ThyroiNow™ Digital Thyroid Cancer Panel

GOPATH® LABORATORIES
Global Pathology Services

"Approximately one-third of Thyroid nodules are classified as indeterminate due to atypical cytology findings"

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"GoPath's ThyroiNow™ is a highly sensitive and specific test for "indeterminate" and "suspicious" Thyroid FNA biopsies"

Clinical Utility

Thyroid cancer typically presents as a nodule without clinical symptoms at early stage. Differential diagnosis of thyroid nodules currently relies on Bethesda criteria combined with cytology findings from FNA biopsy. However, approximately one-third of nodules are classified as indeterminate nodules due to atypical cytology findings and only 10-40% of indeterminate nodules prove to be malignant after surgery. Considering the high medical cost and complications caused by surgery such as hypocalcemia, RLN damage, bleeding and infection, an urgent need has emerged for a more accurate and definitive diagnostic test to differentiate between benign thyroid nodules and malignant nodules in FNA indeterminate biopsies to avoid cost and morbidity.

Testing for common somatic mutations in thyroid cancer has been shown to be useful in detecting thyroid cancer in "indeterminate" and "suspicious" thyroid FNA biopsies. Several somatic mutations including BRAF V600E, KRAS G12D, KRAS G13D, and NRASQ61R have been identified in approximately 50% of thyroid cancers with BRAF V600E mutations present in up to 60% of papillary thyroid cancer. Using droplet digital PCR (ddPCR) technology, we have developed the ThyroiNow™ assay which uses a highly sensitive molecular platform currently available for detecting somatic mutations in thyroid FNA samples. Information obtained from this test will greatly improve the accuracy of diagnosis for thyroid FNA biopsies with indeterminate cytology findings.

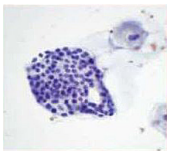
•Lower Medical Costs and Complications
One test provides accurate answers for indeterminate cytology findings to avoid unnecessary surgery for your patients.

•BRAFV600E, KRASG12D, KRASG13D AND NRASQ6
These genes have been identified in approximately 50% of thyroid cancers.

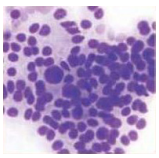
Assay Description and Methodology

DNA from thyroid FNA samples is prepared from either fresh cell pellets or FFPE cell blocks using designated Qiagen DNA kits and is quantified by a measuring instrument (Life Technologies Inc.). BRAF V600E, KRAS G12D, KRAS G13D, and NRAS Q61R mutations are analyzed by a digital PCR approach using assay reagents and a QX200 digital PCR System from BioRad Inc. Sensitivity cut-off established by our laboratory for detection of BRAF V600E and NRAS Q61R mutations is 0.1%. Sensitivity cut-off established by our lab for detection of KRAS G12D and KRAS G13D mutations is 1.0%.

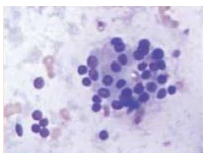
Figure 1. Indeterminate nodules show a follicular growth pattern and therefore FNA is often not sufficient to distinguish between benign and malignant lesions



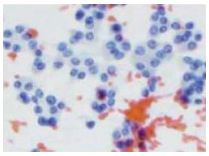
Bethesda II: benign



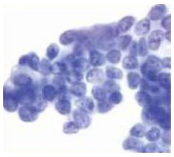
Bethesda III: follicular lesion of indeterminate significance



Bethesda IV: suspicious for Hurthle cell neoplasm



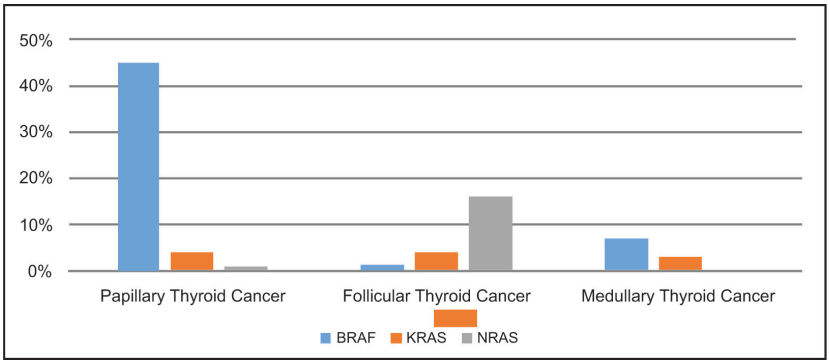
Bethesda IV: suspicious for papillary thyroid cancer



