



## Clinical Validity of RosettaGX Reveal for the Accurate Diagnosis of Pre-Operative Medullary Thyroid Carcinoma Utilizing FNA Smears

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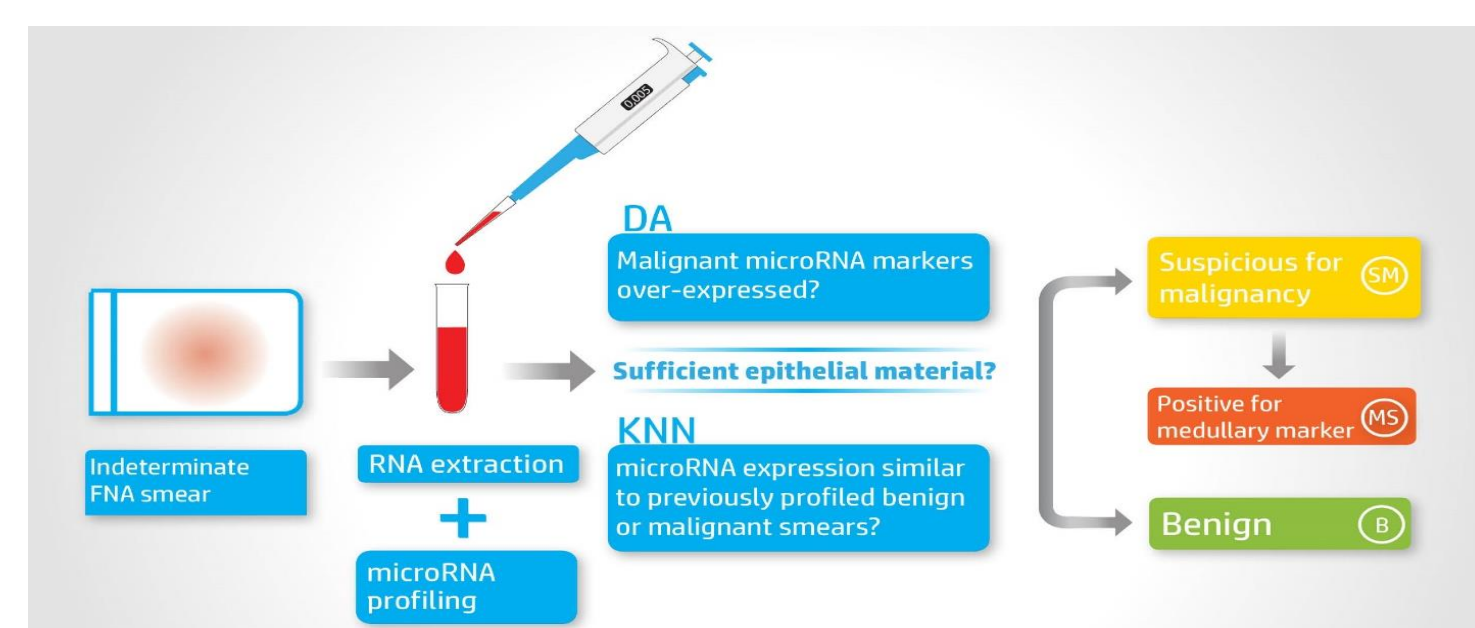
### 1. BACKGROUND

Medullary Thyroid Carcinoma (MTC) is a relatively uncommon disease, accounting for less than 5% of all thyroid malignancies, but it causes a disproportionate number of thyroid cancer-related deaths. While histologic identification of MTC is quite reliable, early clinical detection, which is important for patient management, can be challenging. Fine-needle aspiration (FNA) is an important tool for the diagnosis of thyroid cancer, however, in some cases an MTC diagnosis is not suspected based on FNA cytology alone.

MicroRNAs constitute a class of short, non-coding RNAs and their expression profile has been shown to be a highly reliable tool for cancer subtype classification. We developed a microRNA-based assay, RosettaGX Reveal™, which utilizes FNA smears to classify thyroid nodules with indeterminate cytology as benign, suspicious for malignancy, or positive for medullary carcinoma. The objective of this study was to characterize Reveal's performance in the clinical setting with regard to MTC and to present specific clinical cases, demonstrating the benefits of using the Reveal assay.

