

# Droplet digital polymerase chain reaction testing of BRAF and TERT for pre-operative molecular testing of thyroid nodules

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## BACKGROUND

- Mutations are central to pathogenesis of well differentiated thyroid cancer
- Pre-operative molecular testing of gene mutations can provide important diagnostic and prognostic information
- Current molecular tests are costly and may lack accuracy when limited nucleic acid is obtained from fine needle aspirate biopsy (FNAB)
- These limitations can be addressed by using droplet digital PCR (ddPCR), an ultrasensitive method of detecting gene targets

## OBJECTIVE

- Clinically validate the use of BRAF and TERT mutation testing by ddPCR as a diagnostic adjunct for thyroid FNAB.

## METHODS

- A prospective cohort clinical validation study was conducted
- Patients with thyroid nodules meeting indication for FNAB between March 2015 and September 2018 were considered
- Pre-operative FNAB was collected for standard cytology and molecular testing
- Results were correlated to final surgical pathology

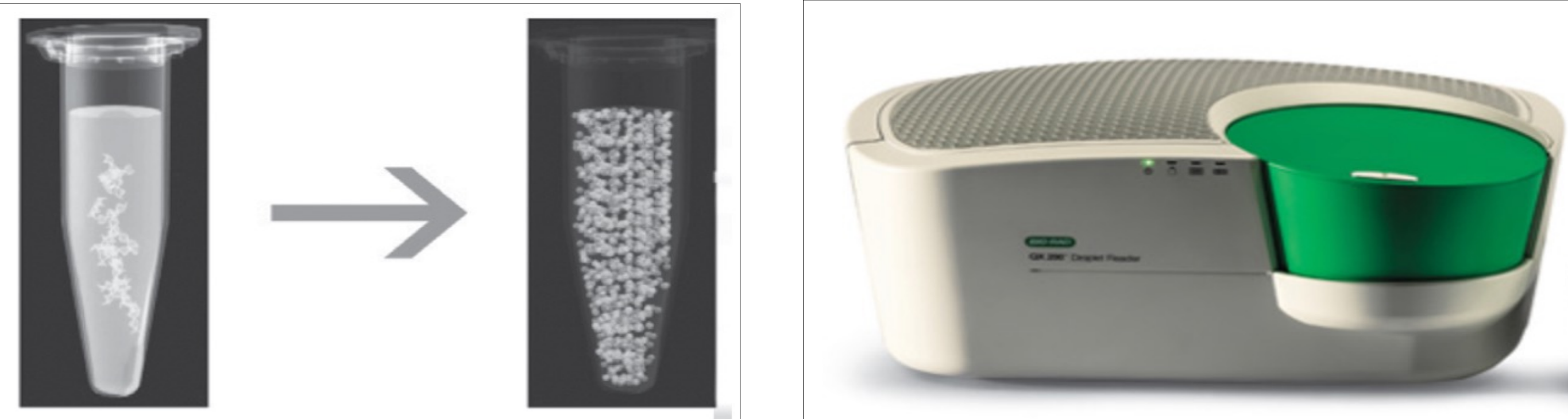


Figure 1. Digital droplet generation and RT-PCR with thyroid biomarker probes

| Characteristics, % | All (%),<br>N=124 | Bethesda Categories (%) |                |                   |                |            |                |
|--------------------|-------------------|-------------------------|----------------|-------------------|----------------|------------|----------------|
|                    |                   | I<br>(non-dx)           | II<br>(benign) | III<br>(AUS/FLUS) | IV<br>(FN/SFN) | V<br>(SFM) | VI (malignant) |
|                    |                   | N=28                    | N=52           | N=9               | N=8            | N=5        | N=23           |
| Age                |                   |                         |                |                   |                |            |                |
| Mean (SD)          | 53.6 (14.6)       | 54.9 (12.0)             | 52.4 (15.8)    | 52.6 (15.1)       | 65.8 (13.3)    | 49.0 (4.6) | 52.1 (15.6)    |
| <55                | 60 (48.4)         | 10 (35.7)               | 24 (46.1)      | 5 (55.5)          | 2 (25.0)       | 5 (100)    | 14 (60.9)      |
| >55                | 64 (51.6)         | 18 (64.3)               | 27 (53.9)      | 4 (44.5)          | 6 (75.0)       | 0          | 9 (39.1)       |
| Sex (female)       | 107 (86.3)        | 27 (96.4)               | 47 (90.3)      | 8 (88.9)          | 4 (50.0)       | 3 (60.0)   | 18 (78.2)      |
| Nodule size        |                   |                         |                |                   |                |            |                |
| 1.0-3.9 cm         | 95 (76.6)         | 28 (100)                | 39 (75.0)      | 0                 | 5 (62.5)       | 3 (60.0)   | 20 (86.9)      |
| 4.0 cm             | 29 (23.4)         | 0                       | 13 (25.0)      | 9 (100)           | 3 (37.5)       | 2 (40.0)   | 3 (13.1)       |
| Surgical pathology |                   |                         |                |                   |                |            |                |
| Benign             | 84 (67.7)         | 24                      | 46             | 7                 | 7              | 0          | 0              |
| PTC                | 23 (18.5)         | 3                       | 4              | 1                 | 1              | 4          | 10             |
| FTC                | 3 (2.4)           | 1                       | 1              | 1                 | 0              | 0          | 0              |
| ATC                | 6 (4.8)           | 0                       | 0              | 0                 | 0              | 1          | 5              |

