No rearrangement detected
+ ___ Rearrangement identified
  + ___ PAX8/PPAR gamma
  + ___ CREB3L2/PPAR gamma
  + ___ Other PPAR gamma rearrangement (specify): ______________
+ ___ Cannot be determined (explain): __________________________

+ Other Markers Tested (if applicable)
+ ___ Specify marker: __________________________
+ ___ Specify results: __________________________

+ Data elements preceded by this symbol are not required.
+ METHODS

+ Dissection Method(s) (select all that apply)
  + ___ Laser capture microdissection
    + Specify test name*: __________________
  + ___ Manual under microscopic observation
    + Specify test name*: __________________
  + ___ Manual without microscopic observation
    + Specify test name*: __________________
  + ___ Cored from block
    + Specify test name*: __________________
  + ___ Whole tissue section (no tumor enrichment procedure employed)
    + Specify test name*: __________________
  # If more than 1 dissection method used, please specify which test was associated with each selected dissection method.

+ BRAF Mutational Analysis (select all that apply)
  + ___ Direct (Sanger) sequencing
  + ___ High-resolution melting analysis
  + ___ Next-generation (high-throughput) sequencing
  + ___ Immunohistochemistry
    + ___ VE1 clone
    + ___ Other (specify): __________________
  + ___ Other (specify): __________________

+ TERT Mutational Analysis
  + ___ Direct (Sanger) sequencing
  + ___ Next-generation (high-throughput) sequencing
  + ___ Other (specify): __________________

+ NRAS, HRAS, KRAS, AKT1, TP53, and PIK3CA Mutational Analysis (select all that apply)
  + ___ Direct (Sanger) sequencing
  + ___ High-resolution melting analysis
  + ___ Next-generation (high-throughput) sequencing
  + ___ Immunohistochemistry
    + ___ Clone (specify): __________________
  + ___ Other (specify): __________________

+ NRAS Codons Assessed (select all that apply)
  + ___ Codon 12
  + ___ Codon 13
  + ___ Codon 61
  + ___ Other (specify): __________________

+ HRAS Codons Assessed (select all that apply)
  + ___ Codon 12
  + ___ Codon 13
  + ___ Codon 61
  + ___ Other (specify): __________________

+ KRAS Codons Assessed (select all that apply)
  + ___ Codon 12
  + ___ Codon 13
  + ___ Codon 61
  + ___ Other (specify): __________________

+ Data elements preceded by this symbol are not required.
+ **ALK Rearrangement**
  + ___ In situ hybridization
  + ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  + ___ Immunohistochemistry
    + ___ **ALK** 5A4 clone
    + ___ **ALK** D5F3 clone
    + ___ Other (specify): _________________________
  + ___ Next-generation (high-throughput) sequencing

+ **PPAR gamma Rearrangement**
  + ___ In situ hybridization
  + ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  + ___ Immunohistochemistry
    + ___ Clone (specify): _________________________
  + ___ Next-generation (high-throughput) sequencing

+ **RET/PTC1, RET/PTC3, NTRK1, and NTRK3 Rearrangement**
  + ___ In situ hybridization
  + ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  + ___ Immunohistochemistry
    + ___ Clone (specify): _________________________
  + ___ Next-generation (high-throughput) sequencing

+ **CTNNB1 Mutational Analysis**
  + ___ Direct (Sanger) sequencing
  + ___ Next-generation (high-throughput) sequencing
  + ___ Immunohistochemistry
    + ___ Clone (specify): _________________________

+ **Sensitivity/Limit of Mutation Detection (Note A)**
  + ___ ≥20%
  + ___ ≥10%
  + ___ ≥5%
  + ___ Other (specify): ________%

+ **Other Methods Used (if applicable)**
  + ___ Specify method: __________________________

+ **COMMENT(S)**
  _______________________________________________________________________
  _______________________________________________________________________

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Note: Fixative type, time to fixation (cold ischemia time), and time of fixation should be reported if applicable in this template or in the original pathology report.

Gene names should follow recommendations of The Human Genome Organisation (HUGO) Nomenclature Committee (http://hugo-international.org; accessed February 10, 2015).

All reported gene sequence variations should be identified following the recommendations of the Human Genome Variation Society (http://www.hgvs.org; accessed February 10, 2015).
Explanatory Notes

A. Specimen Adequacy
The collection of material for molecular studies should not affect the morphologic cytologic assessment. For fine-needle aspirates (FNA), at the time of the FNA procedure, a small portion of the (residual) aspirated material may be collected into nucleic acids preservative. The material may represent a part of the first needle pass or a separate pass dedicated for the molecular analysis. The storage and transportation conditions (time, temperature) have to be specified by laboratories.

The quantity of isolated nucleic acids is the total amount of extracted nucleic acids. The minimal acceptable amount of nucleic acids will depend on the methodology and should be determined by laboratories. The quality of DNA and RNA can be assessed by amplification of housekeeping genes (eg, GAPDH, PGK1). The trouble-shooting procedure for suboptimal specimens should be specified (eg, increasing and decreasing the amount of nucleic acid template).

The proportion of follicular thyroid epithelial cells in an FNA sample can be assessed by comparing the expression of the housekeeping gene and a gene known to be expressed predominantly in thyroid follicular cells (eg, keratin 7, thyroid transcription factor 1 [NK2 homeobox 1]), genes expressed in mimics of thyroid nodule (eg, parathyroid hormone), or genes expressed in medullary thyroid carcinoma (ie, calcitonin).

