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 116 point mutations and 21 gene fusions reported in thyroid cancer in the following genes:

Point Mutations	Gene Rearrangements
AKT1	AGK::BRAF
BRAF	AKAP::BRAF
CTNNB1	CRTC1::MAML2
DICER1	EML4::ALK
EGFR	ETV6::NTRK3
EIF1AX	IGF2BP3::THADA
EZH1	PAX8::PPARG
HRAS	RET::PTC
IDH1	RPS2P32::THADA
KRAS	SND1::BRAF
NRAS	SQSTM1::NTRK3
PIK3CA	STRN::ALK
PTEN	TFG::NTRK1
RET	TMEM233::PRKAB1
SPOP	TPM3::NTRK1
TERT	TPR::NTRK1
TP53	
TSHR	

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

A prospective, observational validation study was conducted in the AHS Calgary Health Care Region, where a guidelines-based thyroid nodule pathway (PCN pathway) including thyroid nodule ultrasound malignancy risk stratification (EFN white paper) and determination of local malignancy risk for each Bethesda category[1] (Ghaznavi et al., Acta Cytologica, 2022) has recently been implemented.

All 615 patients in Southern Alberta with an AUS/FLUS or FN/SFN thyroid nodule diagnosed from July 30, 2020, until July 31, 2022, were included. Electronic health records were used for this study (IRB approval: HREBA.CHC-20-0068) to determine demographics, laboratory results, clinical history, surgical decision making, ultrasound findings, cytology diagnoses, molecular test results, outcomes, surgical procedure, histopathologic findings based on standardized synoptic reporting, and follow-up.

Test performance per Bethesda and US subcategories was determined in this study[2] (Stewardson et al., Thyroid, 2023), noting that in Calgary the pre-test risk of malignancy was 26% in the AUS/FLUS category and 43% in the FN/SFN category prior to implementation of molecular diagnostics[1]. ThyroSPEC™ test performance ranges from resected nodules only, to all resected nodules and unresected nodules with more than 1 year follow-up[2]. TR refers to ACR-TIRADS classification. The limit of detection threshold during validation was a 10% allelic frequency.

	Prevalence of malignancy	Sensitivity	Specificity	NPV	PPV
Bethesda III (AUS/FLUS)	17-41%	74%	67-78%	79-93%	42-61%
Bethesda IV (FN/SFN)	42-53%	67%	79-84%	70-77%	70-82%
ATA Low Suspicion, TR3	20-35%	72%	56-83%	79-92%	46-52%
ATA Intermediate Suspicion, TR4	22-49%	80%	69-79%	78-93%	52-72%
ATA High Suspicion, TR5	17-41%	46%	83-84%	70-89%	35-67%

Detected mutations are classified as follows:

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

Mutation-specific malignancy risks

- **TSHR, EZH1, SPOP, PTEN.** Mostly benign, very few malignant[2].
- **No mutation detected.** Bethesda III (AUS/FLUS) post-test risk of malignancy up to 27%[2]; Bethesda IV (FN/SFN) post-test risk of malignancy up to 35%[2].
- **IDH1, DICER1, $BRAF^{K601E}$, EIF1AX, TP53, or rearrangements in PPARG, THADA.** Post-test risk of malignancy is higher than pre-test risk of malignancy, not necessarily malignant as mutation specific risk remains unclear.
- **NRAS, HRAS, KRAS.** 58% malignancy risk for resected Calgary RAS positive nodules[2].
- **$BRAF^{V600E}$, TERT, or rearrangements in BRAF, RET, NTRK1, NTRK3.** Indicate a malignant tumour (90% risk of malignancy[2]).
- **BRAF + TERT, RAS + EIF1AX, RAS + TERT, AKT1, PIK3CA, CTNNB1, EGFR, or rearrangements in ALK.** Indicate a malignant tumour (100% risk of malignancy[2]).
- **RET mutations.** Medullary thyroid carcinoma.

Management options based on the mutation-specific malignancy risks

- **TSHR, EZH1, SPOP, PTEN.** No molecular indication for malignancy.
- **Bethesda III (AUS/FLUS) no mutation detected.** Lobectomy or observation depending on further malignancy risk assessment including ultrasound, cytology and clinical assessment[3,4,5]. A ThyroSPEC-negative result does not rule out cancer, there is a residual risk of malignancy of up to 27% for ThyroSPEC negative Bethesda III (AUS/FLUS) nodules[2].
- **Bethesda IV (FN/SFN) no mutation detected**. Molecular testing has not changed management recommendations based on ultrasound, cytology and clinical assessment[3,4,5]. A ThyroSPEC-negative result does not rule out cancer, there is a residual risk of malignancy of up to 35% for ThyroSPEC-negative Bethesda IV (FN/SFN) nodules[2].

