XXVIIè Congrés de la Societat Catalana d'Endocrinologia i Nutrició
Barcelona, 21 de Febrer de 2025

Estudios moleculares en nódulos tiroideos de citología indeterminada

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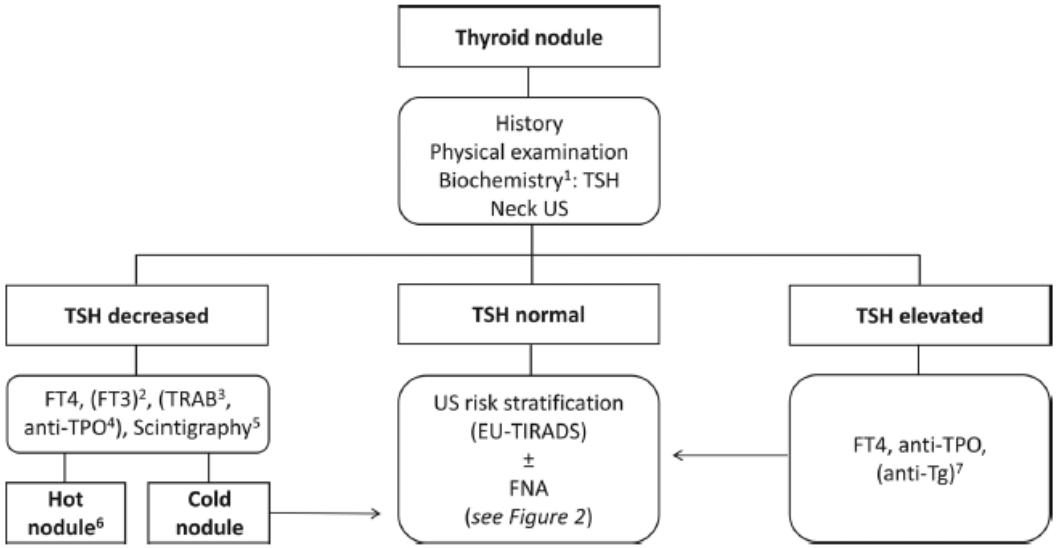
Hospital Clínico Universitario de Valencia

Vocal de TIROSEEN

ÍNDICE

EVALUACIÓN INICIAL DEL NÓDULO TIROIDEO



European Thyroid JOURNAL | C Durante, L Hegedüs *et al.* | 12:5 | e230067

GUIDELINES

2023 European Thyroid Association Clinical Practice Guidelines for thyroid nodule management

Cosimo Durante[1,*], Laszlo Hegedüs[2,*], Agnieszka Czarniecka[3], Ralf Paschke[4], Gilles Russ[5], Fernando Schmitt[6], Paula Soares[7], Tamas Solymosi[8] and Enrico Papini[9]

- Antecedentes: irradiación cervical, cáncer tiroides en familiares, MEN
- Clínica: compresión, parálisis recurrencial, disfagia, disnea, disfonía, estridor…
- Crecimiento rápido, modo de detección
- Palpación
- TSH y Ecografía

