Thyroid: Molecular Testing

The role of molecular testing of thyroid FNA samples, as an adjunct to cytologic examination, has been evaluated in recent literature and is included in guidelines published by the American Thyroid Association.

We are pleased to announce that thyroid molecular testing is now available through TOPA.

If molecular testing is desired on a fine needle aspiration specimen, one additional pass should be performed, and the contents should be placed in a vial of preservative solution (provided by TOPA).

Molecular testing can be ordered at your discretion or as a reflex test (to be performed automatically for indeterminate cytologic findings).

TOPA will store the sample in preservative solution for up to 5 weeks, and will forward the sample (if indicated) to a reference laboratory for testing. Molecular results will be correlated with morphologic findings and integrated into the cytology report.

We will continue to perform comprehensive cytologic examinations, utilizing direct smears, ThinPrep slides, and cell block preparations. We believe that this extensive protocol, along with 25 years of experience in the field of thyroid cytopathology, allows us to optimize our morphologic evaluations, and distinguishes TOPA from other clinical laboratories.

A TOPA representative is available to visit your office and customize the TOPA requisition to include your preferences for reflex testing options.

For additional information, please see the attached TOPA Update on molecular testing.
TOPA Update on Mutation Testing of Thyroid FNA Specimens

BRAF and RAS mutations and RET/PTC and PAX8/PPARγ rearrangements are found in greater than 70% of differentiated thyroid carcinomas.

The most common genetic alterations in papillary carcinomas are:
- BRAF: 40-45%
- RAS: 10-20%
- RET/PTC: 10-20%

The most common genetic alterations in follicular carcinomas are:
- RAS: 40-50%
- PAX8/PPARγ: 30-35%

Of these genetic alterations, BRAF and RAS mutations are the most well established in the literature. The BRAF V600E mutation is commonly found in papillary carcinoma (with classic or tall-cell histology), and is also found in poorly-differentiated and anaplastic carcinoma. This mutation is rarely found in follicular variant of papillary carcinoma, and is not present in follicular carcinoma or benign nodules.

When present in FNA specimens, the BRAF V600E mutation has high specificity for malignancy (with a positive predictive value of 99.8% according to a recent meta-analysis). This mutation is also useful as a prognostic marker. The presence of this mutation is associated with increased risk for extra-thyroidal extension, lymph node metastasis, and recurrence; and it may be an indication for more extensive surgery.

RAS mutations (including KRAS, HRAS, and NRAS mutations) are commonly found in follicular carcinoma and the follicular variant of papillary carcinoma, and may also occur in follicular adenoma. When a RAS mutation is present in an FNA sample, the risk of malignancy is 74-88%. The significance of RAS mutations in follicular adenomas is not well established, but adenomas that carry these mutations may be at increased risk for progression to malignancy.

The risk of malignancy, based on cytologic and molecular findings is outlined below:

<table>
<thead>
<tr>
<th>Cytologic and Molecular findings</th>
<th>Risk of Malignancy</th>
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</thead>
<tbody>
<tr>
<td>Cytology: follicular lesion of undetermined significance</td>
<td></td>
</tr>
<tr>
<td>Molecular testing: positive</td>
<td>88%</td>
</tr>
<tr>
<td>Molecular testing: negative</td>
<td>6%</td>
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<tr>
<td>Cytology: Follicular neoplasm/ suspicious for follicular neoplasm</td>
<td></td>
</tr>
<tr>
<td>Molecular testing: positive</td>
<td>87%</td>
</tr>
<tr>
<td>Molecular testing: negative</td>
<td>14%</td>
</tr>
<tr>
<td>Cytology: Suspicious for malignancy</td>
<td></td>
</tr>
<tr>
<td>Molecular testing: positive</td>
<td>95%</td>
</tr>
<tr>
<td>Molecular testing: negative</td>
<td>28%</td>
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</tbody>
</table>
For cytologic diagnoses of “Follicular neoplasm/ suspicious for follicular neoplasm” and “Suspicious for malignancy”, surgery is generally indicated, regardless of the molecular findings. For these cytologic diagnoses, molecular testing may be useful to determine the extent of surgery (lobectomy for patients with negative molecular results, vs thyroidectomy for patients with positive molecular results).

For cytologic diagnoses of “follicular lesion of undetermined significance”, positive molecular results would be an indication for surgery. The management of patients with a cytologic diagnosis of “follicular lesion of undetermined significance” and negative molecular results is less well established, but conservative management, rather surgery, may be indicated for these patients.

According to the “Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer”, the use of molecular markers (eg. BRAF, RAS, RET/PTC, pax8-PPARγ, or galectin-3) may be considered for patients with indeterminate cytology on FNA to help guide management.

In summary, mutation analysis of FNA samples may offer the following benefits:

- Risk stratification of cases with indeterminate cytologic diagnoses.
- Confirmation of malignant diagnoses to facilitate total thyroidectomy, rather than lobectomy.
- Identification of malignancies with poor prognosis, influencing extent of surgery.

References:
Thyroid 2009; 19: 1167-1214.
Arch Pathol Lab Med 2011; 135: 569-577