- **IDH1, DICER1, NRAS, HRAS, KRAS, BRAF^{K601E}, EIF1AX, TP53, or rearrangements in PPARG, THADA.** Refer to Endocrinology to discuss lobectomy or observation depending on combined risk assessment ultrasound, cytology and clinical assessment[3,4,5].
- **BRAF^{V600E}, TERT, RET, EGFR, BRAF + TERT, RAS + TERT, RAS + EIF1AX, AKT1, PIK3CA, CTNNB1 or rearrangements in BRAF, RET, NTRK1, NTRK3, ALK.** Total thyroidectomy[3,4,5]. Refer to surgery.
- Less aggressive treatment is recommended for nodules 1cm or less according to current guidelines[3].

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

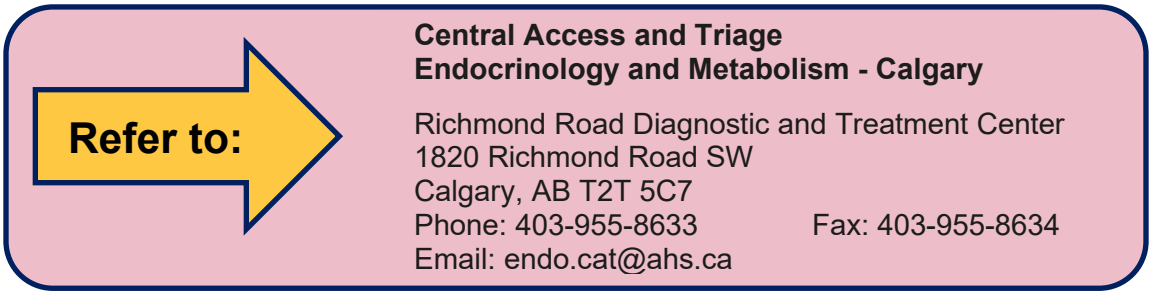
Disclaimer: Interpret the above results within the context of other clinical data such as ultrasound, with clinical management decision making according to the independent medical judgement of the responsible physician and patient preferences.
ThyroSPEC™ was not created to identify germline variants, 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 SA, Clayton H, Eszlinger M, Khalil M, Symonds CJ, Paschke R. Accuracy of thyroid fine-needle aspiration cytology: a cyto-histologic correlation study in an integrated Canadian health care region with centralized pathology service. Acta Cytologica. 2022 Feb 2;66(3):171-8.
[2] Stewardson P, Eszlinger M, Wu J, Khalil M, Box A, Perizzolo M, Punjwani Z, Ziehr B, Sanyal R, Demetrick DJ, Paschke R. Prospective Validation of ThyroSPEC Molecular Testing of Indeterminate Thyroid Nodule Cytology Following Diagnostic Pathway Optimization. Thyroid. 2023 Dec 1;33(12):1423-33.
[3] Durante C, Hegedüs L, Czarniecka A, Paschke R, Russ G, Schmitt F, Soares P, Solymosi T, Papini E. 2023 European Thyroid Association clinical practice guidelines for thyroid nodule management. European Thyroid Journal. 2023 Oct 1;12(5).
[4] Valderrabano P, Eszlinger M, Stewardson P, Paschke R. Clinical value of molecular markers as diagnostic and prognostic tools to guide treatment of thyroid cancer. Clinical Endocrinology. 2023 Jun;98(6):753-62.
[5] Hu XY, Wu J, Seal P, Ghaznavi SA, Symonds C, Kinnear S, Paschke R. Improvement in thyroid ultrasound report quality with radiologists' adherence to 2015 ATA or 2017 TIRADS: a population study. European Thyroid Journal. 2022 Jun 1;11(3).

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

Intermediate Malignancy Risk Molecular Findings

- **NRAS, HRAS, KRAS, BRAF^{K601E}, EIF1AX, TP53, IDH1, DICER1, or rearrangements in PPARG, 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]



High Malignancy Risk Molecular Findings

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

[1] Ghaznavi SA, Clayton H, Eszlinger M, Khalil M, Symonds CJ, Paschke R. Accuracy of thyroid fine-needle aspiration cytology: a cyto-histologic correlation study in an integrated Canadian health care region with centralized pathology service. Acta Cytologica. 2022 Feb 2;66(3):171-8.
[2] Stewardson P, Eszlinger M, Wu J, Khalil M, Box A, Perizzolo M, Punjwani Z, Ziehr B, Sanyal R, Demetrick DJ, Paschke R. Prospective Validation of ThyroSPEC Molecular Testing of Indeterminate Thyroid Nodule Cytology Following Diagnostic Pathway Optimization. Thyroid. 2023 Dec 1;33(12):1423-33.
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[5] Hu XY, Wu J, Seal P, Ghaznavi SA, Symonds C, Kinnear S, Paschke R. Improvement in thyroid ultrasound report quality with radiologists' adherence to 2015 ATA or 2017 TIRADS: a population study. European Thyroid Journal. 2022 Jun 1;11(3).