INDICACIONES DE PAAF: ATA vs TIRADS

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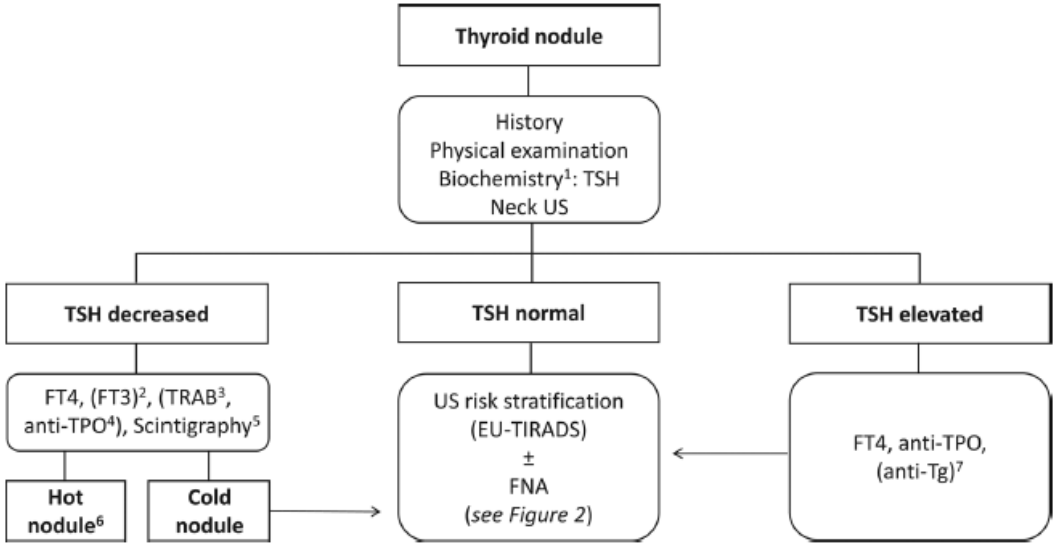