### 2. MATERIALS & METHODS

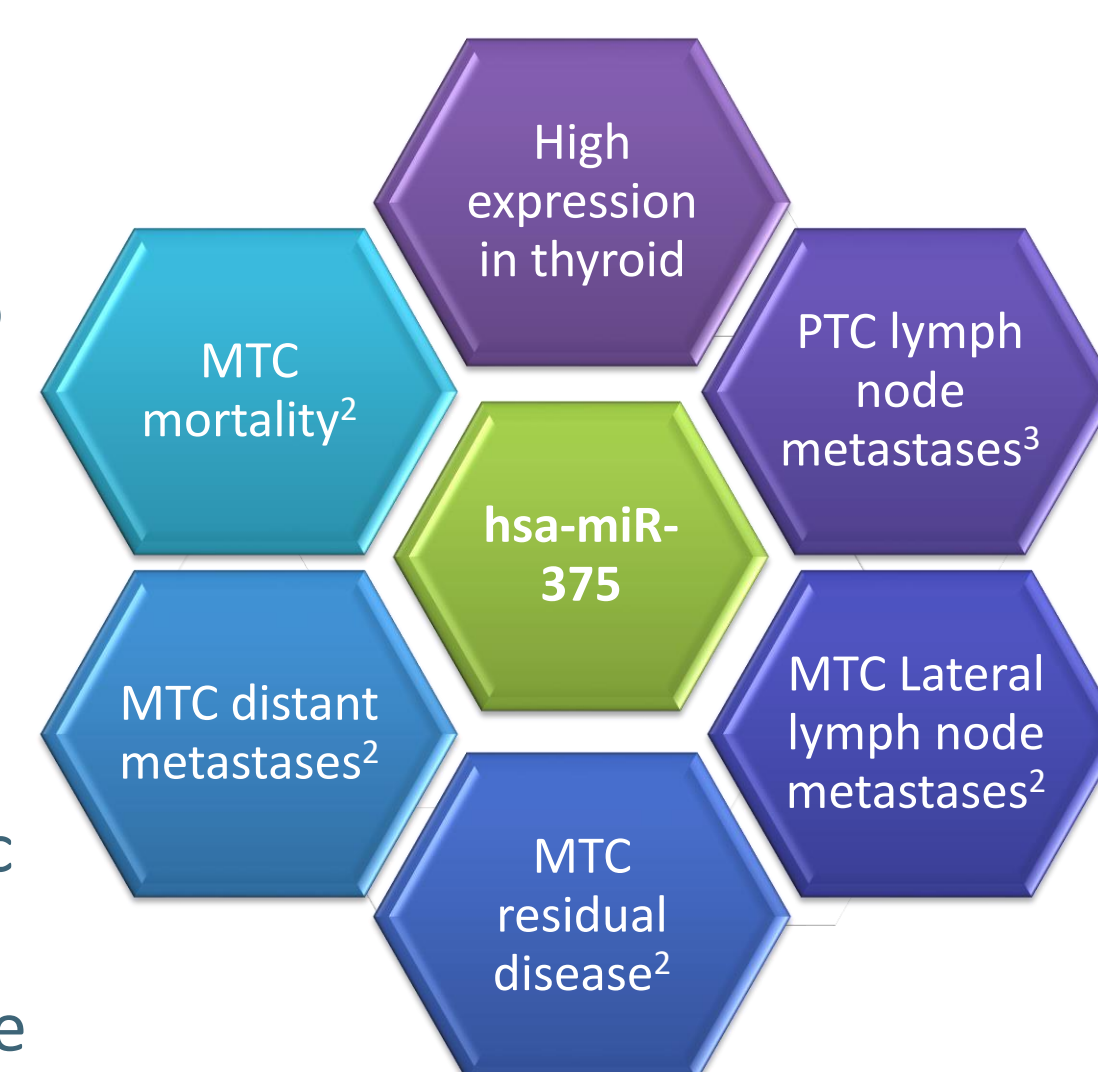
- A set of 17 MTC FNA stained smears were collected for the training and validation of RosettaGX Reveal<sup>1</sup>.
- Additionally, between November 2015 and February 2017, over 1000 cases were referred for testing using the Reveal assay in the Rosetta Genomics Laboratory in Philadelphia, and 10 samples were classified by Reveal as positive for medullary carcinoma.



RosettaGX Reveal distinguishes benign from malignant thyroid nodules using a single FNA stained smear or ThinPrep sample, and does not require fresh FNA tissue nor special collection and shipment conditions.