Table 1. Baseline characteristics by Bethesda category

| FNAB Results           |                     | Surgical Pathology |                     |                  |
|------------------------|---------------------|--------------------|---------------------|------------------|
|                        |                     | Benign (%), N=84   | Malignant (%), N=40 | Total (%), N=124 |
| Cytology<br>(Bethesda) | I – Non-diagnostic  | 24 (28.6)          | 4 (10)              | 28 (22.6)        |
|                        | II – Benign         | 46 (54.8)          | 5 (12.5)            | 51 (41.1)        |
|                        | III – AUS/FLUS      | 7 (8.3)            | 2 (5)               | 9 (7.3)          |
|                        | IV – FN/SFN         | 7 (8.3)            | 1 (2.5)             | 8 (6.5)          |
|                        | V – SFM             | 0                  | 5 (12.5)            | 5 (4.0)          |
|                        | VI – Malignant      | 0                  | 23 (57.5)           | 23 (18.5)        |
| ddPCR<br>Result        | BRAFV600E           | 0                  | 20 (50)             | 20 (16.1)        |
|                        | TERT overexpression | 0                  | 10 (25)             | 10 (8.1)         |
|                        | BRAFV600E + TERT    | 0                  | 6 (15)              | 6 (4.8)          |

Table 2. Pre-operative FNAB cytology and ddPCR results according to final surgical pathology

| Measure, % (95% CI) | Bethesda IV-VI      | Bethesda V-VI         | BRAF V600E            | TERT                  | BRAFV600E +TERT       | BRAFV600E +TERT +Bethesda IV-VI | BRAFV600E +TERT +Bethesda V-VI |
|---------------------|---------------------|-----------------------|-----------------------|-----------------------|-----------------------|---------------------------------|--------------------------------|
| Sensitivity         | 72.5<br>(56.1-85.4) | 70<br>(53.5-83.4)     | 50.0<br>(33.8-66.2)   | 25.0<br>(12.7-41.2)   | 60.0<br>(43.3-74.1)   | 80<br>(64.4-91.0)               | 80<br>(64.4-91.0)              |
| Specificity         | 91.7<br>(83.6-96.6) | 100.0<br>(95.7-100.0) | 100.0<br>(93.8-100.0) | 100.0<br>(95.7-100.0) | 100.0<br>(95.7-100.0) | 91.7<br>(83.6-96.6)             | 100.0<br>(95.7-100.0)          |

Table 3. Comparative diagnostic performance of pre-operative standard cytology and ddPCR testing

| Pathology                | BRAFV600E<br>N=20 | P-value | TERT<br>N=10 | P-value |
|--------------------------|-------------------|---------|--------------|---------|
| Tumor type<br>Anaplastic | 2                 | 0.69    | 4            | <0.001  |
| Stage<br>T3/4            | 8                 | 0.626   | 7            | 0.025   |
| N1 vs N0                 | 19                | <0.001  | 9            | 0.079   |
| LVI or PNI present       | 10                | 0.047   | 3            | 0.702   |
| Extrathyroidal extension | 8                 | 0.311   | 6            | 0.032   |
| Multifocal               | 11                | 0.008   | 4            | 0.702   |
| Recurrence               | 4                 | 0.035   | 1            | 1.000   |

Table 4. Association of BRAFV600E and TERT overexpression with disease aggressiveness in 40 patients with thyroid cancer

## RESULTS

- FNAB ddPCR results showing BRAFV600E or TERT positivity was 100% specific and 60% sensitive
- Combining cytology (Bethesda  $\geq 5$ ) with BRAFV600E and TERT testing increased the sensitivity of a malignant diagnosis to 80%, with accuracy of 94.0% (95% CI 88.0-97.5)
- High TERT levels or dual positivity for TERT and BRAFV600E was associated with aggressive or advanced stage pathology
- High TERT levels were found in all four patients with anaplastic thyroid cancer

## CONCLUSION

- Combining cytology with ddPCR analysis of TERT and BRAF mutations can improve the diagnostic accuracy of thyroid FNABs.