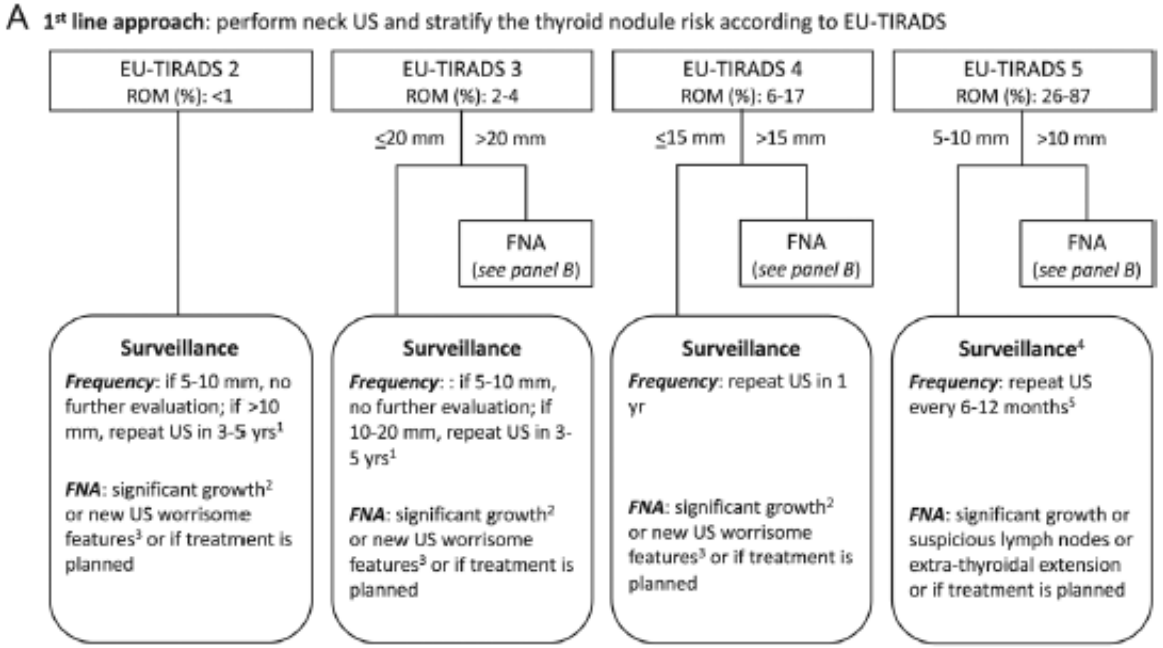
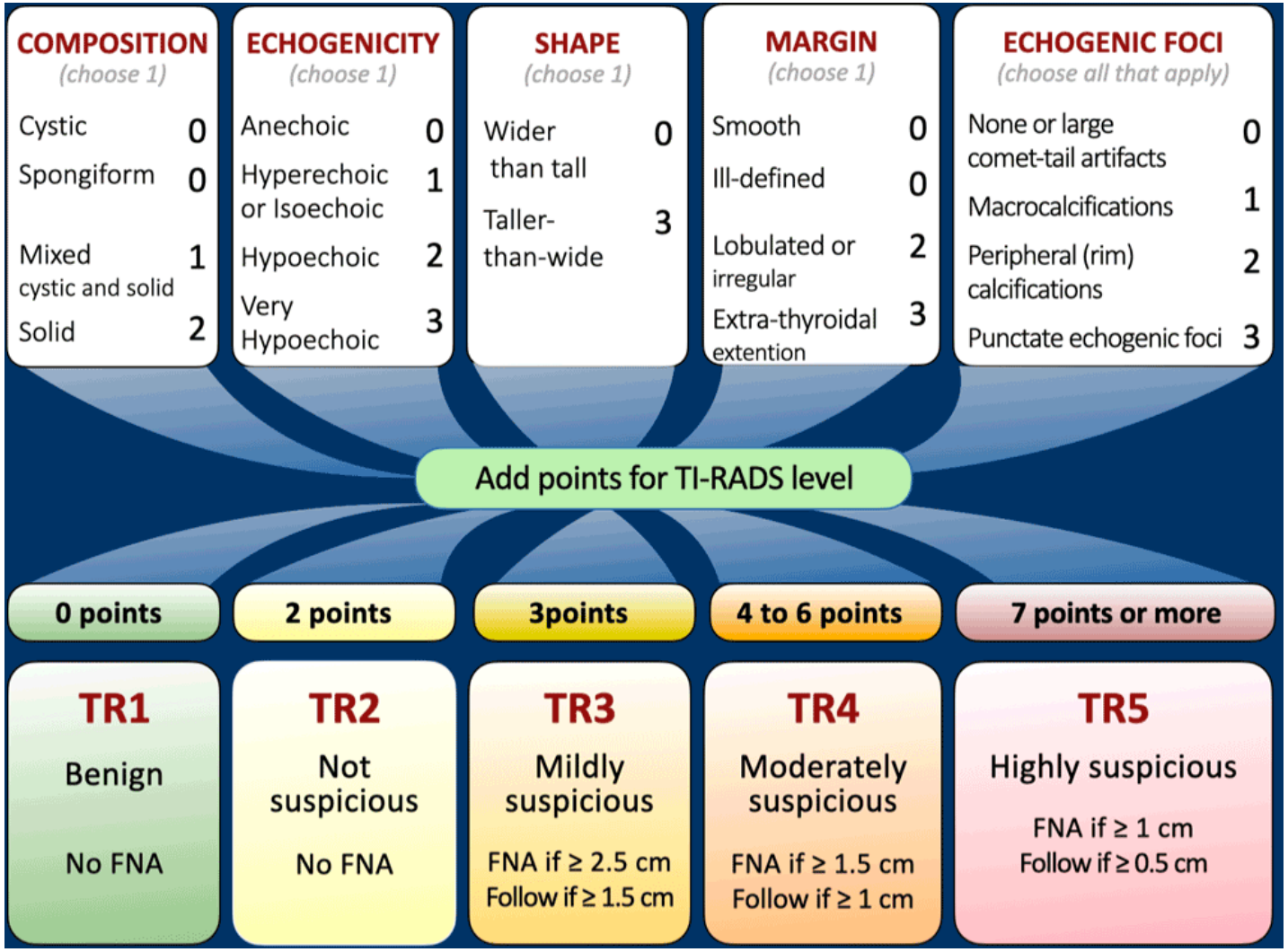
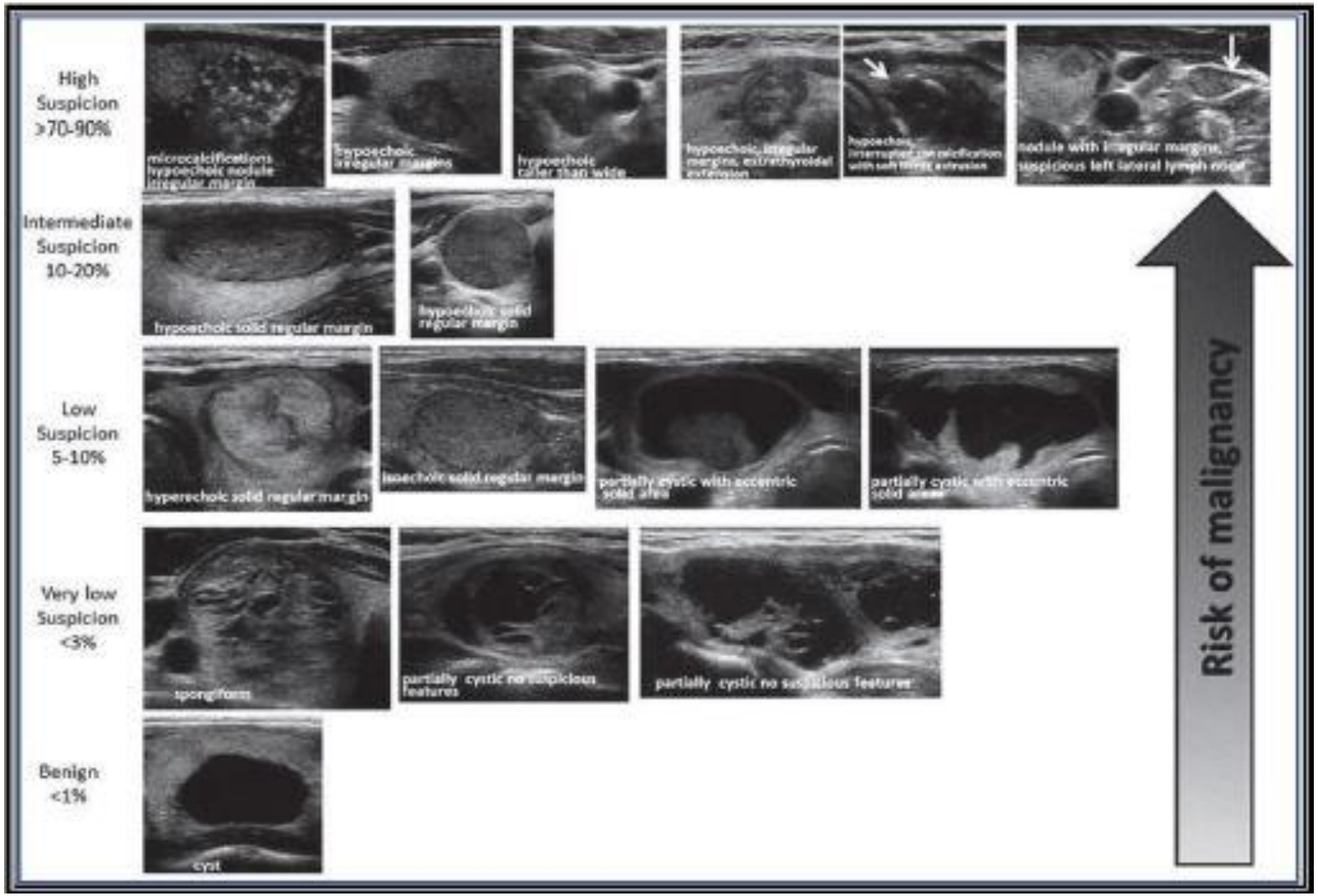




