Molecular diagnostics of cytologically indeterminate thyroid nodule fine-needle aspiration cytologies using the ThyroSPEC™ v1 panel

ThyroSPEC™ is a proprietary, highly accurate, cost-efficient MALDI-TOF mass spectrometry-based mutation detection panel that detects the most prevalent 117 point mutations and 23 gene fusions reported in thyroid cancer in the following genes:

Point Mutations	Gene Rearrangements	Gene Expression Cassette
AKT1	ALK	PGK1
BRAF	BRAF	TTF1
CTNNB1	MAML2	KRT20
DICER1	NTRK1	CALCA
EGFR	NTRK3	PTH
EIF1AX	PPARG	KRT7
EZH1	PRKAB1	PAX8
HRAS	RET	TG
IDH1	THADA	
KRAS		
NRAS		
PIK3CA		
RET		
SPOP		
TERT		
TP53		
TSHR		

The cut-off value for point mutations is 10% variant allele frequency.

ThyroSPEC™ underwent prospective validation on indeterminate FNAC specimens with available histology in the AHS Calgary Health Care Region with centralized FNAC. Data local to the AHS Calgary Health Care Region show a risk of malignancy of 26% in the AUS/FLUS category and 43% in the FN/SFN category[1]. The respective local risk of malignancy affects the NPV and PPV of the molecular test and its knowledge is therefore necessary for proper interpretation of molecular diagnostics.

For pre-test risk of malignancy of 26% in the AUS/FLUS category in Calgary[3]:

Sensitivity	55%
Specificity	88%
Positive Likelihood Ratio	4.5
Negative Likelihood Ratio	0.5
Positive Predictive Value (PPV) at 26% risk of malignancy*	61%
Negative Predictive Value (NPV) at 26% risk of malignancy*	85%
Detection Threshold	10% allelic frequency

For pre-test risk of malignancy 43% in the FN/SFN category in Calgary[3]:

Sensitivity	38%
Specificity	75%
Positive Likelihood Ratio	1.5
Negative Likelihood Ratio	0.8
Positive Predictive Value (PPV) at 43% risk of malignancy*	54%
Negative Predictive Value (NPV) at 43% risk of malignancy*	62%
Detection Threshold	10% allelic frequency

Detected mutations are classified as follows:

Benign molecular markers[2]
TSHR, EZH1, SPOP
Intermediate risk mutations[4,5]
NRAS, HRAS, KRAS, $BRAF^{K601E}$, EIF1AX, IDH1, DICER1, PTEN or rearrangements: PPARG, ALK, THADA
Malignant molecular markers[4,5]
$BRAF^{V600E}$, TERT or rearrangements: BRAF, RET, NTRK1, NTRK3
High-Risk mutations[6]
BRAF + TERT, RAS + TERT, RAS + EIF1AX, TP53, AKT1, PIK3CA, CTNNB1, EGFR
Medullary Thyroid Carcinoma markers
RET mutations

Mutation-specific malignancy risks

- **TSHR, EZH1, SPOP[2]:** risk of malignancy unknown, case reports mostly benign, very few malignant
- **No mutation detected:**
    - Bethesda III (AUS/FLUS) – post-test up to 15% risk of malignancy[3];
    - Bethesda IV (FN/SFN) – post-test up to 38% risk of malignancy[3]
- **NRAS, HRAS, KRAS, $BRAF^{K601E}$, EIF1AX, IDH1, DICER1, PTEN or rearrangements: PPARG, ALK, THADA[4,5]:** post-test risk of malignancy is higher than pre-test risk of malignancy. Variable malignancy risk, with accurate risk assessment depending on ultrasound, cytology and clinical assessment.
- **$BRAF^{V600E}$, TERT or rearrangements: BRAF, RET, NTRK1, NTRK3[4,5]:** indicate a malignant tumour (>90% risk of malignancy)
- **BRAF + TERT, RAS + TERT, RAS + EIF1AX, TP53, AKT1, PIK3CA, CTNNB1, EGFR[6]:** indicate a malignant tumour (100% risk of malignancy)
- **RET mutations:** indicate a medullary thyroid carcinoma

Management options based on the mutation-specific malignancy risks

- **TSHR, EZH1, SPOP:**
  No molecular indication for malignancy.
- **No mutation detected / Bethesda III (AUS/FLUS):** Lobectomy or observation depending on further malignancy risk assessment including ultrasound, cytology and clinical assessment[7,8]. A ThyroSPEC-negative result does not rule out cancer, there is a residual risk of malignancy of up to 15% for ThyroSPEC negative Bethesda III (AUS/FLUS) nodules[3].

