ThyroSeq® - Thyroid Cancer Next-Generation Sequencing Panel

**TEST DETAILS**

**ThyroSeq®** - targeted mutation detection by next generation sequencing in THYROID fine needle aspiration (FNA) and tissue samples. ThyroSeq v.2 next generation sequencing panel offers simultaneous sequencing and detection in >1000 hotspots of 14 thyroid cancer-related genes and for 42 types of gene fusions known to occur in thyroid cancer.

**Gene List for Mutations:** AKT1• BRAF• CTNNB1•GNAS•HRAS•KRAS•NRAS•PIK3CA•PTEN•RET•TP53•TSHR•TERT•EIF1AX
**Gene List for Gene Fusions and Gene Expression:** RET• PPARG• NTRK1•NTRK3•ALK•IGF2BP3•BRAF•MET•CALCA•PTH•SLC5A5• TG•TTF1•KRT7•KRT20

**Indications for testing:**
- Thyroid FNA diagnosed as indeterminate by cytology (Bethesda categories III, IV, V)
- Thyroid FNA diagnosed as malignant by cytology, when molecular testing is expected to affect the decision to perform surgery or determine the extent of surgery
- Thyroid FNA diagnosed as benign by cytology, when strong clinical suspicion for cancer exists based on ultrasonographic features or other imaging and clinical studies.
- Diagnosis of cancer is established in preoperative FNA or surgically excised thyroid tissue, when molecular profiling of cancer will affect clinical decision with regards to administration of radioactive iodine, intensity of follow up, or targeted therapies for advanced cancer.

**Background:**
Thyroid cancer is the most common malignancy of endocrine organs and its incidence is steadily growing in the U.S. and worldwide (1). Thyroid cancer typically occurs in thyroid nodules, which are prevalent in the general population, particularly with increased age. However, most of thyroid nodules are benign and clinical challenge is to accurately identify those nodules that are malignant and need to be surgically removed. Ultrasound-guided fine-needle aspiration (FNA) followed by cytological examination is a standard diagnostic approach that allows to reliably detect cancer or establish a diagnosis of benign nodule in most cases. However, in about 25% of examined thyroid nodules, the presence of cancer cannot be ruled out by FNA cytology and one of the indeterminate cytologic diagnosis is rendered, hampering appropriate clinical management of these patients. According to The Bethesda System for Reporting Thyroid Cytopathology, indeterminate cytologic diagnoses include (i) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) (Bethesda III), (ii) follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) (Bethesda IV), and (iii) suspicious for malignant cells (Bethesda V).