TIRADS ACR


COMPOSITION
(choose 1)
Cystic 0
Spongiform 0
Mixed cystic and solid 1
Solid 2
ECHOGENICITY
(choose 1)
Anechoic 0
Hyperechoic or Isoechoic 1
Hypoechoic 2
Very Hypoechoic 3
SHAPE
(choose 1)
Wider than tall 0
Taller-than-wide 3
MARGIN
(choose 1)
Smooth 0
Ill-defined 0
Lobulated or irregular 2
Extra-thyroidal extention 3
ECHOGENIC FOCI
(choose all that apply)
None or large comet-tail artifacts 0
Macrocalcifications 1
Peripheral (rim) calcifications 2
Punctate echogenic foci 3
Add points for TI-RADS level
0 points
2 points
3points
4 to 6 points
7 points or more
TR1
Benign
No FNA
TR2
Not suspicious
No FNA
TR3
Mildly suspicious
FNA if ≥ 2.5 cm
Follow if ≥ 1.5 cm
TR4
Moderately suspicious
FNA if ≥ 1.5 cm
Follow if ≥ 1 cm
TR5
Highly suspicious
FNA if ≥ 1 cm
Follow if ≥ 0.5 cm

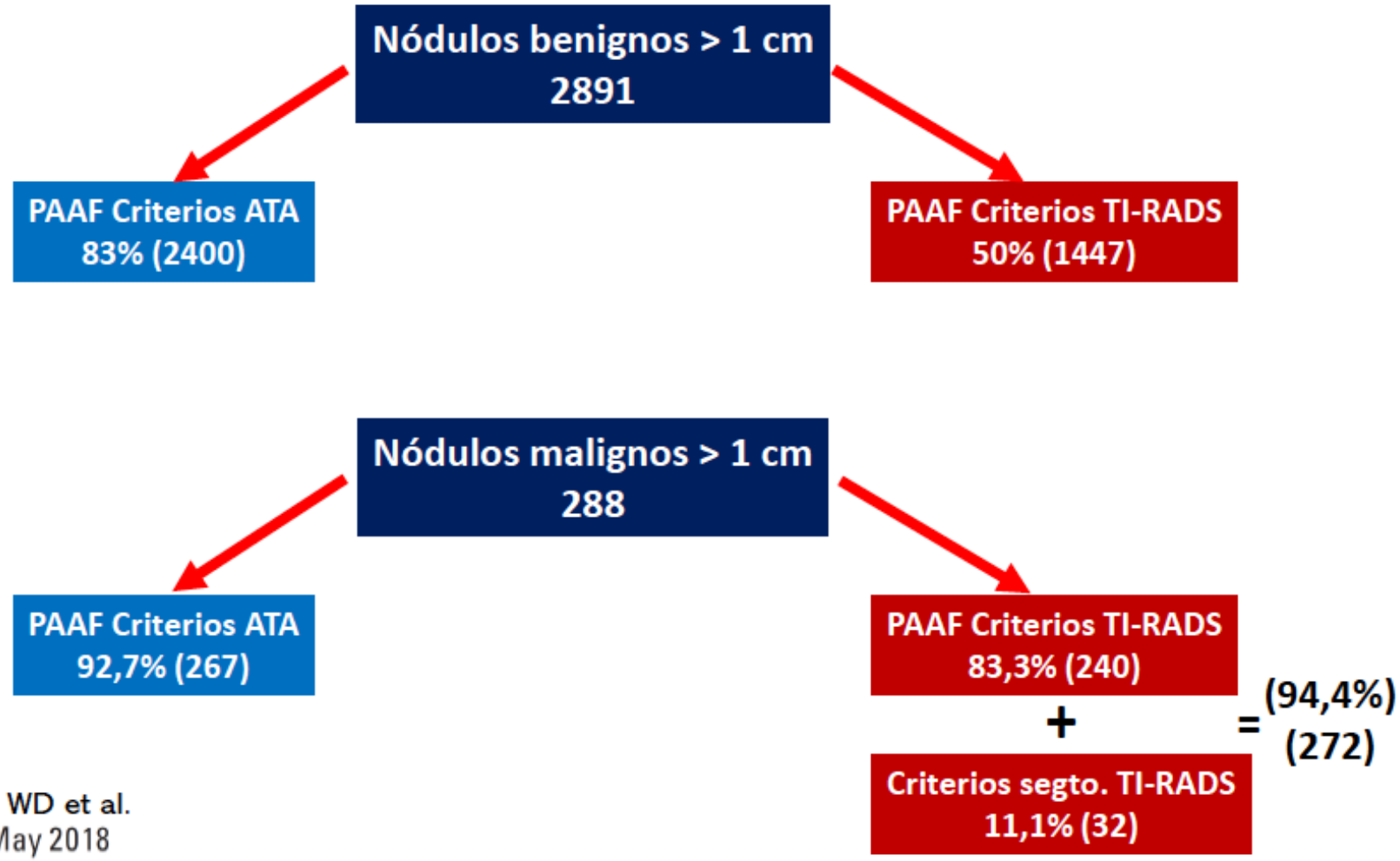
ATA NODULOS TIROIDEOS



Diferencias criterios PAAF Guías ATA / TI-RADS

	ATA	TI-RADS
Nódulos Espongiformes	> 2 cm	No indicada
Nódulos Mixtos (iso o hiperecogénicos) sin criterios de sospecha	> 2cm	No indicada
Nódulos sólidos iso o hiperecogénicos	> 1.5 cm	> 2.5 cm
Moderada Sospecha	> 1 cm	TIRADS 4 > 1.5 cm
Nódulos alta sospecha	> 1 cm	TIRADS 5 > 1 cm

53 % PAAF de Nódulos benignos con TI-RADS



Middleton WD et al.
AJR:210, May 2018

Nódulos tiroideos con PAAF indeterminada

- Entre el 20-30% de las PAAF nos van a salir como citología indeterminada (Bethesda III, IV y V)
- De estos sólo el 10-40% serán carcinomas (no contamos el Bethesda V que suele ser mayor el ROM)

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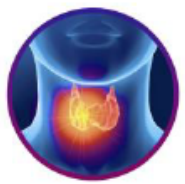
Table 5 Distribution of diagnoses across the Bethesda categories. The third edition of the Bethesda system has been released after the first online appearance of the current manuscript (131). It provides an updated summary of the reporting system and refined estimates of the risk of malignancy, which are therefore slightly different from those reported in Table 5.

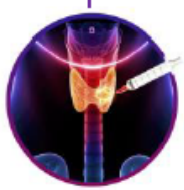
Bethesda categories	Definition of Bethesda categories	Subclassification		Expected frequency (range)	Estimated malignancy risk (NIFTP not cancer)
		Benign entities	Malignant entities		
Bethesda I	Non-diagnostic	NA	NA	3–11%	5–10%
Bethesda II	Benign	Adenomatoid/hyperplastic/colloid nodule Lymphocytic thyroiditis Subacute granulomatous thyroiditis Acute thyroiditis Graves' disease	PTC microcarcinomas in benign nodules	55–74%	0–3%
Bethesda III	Atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS)	Cyst lining cells Hashimoto's thyroiditis with cellular atypia (both follicular and lymphocytic atypia) Adenomatoid nodule (cellular with microfollicular proliferation) Parathyroid adenoma (microfollicular structures) Hürthle cell hyperplasia with lack of colloid	PTC, especially follicular variant; well-differentiated follicular carcinoma; Hürthle cell carcinoma; lymphoma	5–15%	10–30%
Bethesda IV	Follicular neoplasm or suspicious for follicular neoplasm (FN/SFN)	Adenomatoid nodule (cellular with microfollicular proliferation) Parathyroid adenoma (microfollicular structures) Hürthle cell hyperplasia with lack of colloid Follicular-patterned cases with mild nuclear changes (increased nuclear size, nuclear contour irregularity, and/or chromatin clearing), and lacking true papillae and intranuclear pseudo-inclusions	PTC, especially follicular variant; well-differentiated follicular carcinoma; Hürthle cell carcinoma	2–25%	25–40%
Bethesda V	Suspicious of malignancy	Hashimoto's thyroiditis with cellular atypia	Features suspicious for PTC, MTC, lymphoma, or other malignancy	1–6%	50–75%
Bethesda VI	Malignant	Hashimoto's thyroiditis with cellular atypia	Features *conclusive* for malignancy: PTC (true papillae, psammoma	2–5%	97–99%

Indeterminate Thyroid nodules

Current testing options EU



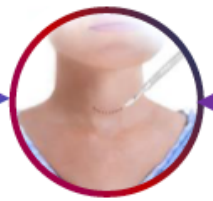
Thyroid Nodule detected by ultrasound



Fine Needle Aspiration (FNA)

Microscopic Analysis

Cancer (10%)



Surgery

Does not allow to avoid Surgery

Rule- In Test or confirm Cancer

Indeterminate (20%)

Risk of cancer 25%

- BRAF-V600
- RET
- TERT

Benign (70%)

Follow up

Nódulos tiroideos con PAAF indeterminada

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- En las guías de manejo de NT ya se incluyen los marcadores moleculares para intentar diferenciar los malignos de los benignos
- El precio (3200-6000 euros por determinación), hacían poco coste-eficaz el usarlos en nuestro medio

