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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 22 gene fusions reported in thyroid cancer in the following genes:

Point Mutations	Gene Rearrangements
AKT1	AGK::BRAF
BRAF	AKAP9::BRAF
CTNNB1	CCDC6::RET
DICER1	CRTC1::MAML2
EGFR	EML4::ALK
EIF1AX	ETV6::NTRK3
EZH1	IGF2BP3::THADA
HRAS	NCOA4::RET
IDH1	PAX8::PPARG
KRAS	PRKAR1A::RET
NRAS	RET::GOLGA5
PIK3CA	RPS2P32::THADA
PTEN	SND1::BRAF
RET	SQSTM1::NTRK3
SPOP	STRN::ALK
TERT	TFG::NTRK1
TP53	TMEM233::PRKAB1
TSHR	TPM3::NTRK1
	TPR::NTRK1

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

Prior to the implementation of routine reflexive ThyroSPEC™ molecular testing in the AHS Calgary Health Care Region, a guidelines-based thyroid nodule pathway (PCN pathway) with lobectomy criteria (Criteria for Extent of Surgery in Differentiated Thyroid Cancer: Lobectomy and Completion Thyroidectomy) was organized and implemented, including thyroid nodule ultrasound malignancy risk stratification and determination of local malignancy risk for each Bethesda category.[1-3]

ThyroSPEC™ was prospectively validated in the AHS Calgary Health Care Region in Southern Alberta with Bethesda III (AUS/FLUS) or Bethesda IV (FN/SFN) thyroid nodules diagnosed from July 30, 2020, until July 31, 2022.[3] The integration of ThyroSPEC™ results with additional clinical variables for 1024 patients facilitated the creation of a nomogram which allows further improvement of malignancy risk prediction for patients with intermediate malignancy risk mutations and no mutation detected.[4] 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 with respective resection rates of 21% and 56%.[2]

Table 1. Range of ThyroSPEC™ Test Performance as calculated by including only resected nodules and by including both resected and unresected nodules that are assumed benign with at least one year follow-up and stable/improved ultrasound (3 year ThyroSPEC™ outcomes[4]).

	Prevalence of malignancy	n	Sensitivity	Specificity	NPV	PPV
All indeterminate	42–28%	357–548	72% [CI 64–79%]	69–76% [CI 62–75%] [CI 71–80%]	77–88% [CI 70–83%] [CI 84–91%]	63–53% [CI 55–70%] [CI 46–60%]
Bethesda III (AUS/FLUS)	41–25%	278–459	74% [CI 65–82%]	67–75% [CI 59–74%] [CI 70–80%]	79–90% [CI 71–86%] [CI 86–93%]	60–49% [CI 52–69%] [CI 42–57%]
Bethesda IV (FN/SFN)	48–43%	79–89	63% [CI 46–78%]	78–80% [CI 62–89%] [CI 67–90%]	70–75% [CI 54–82%] [CI 61–85%]	73–71% [CI 54–87%] [CI 53–85%]
ATA Low Suspicion or ACR-TIRADS 3	30–23%	110–144	79% [CI 61–91%]	66–71% [CI 55–77%] [CI 62–79%]	88–92% [CI 77–95%] [CI 84–97%]	50–45% [CI 36–64%] [CI 32–58%]
ATA Intermediate Suspicion or ACR-TIRADS 4	47–29%	150–246	77% [CI 66–87%]	66–76% [CI 54–76%] [CI 69–82%]	76–89% [CI 65–86%] [CI 83–94%]	67–57% [CI 56–77%] [CI 46–67%]
ATA High Suspicion or ACR-TIRADS 5	48–27%	69–121	52% [CI 34–69%]	78–80% [CI 61–90%] [CI 70–87%]	64–81% [CI 48–78%] [CI 72–89%]	68–49% [CI 46–85%] [CI 31–66%]

ACR-TIRADS, American College of Radiology-Thyroid Imaging Reporting and Data System; PPV, positive predictive value; NPV, negative predictive value; ATA, American Thyroid Association.