- **No mutation detected / Bethesda IV (FN/SFN):**
  Molecular testing has not changed management recommendations based on ultrasound, cytology and clinical assessment[7,8]. A ThyroSPEC-negative result does not rule out cancer, there is a residual risk of malignancy of up to 38% for ThyroSPEC-negative Bethesda IV (FN/SFN) nodules[3].
- **NRAS, HRAS, KRAS, BRAFK^{601E}, EIF1AX, IDH1, DICER1, PTEN or rearrangements: PPARG, ALK, THADA[4,5]:**
  Refer to endocrinology to discuss lobectomy or observation depending on combined risk assessment by ultrasound, cytology, molecular findings and clinical assessment.
- **BRAF^{V600E}, TERT or rearrangements: BRAF, RET, NTRK1, NTRK3:** Total thyroidectomy. Refer to surgery.
- **BRAF + TERT, RAS + TERT, RAS + EIF1AX, TP53, AKT1, PIK3CA, CTNNB1, EGFR[6]:** Total thyroidectomy. Refer to surgery.
- **Medullary thyroid carcinoma molecular markers:** Total thyroidectomy. Refer to surgery.
- Less aggressive treatment is recommended for nodules 1cm or less according to current guidelines[7].

Questions concerning the further clinical interpretation of ThyroSPEC results can be addressed to Dr. Ralf Paschke (ralf.paschke@albertahealthservices.ca)

Disclaimer: Interpret the above results within the context of other clinical data such as ultrasound, with clinical management decision making according to the independent medical judgement of the responsible physician and patient preferences.
ThyroSPEC™ was not created to identify germline variants, nonetheless it is possible that ThyroSPEC™ will discover a germline mutation incidentally. If a germline variant is reported, referral to medical genetics may be advisable.

[1] Ghaznavi, S. et al., 2018, Thyroid Nodule Malignancy Rates Within A Health Care Region with Centralized Pathology. Annual Meeting of the American Thyroid Association, Washington, DC.
[2] Ye, L. et al., 2017, The genetic landscape of benign thyroid nodules revealed by whole exome and transcriptome sequencing. *Nature Communications*, 8:15533. doi: 10.1038/ncomms15533.
[3] Stewardson, P. et al., 2019, Prospective Evaluation of the ThyroSPEC™ Mutation Panel for the Diagnosis of Indeterminate Thyroid Fine Needle Aspiration Cytologies (FNAC) in the Southern Alberta Health Care Region. Annual Meeting of the American Thyroid Association, Chicago, IL.
[4] Yoo, S.-K. et al., 2016, Comprehensive Analysis of the Transcriptional and Mutational Landscape of Follicular and Papillary Thyroid Cancers. *PLOS Genetics*, 12(8):e1006239
[5] The Cancer Genome Atlas Research Network, 2014, Integrated Genomic Characterization of Papillary Thyroid Carcinoma, *Cell*, 159(3):676-90
[6] Ricarte-Filho, J.C., Ryder, M., et al. (2009). Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1, *Cancer Research*, 69(11):4885-93
[7] Haugen, B. R. et al., 2016, 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid*, *26*(1), 1-133.
[8] Paschke, R. et al., 2017, European Thyroid Association guidelines regarding thyroid nodule molecular fine-needle aspiration cytology diagnostics. European thyroid journal, 6(3), 115-129.

Referral Possibilities for Patients with Indeterminate FNA Cytology and Additional ThyroSPEC™ Testing

Intermediate Malignancy Risk Molecular Findings

- **NRAS, HRAS, KRAS, BRAF^{K601E}, EIF1AX, IDH1, DICER1, PTEN or rearrangements: PPARG, ALK, THADA**[1,3]**:** post-test risk of malignancy higher than pre-test risk of malignancy. Variable malignancy risk, with accurate risk assessment depending on ultrasound, cytology and clinical assessment[4]

Refer to:

Central Access and Triage
Endocrinology and Metabolism - Calgary

Richmond Road Diagnostic and Treatment Center
1820 Richmond Road SW
Calgary, AB T2T 5C7
Phone: 403-955-8633
Fax: 403-955-8634
Email: endo.cat@ahs.ca

High Malignancy Risk Molecular Findings

- **BRAF^{V600E}, TERT or rearrangements: BRAF, RET, NTRK1, NTRK3**[1,3]**:** indicate a malignant tumour (>90% risk of malignancy)
- **BRAF + TERT, RAS + TERT, RAS + EIF1AX, TP53, AKT1, PIK3CA, CTNNB1, or EGFR**[5]**:** indicate a malignant tumour with poor prognosis (100% risk of malignancy)
- **RET Mutations:** indicate a medullary thyroid carcinoma

Refer to:

Endocrine Surgeons Offices - Calgary
Dr. Janice Pasieka, Phone: 403-944-2491, Fax: 403-283-4130
Dr. Adrian Harvey, Phone: 403-944-0966, Fax: 403-944-6008

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Dr. Shamir Chandarana, Phone: 403-944-3954, Fax: 403-210-9828
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Dr. Arone Pabbies, Phone: 403-342-0494, Fax: 403-343-0304

[1] Yoo SK et al., 2016 *PLOS Genetics*, 12(8):e1006239
[2] Ye L et al., 2017, *Nature Communications*, 8:15533. doi: 10.1038/ncomms15533.
[3] The Cancer Genome Atlas Research Network, 2014, *Cell*, 159(3):676-90
[4] Stewardson P et al., American Thyroid Association Annual Meeting in Chicago, IL, November 2019
[5] Ricarte-Filho JC et al., 2009, *Cancer Research*, 69(11):4885-93