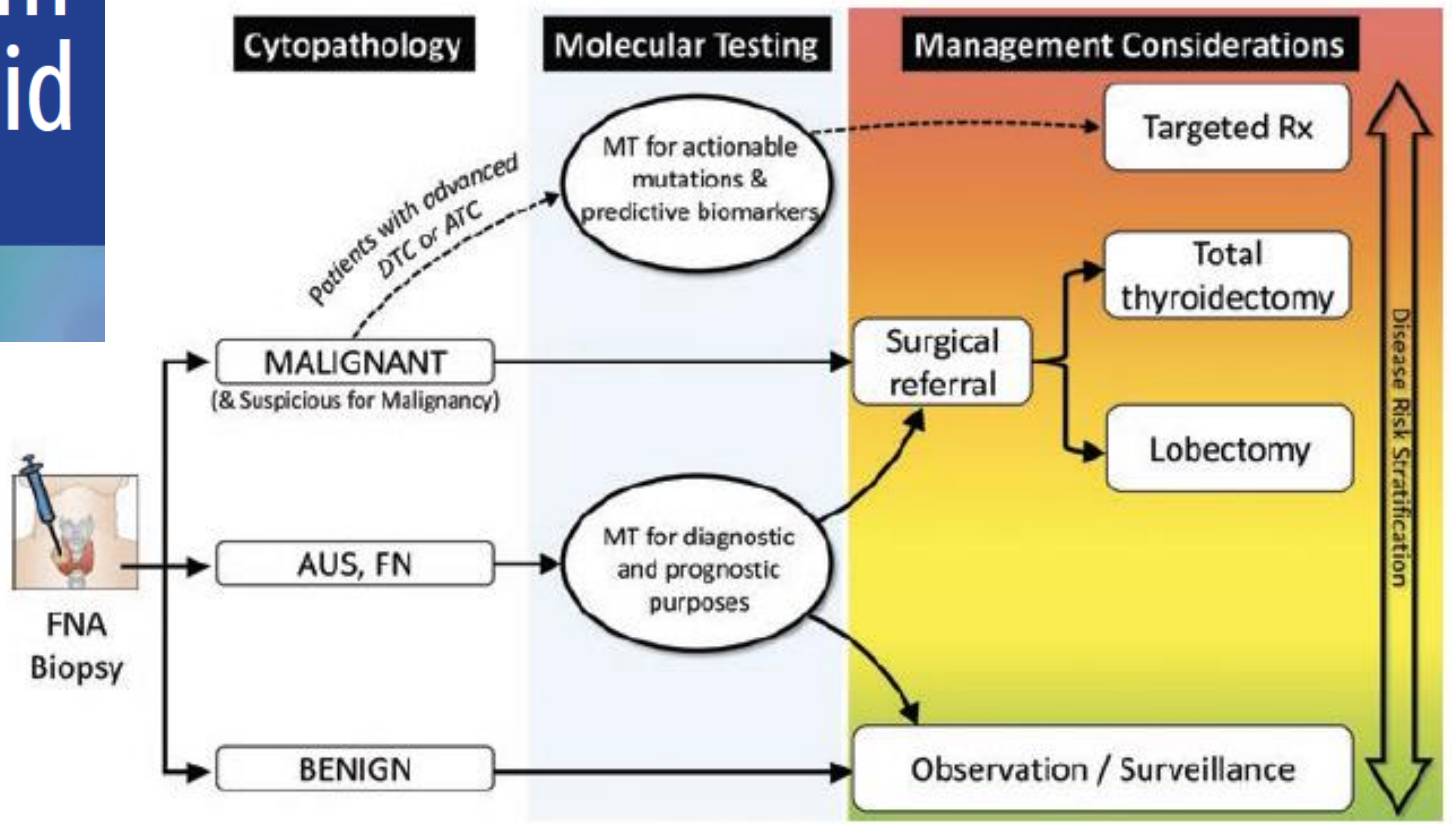
Table 6 Summary of genetic tests for aiding diagnosis of thyroid cancer in FNA cytology.

	Afirma GSC	ThyroSeq v3	ThyGeNEXT/ThyraMIR	ThyroidPrint
Type of test	RNA NGS (mRNA expression)	Targeted DNA and RNA NGS	Targeted NGS + miRNA expression	Quantitative real-time PCR (mRNA expression)
Biomarkers	1115 genes (expression) + mutation hotspots + fusions + LOH	112 genes + >120 fusions + 10 CNA + 19 genes (expression)	10 genes + 28 fusions + 10 miRNA (expression)	10 genes
NPV in marketing study (%)	96%	97%	95%	95%
PPV in marketing study (%)	47%	66%	74%	78%
Sensitivity in marketing study (%)	91%	94%	93%	91%
Specificity in marketing study (%)	68%	82%	90%	88%
Sample size Bethesda III, IV (n)	114, 76	154, 93	92, 86	117, 153

Precio.	6400	3200	2900	1500

The Bethesda System for Reporting Thyroid Cytopathology

- En la nueva edición del Bethesda 2023 incluyen un amplio capítulo sobre Test moleculares



Molecular and Other Ancillary Tests **14**

Michiya Nishino, Paul VanderLaan, Giancarlo Troncone, Claudio Bellevicine, N. Paul Ohori, Tetsuo Kondo, and Camille Buffet

S. Z. Ali, P. A. VanderLaan (eds.), *The Bethesda System for Reporting Thyroid Cytopathology*, https://doi.org/10.1007/978-3-031-28046-7_14

TEST MOLECULARES PARA NODULOS TIROIDEOS

- Paneles mutacionales (alteraciones DNA: mutaciones /fusiones/ alteración número copias):
  - ThyroSeq®
- Test que clasifican por expresión génica (PCR de muy pocos genes, mRNA)
  - Afirma®
  - Thyroidprint®

ThyroSeq®
Thyroid Genomic Classifier

Afirma®
Thyroid FNA Analysis

TABLE 1 The multiplatform test (MPTX) showing mutations and messenger RNA fusion transcripts (ThyGeNEXT) and microRNAs (ThyraMIR)

Expanded mutation panel (ThyGeNEXT)		microRNA risk classifier(ThyraMIR)
DNA variant	**Fusions (n) and mRNA**	**microRNA**
BRAF[a]	*BRAF* (3)[b]	miR-31-5p
ALK	*ALK* (2)	miR-29b-1-5p
GNAS	*NTRK* (8)	miR-138-1-3p
HRAS	*PPARg* (5)	miR-139-5p
KRAS	*RET* (14)[b]	miR-146b-5p
NRAS	*THADA* (5)	miR-155
PIK3CA	*NKX2.1*	miR-204-5p
PTEN	*PAX8*	miR-222-3p
RET[b]	*TBP*	miR-375
TERT promoter[b]	*USP33*	miR-551b-3p

Abbreviation: mRNA, messenger RNA.
[a]*BRAFV600E* is a strong driver mutation, while *BRAFK601E* is a weak driver mutation.
[b]Strong driver mutation.

ThyroSeq®
Thyroid Genomic Classifier

TEST MOLECULARES PARA NODULOS TIROIDEOS

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Association of comprehensive thyroid cancer molecular profiling with tumor phenotype and cancer-specific outcomes

Jason B. Liu, MD, MS[a], Kimberly M. Ramonell, MD[a], Sally E. Carty, MD[a], Kelly L. McCoy, MD[a], Barry M. Schaitkin, MD[b], Esra Karslioglu-French, MD[c], Elena M. Morariu, MD[c], N. Paul Ohori, MD[d], Raja R. Seethala, MD[d], Simion I. Chiosea, MD[d], Marina N. Nikiforova, MD[d], Yuri E. Nikiforov, MD, PhD[d], Linwah Yip, MD[a,*]

