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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

Acording to the received FNAcytology report

Sample type:

Received Date:

Analyzed Nodule ID:

Supplementary Comments

FINAL RESULTS

The expression profile of microRNAs[1] 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:
<4%

Detailed results:

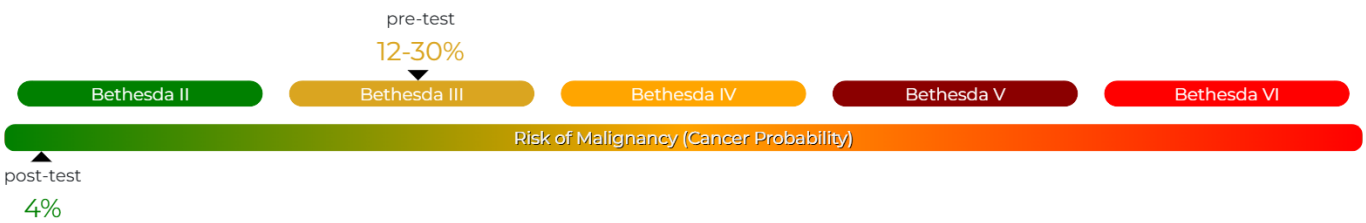
microRNA Expression:

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-[2] and post-test risk of malignancy



TECHNICAL NOTES

Note 1: Test developed and validated in-house (LDT). At clinical descrition, 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 are in accordance with the validated parameters and were considered approved(1).

Note 3: The probability of a "negative for malignancy" result actually corresponding to a benign nodule (a parameter known as the negative predictive value) is 96%. This value was calculated based on the evidence observed in the diagnostic performance validation study (1). This means that, given this result, there is still a residual probability (risk of residual malignancy) of 4% of the analyzed nodule being positive for malignancy (cancer or PTLNC). The 4% risk of residual malignancy assigned to the sample analyzed in this molecular test is statistically similar to the risk of malignancy assigned to samples classified as "Bethesda 2 - Benign" in the cytological examination (2-7%), and therefore suggests a similar clinical approach.

Note 4: In the investigation of the status of the *BRAF* V600E and *pTERT* C228T and C250T mutations, the pre- and post-test quality controls are in line with the validated parameters and were considered approved.

Note 5: In the case of evaluating the status of *BRAF* V600E and *pTERT* C228T/C250T mutations, non-detectable results mean the absence of mutant alleles or, although unlikely, but possible, their presence below the detection limit. The validated detection limits are 10 and 100 copies of mutant alleles respectively.

SUPPLEMENTARY COMMENTS

***BRAF* V600E isolated**

This mutation, when isolated, is associated with a high probability (>98%)[3] of Papillary Thyroid Carcinoma. The risk of recurrence is classified by the ATA[4] 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[5], there is still no consensus in the literature about its real prognostic power (when isolated).[4,6] Recently it was observed that patients over 55 years of age with this mutation had a significantly lower recurrence-free survival.[7] The American Health Agency (FDA) has already approved[8] the use of Dabrafenib in association with Tramatinib 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.[5]

***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[4] 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.[5, 9, 10]

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[11]and an increased risk of central lymph node metastasis.[12]

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[1] that compared the results obtained by the mir-THYpe® 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[11, 12]) and the miR-375 (a biomarker of medullary thyroid carcinoma[13]). 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.

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