Bethesda V: papillary thyroid cancer

Bethesda classification for thyroid nodule cytology (according to The NCI Thyroid Fine-Needle-Aspiration State of the Science Conference scheme). Keutgen, X. M., Filicori, F., & Fahey III, T. J. (2013). Molecular Diagnosis for Indeterminate Thyroid Nodules on Fine Needle Aspiration. Retrieved August 05, 2016, from http://www.medscape.com/viewarticle/809127

Figure 2. Frequency of Genetic Alterations in 3 Types of Thyroid Cancer



	Papillary Thyroid Cancer	Follicular Thyroid Cancer	Medullary Thyroid Cancer
BRAF	45%	1.3%	7%
KRAS	4%	4%	3%
NRAS	1%	16%	0%

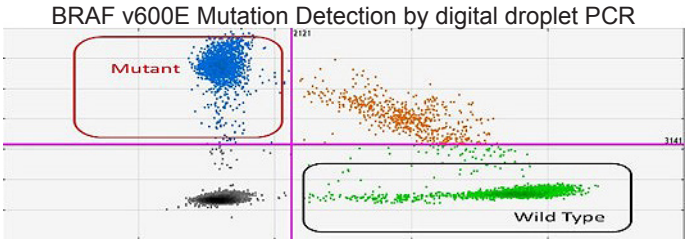
Nucleic Acids Research. (n.d.). Retrieved August 04, 2016, from http://nar.oxfordjournals.org/content/43/D1/D805.abstract?keytype=ref

Interpretation

Positivity of one of the above mutations (see Figure 2.) indicates the likelihood of the presence of neoplastic cells in the tested sample. Negativity of the above mutations indicates that either no neoplastic cell is present in the tested sample or the number of neoplastic cells is below the sensitivity cut-offs of the assays. Therefore, negative results of above mutations do not exclude the existence of neoplastic lesions in this patient.

Figure 3. Example Of A Positive BRAF V600E Mutation Detection Result

Mutation	Location 1	Detection	Location 1	Detection
$BRAF_{V600E}$	H. Nodule	Positive	Left LN	Negative
$KRAS_{G12D}$	H. Nodule	Negative	Left LN	Negative
$KRAS_{G13D}$	H. Nodule	Negative	Left LN	Negative
$NRAS_{Q61R}$	H. Nodule	Negative	Left LN	Negative



Samples For Submission

FNA samples are delivered in the form of either FFPE tissues or freshly collected specimens in 500 µl of ThinPrep® Cytolyt Solution (Hologic Inc.) provided by GoPath Laboratories.

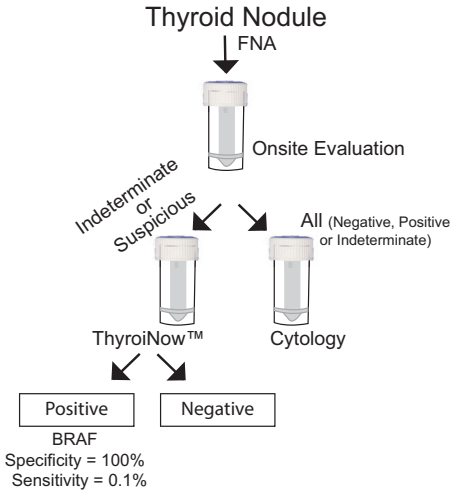
Inadequate Samples

FNA specimens yielding less than 40ng of DNA after extraction are reported as insufficient tissue material for the test.

Assay Limitations

This test is intended for detections of BRAF V600E, KRAS G12D, KRAS G13D and NRAS Q61R mutations in clinical thyroid FNA specimens within sensitivity limits specified above and its performance characteristics were determined by GoPath Laboratories. Mutations other than the above four mutations are not tested and the mutations present below the sensitivity limits of the assay in samples are not detected.

Figure 4. Indications For Testing



References

1. Cohen Y, et al (2004) Mutational analysis of BRAF in fine needle aspiration biopsies of the thyroid: a potential application for the preoperative assessment of thyroid nodules. Clin Cancer Res 10(8):2761–2765
2. Xing M: BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005 Jun;12(2):245-62.
3. Park SJ. et al: Application of BRAF, NRAS, KRAS mutations as markers for the detection of papillary thyroid cancer from FNAB specimens by pyrosequencing analysis. Clin Chem Lab Med. 2013 Aug;51(8):1673-80.
4. Pohl G. et al: Principle and applications of digital PCR. Expert Rev Mol Diagn. 2004 Jan;4(1):41-7.

GOPATH® LABORATORIES
Global Pathology Services

1351 Barclay Blvd, Buffalo Grove, IL 60089
Toll Free: 1- 855- GOPATH9 (855.467.2849)
Fax: 224.588.9941
E-mail: sales@gopathlabs.com