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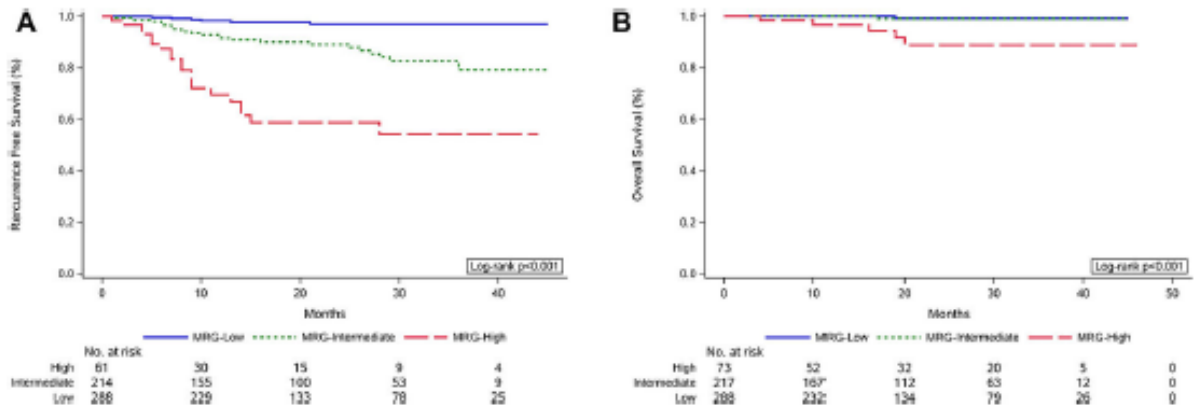
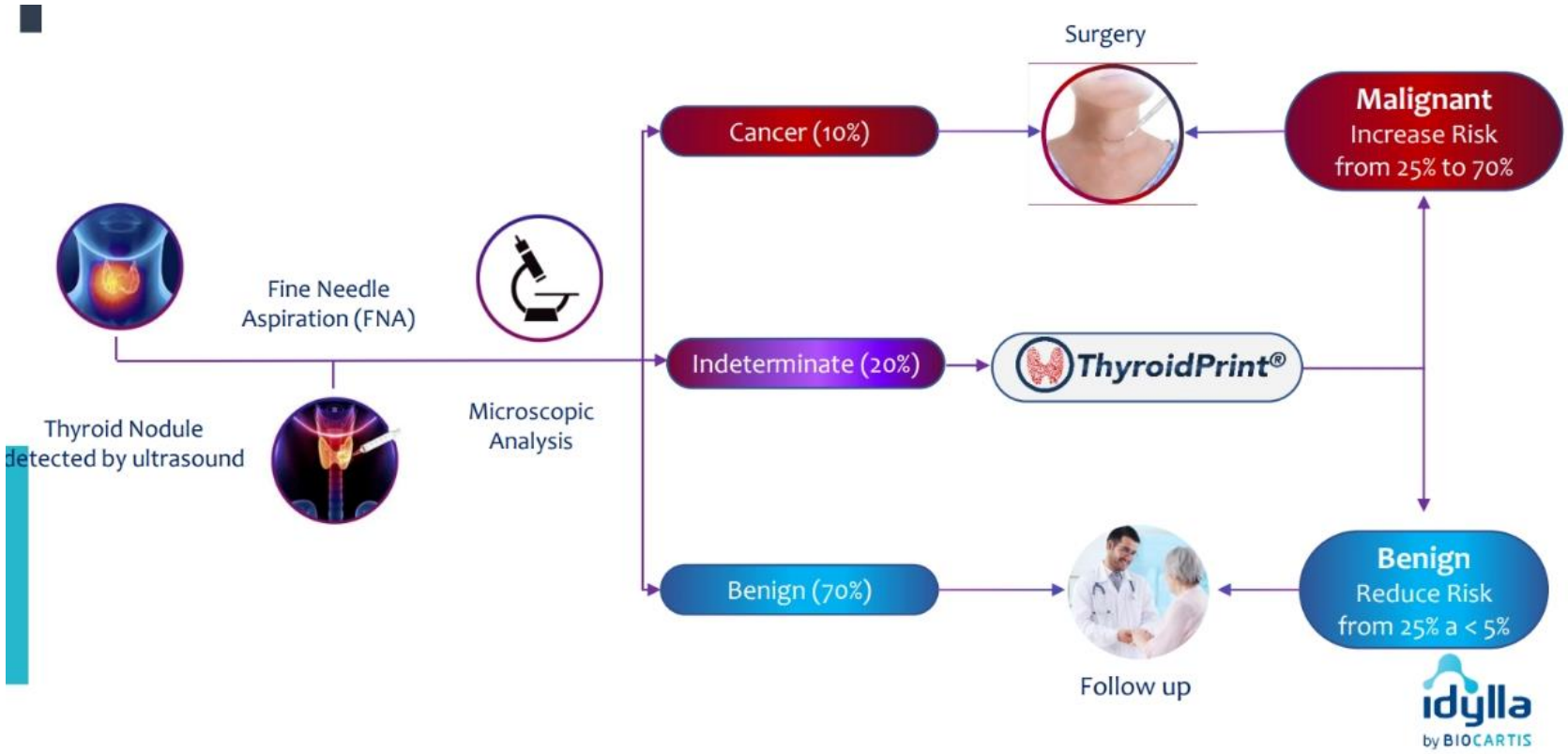


Figure 2.
(*A*) Recurrence-free survival and (*B*) overall survival by molecular risk group. MRG, molecular risk group.

TEST MOLECULAR THYROIDPRINT®

ThyroidPrint®

- Test molecular
- Panel genético de diez genes



FEATURES

The Idylla™ ThyroidPrint® Assay measures gene expression levels in 10 epithelial and stromal cell target genes relative to two reference genes listed in the table below.

Expressed Genes Detected by Idylla™ ThyroidPrint® Assay (RUO).*

Gene Name	Gene Name Abbreviation	Chromosome # Transcript ID Number	Gene Function and Role
C-X-C motif chemokine receptor 3	CXCR3	Chromosome X ENST00000373693.4	Tumor Inflammatory Microenvironment Target Genes
C-X-C motif chemokine ligand 10	CXCL10	Chromosome 4 ENST00000306602.3	
C-C motif chemokine receptor 7	CCR7	Chromosome 17 ENST00000246657.2	
Coxsackie virus and adenovirus receptor	CXADR	Chromosome 21 ENST00000284878.12	
C-C motif chemokine receptor 3	CCR3	Chromosome 3 ENST00000395940.3	
Keratin 19	KRT19	Chromosome 17 ENST00000361566.7	Tumor Epithelial Target Genes
Claudin 1	CLDN1	Chromosome 3 ENST00000295522.4	
TIMP metallopeptidase inhibitor 1	TIMP-1	Chromosome X ENST00000218388.9	
Actin Filament Associated Protein 1 Like 2	AFAP1L2	Chromosome 10 ENST00000304129.9	Stabilizing Target Genes
Heme oxygenase 1	HMOX-1	Chromosome 22 ENST00000216117.9	