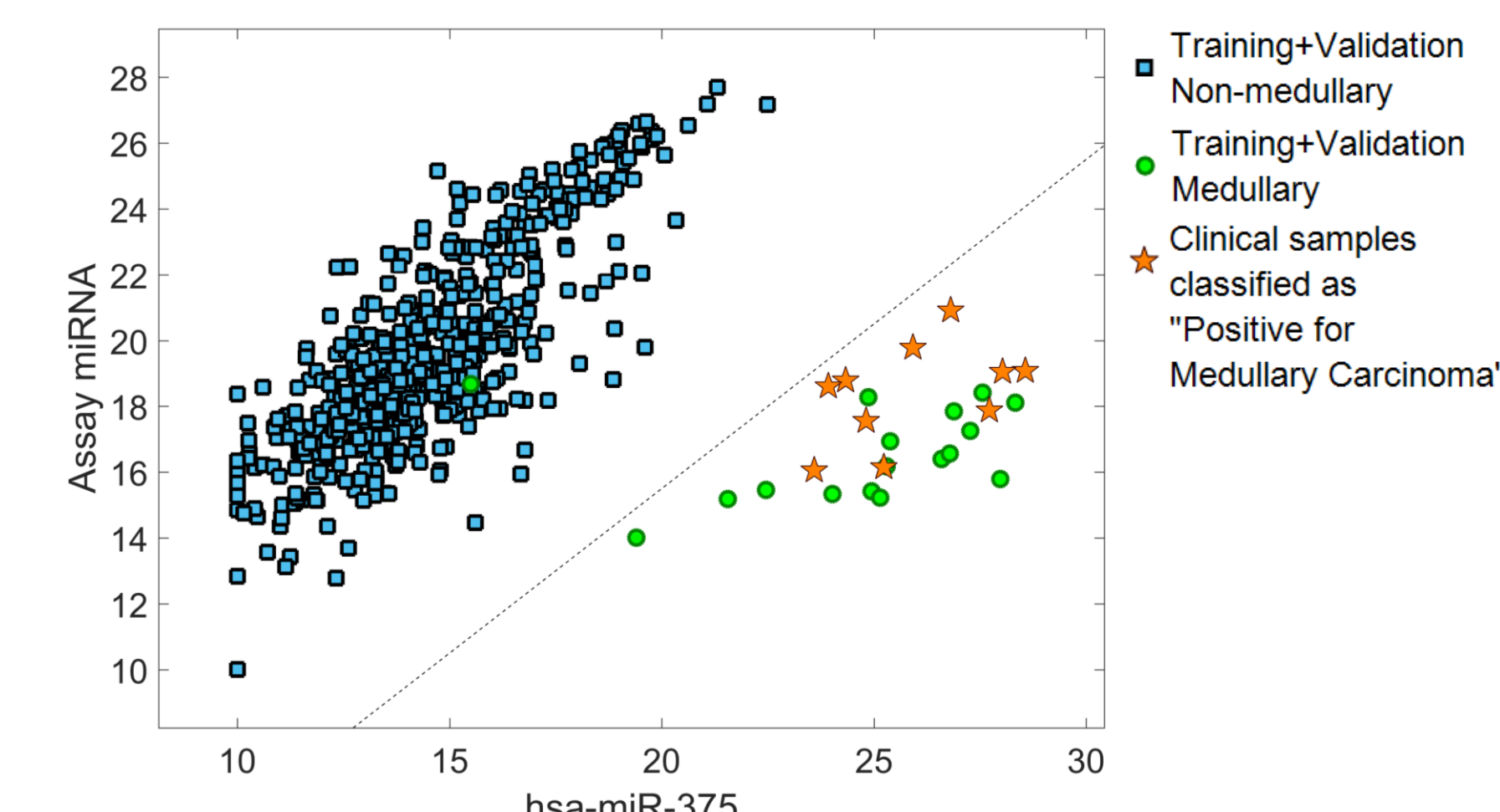
### hsa-miR-375 is a Significant Marker for MTC

hsa-miR-375 has been demonstrated, in several studies, to be overexpressed in MTC<sup>2-4</sup>. Its overexpression may also be associated with various prognostic factors, such as lateral lymph node metastases and residual disease<sup>2,3</sup>.



### 3. RESULTS

#### Accurate Diagnosis of MTC Using hsa-miR-375



A classifier to differentiate MTC was developed based on the training samples shown above, and is part of the Reveal test. Ten clinical samples were classified by the Reveal assay as positive for medullary carcinoma and were all confirmed as medullary, based on surgical diagnosis.

#### Detection of MTC with RosettaGX Reveal

	Histologically confirmed MTC (n)	Bethesda classification	Positive for MTC by Reveal (n)	Suspicious for malignancy by Reveal (n)	PPV
<b>Training &amp; Validation (n=576)</b>	17	IV: 2; V: 6; VI: 9;	16/17	1/17	100%
<b>Clinical samples (n=1170)</b>	10	III: 2; IV: 3; V: 2; VI: 3	10/10	ND	100%

### 4. SUMMARY

- Detection of Medullary Thyroid Carcinoma by RosettaGX Reveal™ was found to be accurate in previous training and validation studies.
- All ten clinical samples identified as positive for medullary carcinoma were confirmed as medullary based on surgical specimens.
- Three case studies demonstrate that the assay can identify MTC in cytology specimens where MTC were indeterminate.