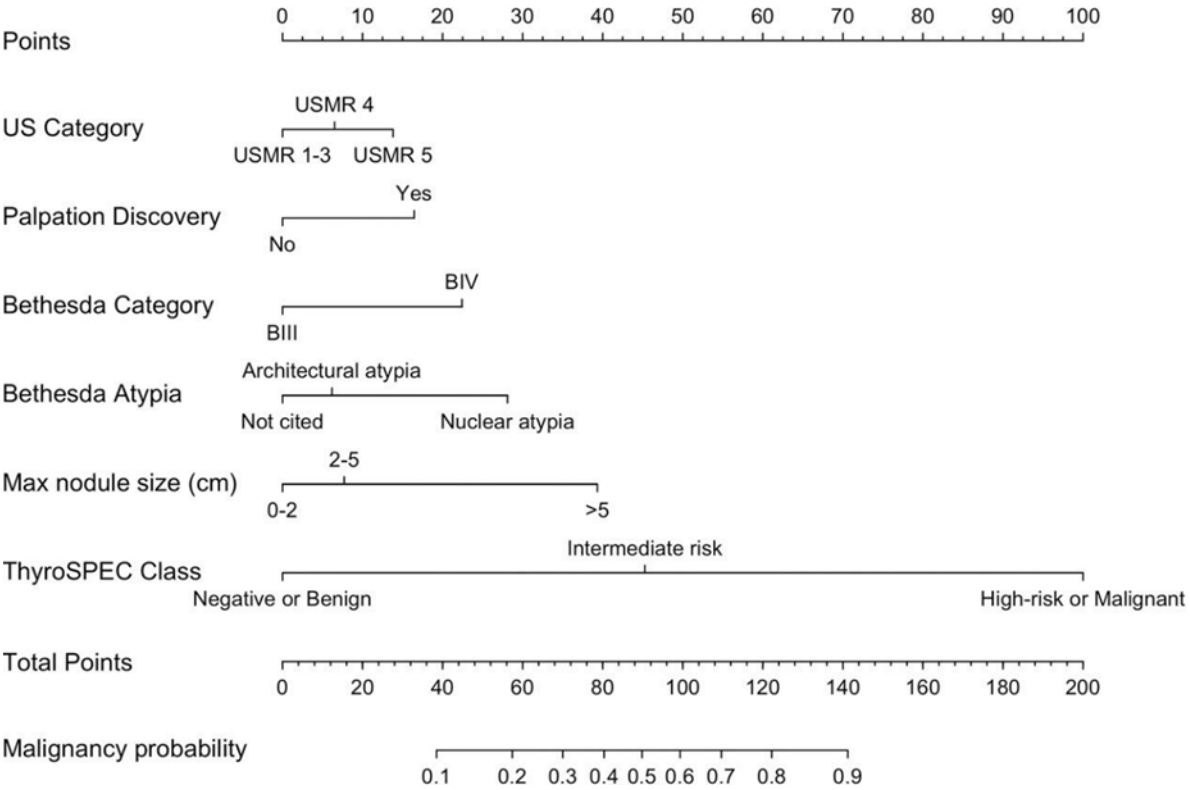
Table 2. Revised Thyroid Nodule Molecular Diagnostic Test Results: Interpretation and Management Guide (implemented April 2025). Range of ThyroSPEC™ ROM as calculated by including only malignant cases defined as nodules that were malignant on surgical histology and by including both resected and unresected nodules that are assumed benign with at least one year follow-up and stable/improved ultrasound (3 year ThyroSPEC™ outcomes[4]).