Ca. Papilar

Gene Name	Gene Name Abbreviation	Chromosome # Transcript ID Number	Gene Function and Role in TP Assay
ERCC excision repair 3	ERCC3	Chromosome 2 ENST00000285398.7	Reference Genes
Glucuronidase beta	GUSB	Chromosome 7 ENST00000304895.9	

Papilares y anaplásicos

Microambiente tumor maligno

Malignos

Metástasis linfática

Invasión y metástasis

Sobreexpresados en malignos

Estos 2 últimos genes de han de amplificar correctamente para saber que tenemos una muestra correcta

No muestran expresión diferencial entre muestras benignas y malignas (estables)

THYROID
Volume 27, Number 8, 2017
Mary Ann Liebert, Inc.
DOI: 10.1089/thy.2017.0067

A 10-Gene Classifier for Indeterminate Thyroid Nodules: Development and Multicenter Accuracy Study

Hernán E. González,[1,*] José R. Martínez,[1,*] Sergio Vargas-Salas,[1] Antonieta Solar,[2] Loreto Veliz,[3] Francisco Cruz,[4] Tatiana Arias,[4] Soledad Loyola,[4] Eleonora Horvath,[5] Hernán Tala,[5] Eufrosina Traipe,[6] Manuel Meneses,[6] Luis Marín,[6] Nelson Wohlk,[7] René E. Diaz,[7] Jesús Véliz,[7] Pedro Pineda,[8] Patricia Arroyo,[9] Natalia Mena,[10] Milagros Bracamonte,[10] Giovanna Miranda,[10] Elsa Bruce,[10] and Soledad Urra[1]

BACKGROUND 10 TARGET GENES

	Biomarker	Cellular Funtion	Involvement in Cancer	Thyroid Cancer	References
Tumor Epithelial	CLDN-1	Belongs to the family of transmembrane tight junction proteins tightening the paracellular cleft of epithelial cells. Claudins perform crucial roles in maintaining cell polarity in epithelial and endothelial cell sheets and controlling paracellular permeability.	In human malignancies, CLDN-1 is often dysregulated and located in subcellular compartments, particularly in the nucleus. Altered expression of CLDN-1 has been reported in several tumor types including endometrial, papillary renal cell and colonic carcinoma, and increased claudin-1 mRNA levels have been observed in papillary thyroid carcinoma (PTC). Over expression of nuclear CLDN-1 in thyroid cancer cells results in increased cell migration and invasion. Epithelial-to-mesenchymal transition is one of the most important functions of claudin proteins in disease progression.	Increased ↑↑↑	*Zwanziger D et al. Endocrine Related Cancer, 22 (5), 2015., *Nemeth J et al., Pathology and Oncology research. 2009, volume 16, pages 19–27 (2010), *Wang D. et al. Frontiers in Oncology. Volumen 12, 2022.
	KRT-19	Keratin 19 is a member of the keratin family. The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells. It is well known as a marker of epithelial cells and tissues.	Due to its high sensitivity, KRT19 is one of the most used marker for the RT-PCR-mediated detection of tumor cells. KRTs may interact with several signal transduction molecules, such as adaptors, effectors, kinases, and receptors, which may regulate signaling pathways and mediate cell apoptosis, cell cycle arrest, invasion, and metastasis. KRT19 expression is higher in thyroid cancer when compared with normal thyroid tissues, and was associated with lymph node metastasis, tumor stage and tumor-node-metastasis stage.	Increased ↑↑	*Wang X., et al. ONCOLOGY LETTERS 18: 927-935, 2019. *Saha S., et al. Int. J. Mol. Sci. 2018, 19, 1423
	TIMP-1	This protein is a member of the TIMP family, a tissue inhibitor of metalloproteinases. This glycoprotein is a natural inhibitor of the matrix metalloproteinases (MMPs), a group of peptidases involved in degradation of the extracellular matrix. In addition to its inhibitory role against most of the known MMPs, the encoded protein is able to promote cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function.	TIMP-1 mRNA levels correlated directly with thyroid aggressiveness: the highest number of TIMP-1 transcripts was found in stages III and IV vs benign goitre. In patients with thyroid cancer, TIMP-1 expression levels are found to be highest in the group with metastasis in lateral neck. BRAFV600E mutation occurs selectively in PTC nodules and is associated with hyperactivation of NF-κB and upregulation of both TIMP-1 and its receptor CD63. Findings demonstrate that BRAFV600E causes upregulation of TIMP-1 via NF-κB. TIMP-1 bind to its surface receptor CD63, leading eventually to Akt activation, which in turn confers antiapoptotic behavior and promotion of cell invasion.	Increased ↑↑	* Shy L., et al. British Journal of Cancer 79, pages 1234–1239 (1999) *Bumber B et al., Clinical Otolaryngology 45; (1), 2020 Pages 55-62 *Bomarito A., et al. Endocrine-Related Cancer (2011) 18 669–685
	XB130 / AFAP1L2	XB130 (actin filament-associated protein 1-like 2, AFAP1L2) is a thyroid-abundant cytosolic adaptor protein and signal transduction mediator. XB130 regulates cell proliferation, cell survival, cell motility and gene expression. XB130 critically regulates thyrocyte polarization by functioning as a link between the actin filament cortex and microtubule-associated proteins at the apical membrane of thyrocytes. It is a substrate and regulator of multiple tyrosine kinase-mediated signaling.	XB130 has a controversial effect on cancer. Studies have shown that XB130 can promote cancer progression and downregulation of XB130-reduced growth of tumors derived from certain cell lines. A higher mRNA level of XB130 was shown to be associated with a better survival in non-small cell lung cancer. Previous studies have shown that XB130 can regulate cell growth, migration and invasion and possibly has the effect through the cAMP-cSrc-phosphoinositide 3-kinase/Akt pathway.	Decreased ↓	* Zhang R., et al. Biomed Rep. 2016 Mar; 4(3): 300–306.
	HMOX-1	HMOX1 (heme oxygenase 1 gene) is a human gene that encodes for the enzyme heme oxygenase 1. Heme oxygenase (abbreviated HMOX or HO) mediates the first step of heme catabolism, it cleaves heme to form biliverdin. HO-1 plays a pivotal and multifaceted role in cellular protection, which is likely attributable to its antioxidant, anti-inflammatory, and antiapoptotic properties. Nonetheless, the augmented expression of HO-1 in tumor tissues may have detrimental effects as it provides the selective advantage for tumor cells to overcome the increased oxidative stress during tumorigenesis and during treatment	Up-regulation of HO-1 in papillary thyroid tumours in comparison with normal thyroid tissue has been shown. Overexpression of HO-1 in a subset of thyroid cancers is associated with tumour aggressiveness and BRAF V600E expression. HO-1 might have a potential role in prognosis and targeted treatment in patients with thyroid cancer. In this context, it is interesting to note that HO-1 was overexpressed in thyroid cancer and was associated with tumor aggressiveness. Our previous findings were verified by a recent report showing that a 10-gene signature, including significantly overexpressed HO-1, may accurately classify indeterminate thyroid nodules.	Increased ↑	Giaginis C., et al. APMIS 2010; 118: 210–21. *Wang TY., et al. Histopathology. 2015 Feb;66(3):447-56.
Tumor Inflammatory Microenvironment	*CXADR*	Coxsackievirus and adenovirus receptor (CAR) is a protein that in humans is encoded by the CXADR gene.The protein encoded by this gene is a type I membrane receptor for group B coxsackie viruses and subgroup C adenoviruses. CAR is a cell adhesion molecule predominantly associated with epithelial tight junctions in adult tissues. CAR protein is expressed in several tissues, including heart, brain, and, more generally, epithelial and endothelial cells. It functions as a homophilic and heterophilic cell adhesion molecule through its interactions with extracellular matrix glycoproteins such as: fibronectin, agrin, laminin-1 and tenascin-R. In addition, it is thought to regulate the cytoskeleton through interactions with actin and microtubules.	CAR immunoreactivity was significantly increased in malignant compared with that in benign thyroid lesions ($p = 0.00002$). Both malignant and benign thyroid lesions with enhanced follicular cells' proliferative capacity showed significantly increased CAR immunoreactivity ($p = 0.00027$). In malignant thyroid lesions, enhanced CAR immunoreactivity was significantly associated with larger tumor size ($p = 0.0067$).	Increased ↑	*Giaginis C., et al. APMIS 2010; 118: 210–21.
	CHEMOKINES (CCR3, CXCL10, CXCR3, CCR7)	Chemokines are a family of 8–15 kDa molecular weight cyto- kines traditionally defined by their ability to stimulate cellular migration along a chemical gradient. Chemokine signaling through its receptors plays key functions during development, homeostasis, inflammation, infection, and pathological processes. These functions largely reflect the cardinal roles for chemokines in directed cell migration and substrate adhesion (Fig. 1). Chemokines also possess significant functional pleiotropy, with demon- strated roles in cell type-specific proliferation and apopto- sis. Chemotaxis directs cells to move along an increasing concentration gradient of chemokine ligands.	Conventionally, the governing elements for tumorigenesis and metastatic behavior were thought to be oncogenes or tumor suppressors. In cancer, chemokines play paradoxical roles in both the directed migration of metastatic, receptor-expressing cancer cells out of the tumor as well as immigration of tumor-infiltrating immune cells that culminate in a tumor-unique immune microenvironment. Inflammation is a key component of the tumor microenvironment and chemokines are part of the inflammatory network of mediators associated to neoplasia. Frequently, chemokines and chemokine receptors are found in tumors and often their expression and signaling are deregulated. In fact, chemokine receptor signaling in malignant cells promotes tumor growth, invasion and metastasis. Spread of tumor cells to chemokine gradients is restricted to specific patterns of chemokine receptors and to chemokine availability in the tumor microenvironment. Inflammatory cytokines produced by tumor cells and/or by tumor associated leukocytes may contribute to malignant progression	Increased ↓↑	*Drouillard D., et al. Am J Physiol Cell Physiol 324: C167–C182, 2023. *Urra et al. Oncotarget. 2017 Dec 20;9(2):2445-2467