### 5. CONCLUSIONS

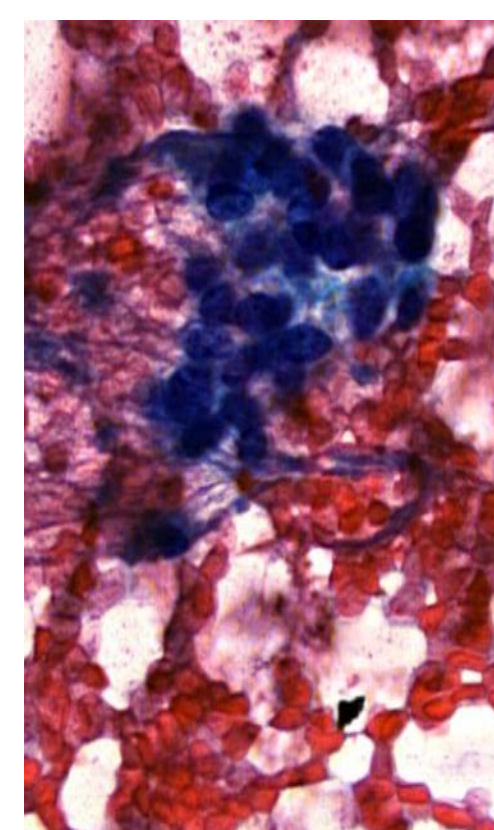
- RosettaGX Reveal can accurately detect medullary carcinoma, using stained FNA smears or ThinPrep samples, even in cytologically indeterminate samples.
- The Reveal assay is suggested in patients with an indeterminate cytology diagnosis.
- The assay can play a valuable role in the detection of MTC, and hopefully, a more favorable outcome for patients.

### 6. REFERENCES

- Lithwick-Yanai G et al. Multicentre validation of a microRNA-based assay for diagnosing indeterminate thyroid nodules utilizing fine needle aspirate smears. J Clin Pathol. 2016
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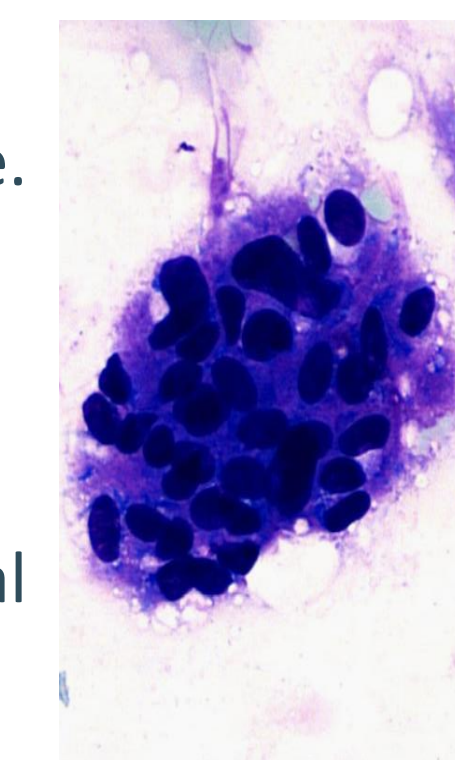
#### Case Study 1

- A 48 year-old female with left 1.8 cm nodule
- FNA diagnosis: Atypia, suspicious for neoplasm (Bethesda V).
- Differential diagnosis included: Follicular neoplasm, benign trabecular adenoma, medullary carcinoma papillary carcinoma, and other possibilities.
- Calcitonin level was not measured
- RosettaGX Reveal test run on the pre-existing FNA smear classified the nodule as "Positive for Medullary Carcinoma".
- A total thyroidectomy, left lateral and central compartment neck dissection was performed. Final surgical pathology determined it was MTC with all lymph nodes negative for metastatic tumor.
- The FNA slide was reviewed post-operative by an additional blinded expert cytologist who confirmed the original cytologist classification.



#### Case Study 2

- A 60 year-old male with a 3.5 cm hyperechoic nodule in the left thyroid lobe.
- FNA diagnosis: Atypia of undetermined significance (Bethesda III).
- RosettaGX Reveal test result was "Positive for Medullary Carcinoma" from the original FNA smear.
- A subsequent serum calcitonin level was 1884 (normal <16).
- A total thyroidectomy with central compartment neck dissection was performed. Final pathology determined it was MTC with all lymph nodes negative for metastatic tumor.
- The FNA slide was reviewed post-operative by an additional independent blinded expert cytologist who classified it as "Follicular neoplasm, possible medullary".



#### Case Study 3

- A 43 year-old female with a 2.3 cm left lobe nodule and previous atypical FNA.
- The specimen had enlarged follicular cells and scant colloid and was classified as Bethesda III.
- The RosettaGX Reveal test result was "Positive for Medullary Carcinoma".
- A subsequent serum calcitonin level was 73 (normal <16).
- The patient underwent total thyroidectomy, and was determined to be MTC based on final pathology.
- The FNA slide was reviewed post-operative by an additional blinded expert cytologist who diagnosed the case as "suspicious for medullary carcinoma".

