

## REPORT - Indeterminate Thyroid Nodule Molecular Classification Test

**Patient:**  
**Patient ID:**      **DOB:**  
**Requesting Physician:**  
**Medical license ID:**      **Country:**      **Results released on:**

**Report sent to:**  
☐ Patient  
☐ Requesting Physician  
☐ Lab Partner

### SAMPLE RECEIVED



#### Nodule Classification:

- ☒ **Bethesda III**  
☐ **Bethesda IV**  
☐ **Bethesda V**

According to the  
received FNACytology  
report

#### Sample type:

#### Received Date:

#### Analyzed Nodule ID:

#### Supplementary Comments

### FINAL RESULTS

The expression profile of microRNAs<sup>1</sup> and the status of mutations in the *BRAF* gene (V600E) and in the *TERT* gene promoter (C228T and C250T) of the submitted sample were classified by our algorithm which evaluated the molecular behavior for malignancy as potentially:

☐ **NEGATIVE**  
☒ **POSITIVE**

Risk of Malignancy:

**>98%**

#### Detailed results:

##### microRNA Expression:

microRNAs profiling ☐ negative ☒ **positive**  
Medullary Carcinoma ☐ negative ☒ **positive**  
miR-146b expression ☐ low ☒ **high**

##### Mutations (DNA):

*BRAF* V600E ☒ not detected ☐ detected  
*pTERT* C228T ☒ not detected ☐ detected  
*pTERT* C250T ☒ not detected ☐ detected

#### Pre-<sup>2</sup> and post-test risk of malignancy

pre-test  
**12-30%**

Bethesda II

Bethesda III

Bethesda IV

Bethesda V

Bethesda VI

Risk of Malignancy (Cancer Probability)

post-test  
**>98%**

### TECHNICAL NOTES

**Note 1:** Real-time PCR analysis developed and validated in-house. At clinical decision, if the patient has other eligible nodule(s), molecular investigation is suggested for diagnostic and/or prognostic complementation.

**Note 2:** In the investigation of the microRNA profile, the pre and post-test quality controls were in accordance with the validated parameters and were considered approved(1).

**Note 3:** The overexpression of microRNA miR-375 in this sample suggests positivity for the Medullary Thyroid Carcinoma tumor subtype. The risk of malignancy observed in samples with the same result is 98%, i.e. there is still a 2% probability of the analyzed nodule being negative for malignancy. This risk of malignancy (98%) is similar to that observed in samples classified as Bethesda 6 in the cytological examination.

**Note 4:** According to the American Thyroid Association (ATA), the investigation of germline mutations in the *RET* gene is suggested for the investigation of MEN2A syndrome and Familial Medullary Carcinoma. The mir-THYpe RET-Hereditary test investigates mutations in all exons of the *RET* gene and is available throughout Brazil.

**Note 5:** Considering the limited amount of DNA available, it was not possible to investigate somatic mutations in the *RET* gene.

**Note 6:** In the case of evaluation of somatic mutation status, non-detectable results mean absence of mutant alleles or, although unlikely, but possible, presence below the detection limit.

## SUPPLEMENTARY COMMENTS

### **BRAF V600E isolated**

This mutation, when isolated, is associated with a high probability (>98%)<sup>3</sup> of Papillary Thyroid Carcinoma. The risk of recurrence is classified by the ATA<sup>4</sup> as intermediate for tumors > 1cm and low for tumors > 1cm. Although some studies suggest that this mutation is a predictor of aggressiveness and worse clinical prognosis<sup>5</sup>, there is still no consensus in the literature about its real prognostic power (when isolated).<sup>4,6</sup> Recently it was observed that patients over 55 years of age with this mutation had a significantly lower recurrence-free survival.<sup>7</sup> The American Health Agency (FDA) has already approved<sup>8</sup> the use of Dabrafenib in association with Trametinib for patients with this mutation for all unresectable or metastatic solid thyroid tumors that have progressed after prior treatment, in adult and pediatric patients over 6 years of age.

### **pTERT C228T or C250T isolated**

This mutations are associated with a high probability (>88%) of Papillary or Follicular Thyroid Carcinoma and predictor of more aggressive tumor behavior and worse prognosis, including increased risk of recurrence/persistence, lymph node metastasis, extrathyroidal extension, capsule invasion, tumor size, distant metastasis and higher TNM staging.<sup>5</sup>

### **BRAF V600E associated with pTERT C228T or C250T**

The coexistence of these mutations is associated with a high probability (>98%) of Papillary Thyroid Carcinoma. The risk of recurrence is classified by the ATA<sup>4</sup> as high. This association is predictive of more aggressive tumor behavior and worse prognosis (especially with pTERT C228T), including increased risk of recurrence/persistence, lymph node metastasis, extrathyroidal extension, capsule invasion, tumor size, distant metastasis and higher TNM staging and worse response to therapy.<sup>5,9,10</sup>

### **miR-146b highly expressed**

Overexpression of this microRNA is associated with a high probability (>92%) of Papillary Thyroid Carcinoma. The high expression of this microRNA has shown an impact on disease-free survival<sup>11</sup> and an increased risk of central lymph node metastasis.<sup>12</sup>

## ABOUT THIS TEST

The indeterminate thyroid nodule molecular classification test is indicated only for patients with indeterminate thyroid nodules, which means, those who, in the cytological analysis of the FNA smear slide(s) were classified by the Bethesda System as categories III or IV and, in selected cases, as V. The test analyzes the mutational status of the BRAF V600E and of the promoter region of the TERT gene (C228T and C250T), markers of clinical diagnostic and prognostic utility. The test also analyzes a microRNA expression profile and, through a proprietary algorithm, accurately assists in the classification of indeterminate thyroid nodules by evaluating the molecular behavior of the sample as potentially "positive" or "negative" for malignancy. The test performance was calculated based on a validation study<sup>1</sup> that compared the results obtained by the mir-THYpe<sup>®</sup> test, using genetic material extracted from FNA cytology smear slide samples from patients with indeterminate thyroid nodules, with the results of the post-surgical anatomopathological histology of the same nodules (by consensus of, at least, two independent cytopathologists). The classification algorithm has not been trained with samples of thyroid nodules classified as Bethesda I, II or VI or other tumor types and biological samples. The test also analyzes the isolated expression of the miR-146b (a biomarker that predicts potentially more aggressive behavior in papillary carcinoma<sup>11, 12</sup>) and the miR-375 (a biomarker of medullary thyroid carcinoma<sup>13</sup>). The results obtained using this test should be interpreted together and in context with other diagnostic and clinical findings to decide on the medical/clinical management to be followed, especially on the need or not of any surgical procedure, including the surgical extension and the total or partial removal of the thyroid gland. The results obtained using this test are relevant only to the nodule that was analyzed.

## REFERENCES

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