Thyroid cancer develops and progresses through accumulation of genetic alterations, which can serve as important diagnostic, prognostic, and predictive biological markers (2). The most common mutations that occur in papillary thyroid cancer (PTC) are point mutations of the BRAF and RAS genes and RET/PTC rearrangements, all of which are able to activate the mitogen-activated protein kinase (MAPK) pathway. Two most common types of RET/PTC rearrangement are RET/PTC1 (RET/CCDC6) and RET/PTC3 (RET/ELE1) followed by RET/PTC6 (RET/HTIF1) and >10 other, rare subtypes (2). Follicular thyroid cancer most frequently harbors RAS mutations or PAX8/PPARγ rearrangement. Genetic alterations involving the PI3K/AKT signaling pathway (PIK3CA, PTEN, AKT1 mutations) also occur in thyroid tumors, particularly in advanced and dedifferentiating tumors. Additional mutations known to occur in more aggressive types of well differentiated thyroid cancer as well as in poorly differentiated and anaplastic carcinomas involve the TP53 and CTNNB1 genes. Medullary thyroid carcinomas frequently carry point mutations affecting the RET and RAS genes. Somatic mutations of TSHR and GNAS frequently occur in autonomously functioning benign thyroid nodules, although TSHR mutations located at specific hotspots and present at high allelic frequency are also associated with thyroid follicular carcinomas. More recently identified point mutations and gene fusions in thyroid cancer include EIF1AX mutations (3) and STRN-ALK and ETV6-NTRK3 fusions (4,5). Mutations in the promoter region of the TERT gene, either C228T or C250T, were recently reported in well differentiated thyroid papillary and follicular carcinomas and with higher frequency in dedifferentiated thyroid cancers (6-8).
Finding BRAF or TERT mutation or any type of gene fusion in thyroid FNA samples at high (>10%) allelic frequency correlates with >95% probability of cancer, whereas finding of RAS mutation confers a cancer risk of 74-85% (9-12). In our validation analysis, the presence of TSHR or PTEN mutation correlated with a 20-40% risk of malignancy, and the risk was higher in nodules with a very high (>30%) allelic frequency of TSHR mutations (13). Mutations in the TP53, PIK3CA, CTNNB1, and AKT1 genes are found more commonly in advanced thyroid cancers and rarely in benign nodules (14). EIF1AX-positive tumors were predominantly follicular variants of PTC and low grade at presentation (3), and in our validation analysis EIF1AX mutations were also seen in benign thyroid nodules, predicting a ~50% risk of cancer in thyroid nodules carrying this mutation. Mutations of the RET gene are typically present in sporadic and familial forms of medullary thyroid carcinoma. The residual risk of cancer in nodules negative for all mutations and gene fusions was established in our earlier reported study at UPMC (11), validation analysis of >180 samples with indeterminate cytology and available surgical follow-up (13), and a recent study of 143 consecutive FNA samples with a cytoligic diagnosis of FN/SFN from patients with known surgical outcomes using ThyroSeq v.2 (15). Based on these studies and in populations with similar disease prevalence in each cytology group, the residual risk of cancer in thyroid nodules with AUS/FLUS (Bethesda III) cytology (cancer prevalence 14%) and negative ThyroSeq v.2 test is expected to be 3%, in thyroid nodules with FN/SFN (Bethesda IV) cytology (cancer prevalence 27%) and negative ThyroSeq v.2 test to be 4%, and in thyroid nodules with suspicious for malignant cells (Bethesda V) cytology (cancer prevalence 60%) and negative ThyroSeq v.2 test to be 20%. To illustrate the impact of disease prevalence on cancer risk, if at a given institution the probability of cancer in nodules with FN/SFN cytology is 35%, ThyroSeq v.2 negative samples would be expected to have a 5% residual risk of cancer, and the pre-test probability of cancer at 50% would increase the residual cancer risk in ThyroSeq-negative nodules to 10%. Clinical management of patients with thyroid nodules based on the combination of cytoplogic findings and molecular testing has been proposed (11) and validated in routine clinical practice (16). It was also suggested that mutational testing of thyroid nodules diagnosed as cancer on FNA cytology may be helpful to define the appropriate surgical and post-surgical management of these patients (17,18). Finally, mutational testing allows to decrease the rare of false-negative thyroid cytology (9), and was suggested to be beneficial for samples with benign cytology, but only in the presence of clinical suspicion of malignancy (19).

Mutational markers can also be used for tumor prognostication. The presence of TERT mutations is associated with more invasive tumor phenotype at presentation (6,7) and with a significantly higher risk of distant metastases, disease persistence and cancer-specific mortality (8). BRAF V600E mutation has been associated with higher risk of tumor recurrence and cancer-specific survival (20,21), especially when BRAF mutation is found in combination with other mutations such as TERT (22). In a similar way, the presence of multiple mutations and/or TP53 mutations may predict more aggressive tumor behavior and predisposition to tumor dedifferentiation in TP53-mutant cancers (13).