The sensitivity of mutation detection and the method used to establish sensitivity should be established by the laboratory for each methodology (eg, serial dilutions of the positive controls in normal blood/lymphocytes or normal formalin-fixed paraffin-embedded tissue).

Resection specimens may be inadequate due to improper fixation, decalcification, low tumor content, or small tumor size.

B. BRAF Mutational Analysis
The presence of BRAF V600E mutation in a fine-needle aspirate is indicative of about 99% risk of cancer in the sampled thyroid nodule. When identified alone, BRAF V600E mutation may merely reflect the conventional morphology or tall cell variant of papillary thyroid carcinoma. The combination of BRAF V600E mutation with TERT, AKT1, PIK3CA, or TP53 mutations predicts a more aggressive tumor behavior. BRAF K601E is an unusual BRAF mutation, which had been reported in follicular variant of papillary thyroid carcinoma and rarely in follicular adenomas.

C. RAS Mutational Analysis
The finding of RAS mutation in a fine-needle aspirate is associated with an about 80% risk of cancer in a given nodule. The most common types of cancer with RAS mutations are the encapsulated follicular variant of papillary carcinoma and follicular carcinoma. The remaining RAS-positive thyroid nodules are usually diagnosed as follicular adenomas. Sporadic medullary thyroid carcinomas with wild type RET genes may harbor RAS mutations (HRAS or KRAS).

D. PIK3CA, AKT1, and TP53 Mutational Analysis
PIK3CA, AKT1, and TP53 mutations are usually found in advanced thyroid cancer with propensity for dedifferentiation and distant metastasis.

E. CTNNB1 Mutational Analysis
The presence of CTNNB1 mutation in a given thyroid nodule is expected to confer a >90% risk of cancer. Point mutations in exon 3 of CTNNB1 stabilize the protein by making it insensitive for adenomatous polyposis coli (APC)-induced degradation, leading to the accumulation of β-catenin in the nucleus. In thyroid tumors, mutations in exon 3 of CTNNB1 were also reported in poorly
differentiated and anaplastic carcinomas, but not in well-differentiated carcinomas or benign thyroid nodules.\textsuperscript{16}

**F. RET Mutational Analysis**
The presence of RET rearrangements in thyroid fine-needle aspirate is associated with >95% risk of cancer, most frequently classic papillary thyroid carcinoma. Mutations of the RET gene are typically present in sporadic and familial forms of medullary thyroid carcinoma. Among sporadic medullary carcinomas, RET p.M918T mutation accounts for more than 75% of all somatic RET mutations found in medullary carcinomas.\textsuperscript{19,20}

Laboratories should disclose whether the test was performed on tissue type (tumor versus normal tissue) that allows distinguishing between germline (inherited) and sporadic (acquired) mutation. Nevertheless, the distinction between sporadic and germline mutation can be reliably made only by testing a nontumorous specimen, preferably patient blood. Clinical management of patients based on the presence of specific RET mutations has been defined.\textsuperscript{19,20}

**G. ALK Mutational Analysis**
The identification of ALK fusions (STRN/ALK or EML4/ALK) in a thyroid FNA is associated with a very high risk of thyroid cancer. ALK fusions were identified in \~1.5% of papillary thyroid carcinomas and in 4% to 9% of dedifferentiated thyroid cancers.\textsuperscript{21,22} In advanced papillary thyroid carcinomas and in dedifferentiated thyroid tumors, the presence of an ALK fusion may represent a therapeutic target for crizotinib.\textsuperscript{21,22}

**H. NTRK1 and NTRK3 Mutational Analysis**
Rearrangements of the NTRK1 gene occur in <5% of papillary carcinomas.\textsuperscript{23} Different fusions partners of NTRK1 have been described including TPM3 and TPR genes. Some studies reported that NTRK1 fusion-positive papillary thyroid carcinomas may have more aggressive biological behavior and higher rate of local recurrence.\textsuperscript{24} NTRK3 fusions have been reported in papillary thyroid carcinomas.\textsuperscript{25,26} In vitro studies showed that ETV6/NTRK3 aberrantly activates phosphatidylinositol 3-kinase signaling pathway. A phase 1a/1b clinical trial of the oral TRK Inhibitor LOXO-101 is available.

**I. PPARG Mutational Analysis**
The presence of rearrangements involving the PPARG gene, PAX8/PPARG and less frequently CREB3L2/PPARG, correlate with \~95% risk of cancer, most frequently follicular variant of papillary carcinoma, followed in frequency by follicular carcinoma. Rare cases of follicular adenoma carrying PPARG rearrangements have been reported.\textsuperscript{27} Most of thyroid cancers positive for PPARG rearrangements are low-grade tumors, whereas 5% to 10% of those tumors have aggressive behavior. Of note, PPARG fusions can be exploited as a therapeutic target for advanced thyroid cancer. The presence of PAX8/PPARG or CREB3L2/PPARG rearrangement in thyroid fine-needle aspirates correlated with >95% risk of cancer, most frequently follicular variant of papillary carcinoma or follicular carcinoma.\textsuperscript{28}

**References**