Classification	Molecular Markers	Risk of Malignancy (ROM)	Management Recommendations
Negative	No mutations detected	**Bethesda III (AUS/FLUS):** 10-20% **Bethesda IV (FN/SFN):** 20-35%	**Bethesda III (AUS/FLUS):** - If first AUS/FLUS result: Consider repeat biopsy (clarifies ~50% of cases) - If repeat biopsy declined or second AUS/FLUS result: - Nodules < 4 cm: Discuss observation vs. surgery • If observation: Repeat ultrasound in 6-12 months • If surgery: Refer to Surgery - Nodules ≥ 4 cm: Refer to Surgery **Bethesda IV (FN/SFN):** - Nodules < 4 cm: Discuss observation vs. surgery - If observation: Repeat ultrasound in 6-12 months - If surgery: Refer to Surgery - Nodules ≥ 4 cm: Refer to Surgery
Low risk	EZH1 PTEN SPOP TSHR	<3%	- Consider discontinuation of surveillance or repeat ultrasound in 12-24 months - Management should integrate clinical symptoms, ultrasound findings, cytology, and patient factors - Consider routine referral to Endocrinology if needed for management approach
Intermediate risk	BRAF K601E DICER1 EIF1AX HRAS IDH1 KRAS NRAS TP53 Rearrangements involving PPARG, THADA	**Bethesda III:** 30-50% **Bethesda IV:** 50-60% *When malignant, these are typically very low risk thyroid cancers	**Bethesda III (AUS/FLUS):** - If first AUS/FLUS result: consider repeat biopsy (clarifies ~50% of cases) - If repeat biopsy declined or second AUS/FLUS result: - Nodules < 4 cm: discuss observation vs. surgery • If observation: Repeat ultrasound in 6-12 months • If surgery: refer to Surgery - Nodules ≥ 4 cm: refer to Surgery **Bethesda IV (FN/SFN):** Surgery is typical management. Refer to Surgery. If surgery is not an option: observation under primary care provider or endocrinologist
High risk	AKT1 BRAF V600E BRAF + TERT CTNNB1 EGFR	>90%	- Nodules ≥ 2 cm: surgery is preferred management. Refer to Surgery - Nodules < 2 cm: surgery is typical management, but active surveillance may be

	PIK3CA RAS + TERT RAS + EIF1AX TERT Rearrangements involving BRAF, RET, NTRK1, NTRK3, ALK		an option for some patients (refer to Endocrinology to discuss) - If surgery is not an option: observation under primary care provider or endocrinologist
Insufficient material	Not applicable	Cannot be determined	- Recommend repeat thyroid biopsy if clinically appropriate - Note: repeat biopsy can clarify an initial AUS/FLUS diagnosis in ~50% of cases

For **no mutation** detected and **intermediate risk mutations**, please see **Figure 1** (nomogram) for an integrated malignancy risk assessment[4].

Figure 1. Nomogram for calculation of the integrated malignancy risk based on Calgary data[4].



The integrated malignancy risk stratification of intermediate risk mutations allows to select 40% of RAS-positive nodules for **successful active surveillance**[5].

Using ThyroSPEC™ for Bethesda III and IV nodules in the context of an interdisciplinary lobectomy proposal and in the setting of an optimized thyroid nodule diagnostic pathway, the number of **completion thyroidectomies** can be reduced by increasing the number of appropriate **upfront total thyroidectomies**[6].

An optimized thyroid nodule diagnostic pathway — including reflexive ThyroSPEC™ testing of Bethesda III (AUS/FLUS) and Bethesda IV (FN/SFN) nodules and less aggressive surgical management performed by more high-volume thyroid surgeons — is associated with improved ultrasound malignancy risk assessment reporting quality, a higher rate of reported Bethesda atypia, and a **greater prevalence of malignancy among resected nodules**[7].

Further clinical impacts of ThyroSPEC™ testing are:

- significant decrease in repeat FNAs,
- increase in risk of malignancy for Bethesda III (AUS/FLUS) and IV (FN/SFN) nodules,
- increase in diagnostic yield due to a higher malignancy rate of resected nodules,
- earlier surgery for patients with high-risk mutations: patients with high-risk mutations underwent surgery 78 days after molecular testing, compared with 184 days for ThyroSPEC™-negative patients
- high NPV for the diagnostically most challenging ATA intermediate / ACR-TIRADS 4 nodules[3].

For all categories, referral to Endocrinology or Surgery can be considered if it will aid in determining the best management approach.

For a list of **providers accepting thyroid nodule referrals**, visit:

Endocrinology Services: Thyroid Nodule & Cancer Referrals – Southern Alberta Zone

Surgery Services: Thyroid Nodule & Cancer Referrals – Southern Alberta Zone

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.

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