Mutational status may also inform targeted therapies for advanced thyroid cancer. Clinical trials with BRAF or MEK inhibitors to enhance the radiiodine uptake are available for patients with BRAF-mutant, RAI-refractory thyroid cancer (ClinicalTrials.gov Identifier: NCT02145143) and RAS mutant thyroid cancer (ClinicalTrials.gov Identifier: NCT02152995), and trials with a combination of BRAF and MEK inhibitors to treat patients with BRAF-mutated anaplastic thyroid cancer (ClinicalTrials.gov Identifier: NCT02034110). Furthermore, clinical trials targeting advanced thyroid cancer carrying PAX8/PPARγ fusions (ClinicalTrials.gov Identifier: NCT01655719) and tumors carrying NTRK alterations (ClinicalTrials.gov Identifier: NCT02122913) are currently open. Preclinical studies and single case reports suggest that patients with advanced thyroid cancer, including anaplastic thyroid cancer, carrying ALK fusions can benefit from treatment with ALK inhibitors such as Crizotinib (4,23,24).

References:

ThyroSeq NGS Panel is performed on DNA and RNA isolated from FNA material collected into preservative solution (please contact MGP laboratory at 412-864-6162 for collection protocol and solution tubes), fixed FNA specimens, or formalin-fixed, paraffin-embedded (FFPE) tumor tissue. The analysis is performed using next-generation, semiconductor-based sequencing (Ion Torrent PGM platform). Input DNA and RNA is amplified using the AmpliSeq technology (Ion Torrent), after which the amplicons are modified with adaptors, re-amplified, and subjected to emulsion PCR. The final products are sequenced on a 318 chip. The analytical sensitivity is 3.5% of mutant alleles for detection of mutations and 1% for detection of gene fusions. The minimal required sequencing depth is 500x.

**SPECIMEN REQUIREMENTS AND SHIPPING INSTRUCTIONS**

**Specimen Type:** FNA (Fine Needle Aspiration)

**Requirements:** To collect an FNA sample for molecular testing, during the FNA procedure place small part (1-2 drops) of the first pass into a provided collection tubes and use the rest of the first pass for cytology. Wash the needle using solution in the tube to collect more material if necessary. If the first pass is not cellular (based on cytology) or cellularity cannot be determined immediately and fluid in the tube has not changed color, add one-half of the second pass into the SAME tube. (Please contact lab for collection tubes)

**Shipping Conditions:** After the sample is collected, the specimen should be kept at -20°C. If this is not possible, the specimen can be kept at room temperature for no longer than 6 hours or at 4°C for no longer than 24 hours. Specimens can be shipped at room temperature when using “next business morning” delivery or with ice
packs by overnight mail. Attach a completed requisition form and send the specimen with a surgical pathology and/or cytology report.

**Specimen Type:** FFPE – (Formalin Fixed Paraffin Slides)
**Requirements:**
4-6 unstained 5um FFPE slides containing a minimum of 300 tumor cells to be analyzed with areas of tumor marked.

Transport Temperature: sent at 20-25°C. Protect paraffin tissue from excessive heat. Ship in cooled container during summer months.

Unacceptable conditions: No tumor in tissue, decalcified specimen, alternative fixtures.

**Shipping Conditions:**
Ship at room temperature (20-25°C) in an insulated container by overnight courier. Do not heat or freeze. Attach a completed requisition form and send specimen with a surgical pathology and/or cytology report.

**Specimen Type:** Fresh Frozen Tissue
**Requirements:**
A minimum of 2x2x2 mm (optimal 5x5x5 mm) of fresh tissue snap frozen at -20°C. Store at -20°C. Tissue specimen containing at least 50% of tumor cells can be either placed into cryogenic tube and snap frozen in liquid nitrogen, or placed into a tube with preservative solution provided by the Molecular Anatomic Pathology laboratory (request solution from the lab) and frozen at -20°C.

**Shipping Conditions:**
Ship on dry ice in an insulated container by overnight courier. Attach a completed requisition form and send specimen with a surgical pathology and/or cytology report.

**Specimen Type:** Other
**Requirements:**
Please contact the laboratory to discuss other specimen types that may be acceptable.

**Shipping Conditions:**
Please contact the laboratory to discuss if shipping other specimen types.

**Turn Around Time:** 7 days

**Billing Information**
**List Price:** *For insurance or Institutional Price, please call.

A REQUISITION FORM MUST ACCOMPANY ALL SAMPLES. Please include detailed clinical information.