- **Estudio prospectivo multicéntrico**
- **EEUU y Chile**
- **4061 PAAF**
- **897 (22%) citología indeterminada**

Alto valor predictivo negativo (VPN)

Resultado:

- **HIGH** → 69% malignos
- **LOW** → 95% benignos

THYROID
Volume 30, Number 5, 2020
Mary Ann Liebert, Inc.
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A Thyroid Genetic Classifier Correctly Predicts Benign Nodules with Indeterminate Cytology: Two Independent, Multicenter, Prospective Validation Trials

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New sample-to-result Idylla™-ThyroidPrint® Assay accurately identifies benign thyroid nodules with indeterminate cytology: A multicenter-prospective double-blinded clinical validation trial.

ETA 2024

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Demographics and Clinical Characteristics

Patients	172	
FNAs	172	
Female	143	83%
Age (years)	48	
Range	18 - 76	
Geographic Origin		
Europe	97	56%
North America	29	17%
South America	46	27%
Bethesda Category		
Bethesda III	74	43%
Bethesda IV	98	57%
Nodule Size		
Median Nodule Size (mm)	19	
Range (mm		
Histopathology		
Benign		
Low-risk ne		
Malignant		

Performance Across Histopathological Subtypes

Histopathology Subtype	Nodules	%	Classification Benign / Suspicious	Specificity
Total Cohort	**172**			
Benign	**123**	**72%**	**102 / 21**	**83.0%**
Benign follicular nodule	63	53%	53 / 10	86%
Follicular adenoma	15	12%	13 / 2	80%
Oncocytic adenoma	18	10%	15 / 3	83%
Chronic lymphocytic thyroiditis	1	1%	1 / 0	100%
Parathyroid adenoma	3	2%	3 / 0	100%
Low Risk Neoplasm				
NIFTP *	7	5%	4 / 3	61%
FLUMP **	14	15%	11 / 3	79%
Hyalinizing trabecular tumor	3	2%	2 / 1	76%
				Sensitivity
Malignant	**49**	**28%**	**4 / 45**	**92%**
Papillary Thyroid Carcinoma				
Convential Variant	18	37%	2 / 16	89%
Follicular Encapsulated Variant	8	16%	1 / 7	88%
Follicular Invasive Variant	2	4%	0 / 2	100%
Tall-Cell Variant	1	2%	0 / 1	100%
Solid Variant	2	4%	0 / 2	100%
				100%
				100%
				100%
				75%
				100%
				100%

** Follicular or oncocytic cell lesion of undetermined malignant potential (FLUMP)

Conclusions: The diagnostic performance of ThyroidPrint is preserved in the cartridge-based Idylla™-ThyroidPrint® Assay. The Assay could have potentially spared almost 2 every 3 surgeries with a 3% false negative rate. Data also suggests high test performance in oncocytic neoplasms and medullary thyroid cancer.

Estudio observacional unicéntrico

Chile

Impacto del resultado de Test Thyroidprint en la decision clínica

1272 PAAF, 244 (19%) citología indeterminada

Endocrine-Related Cancer | R Olmos *et al.* | 30:11 | e220409

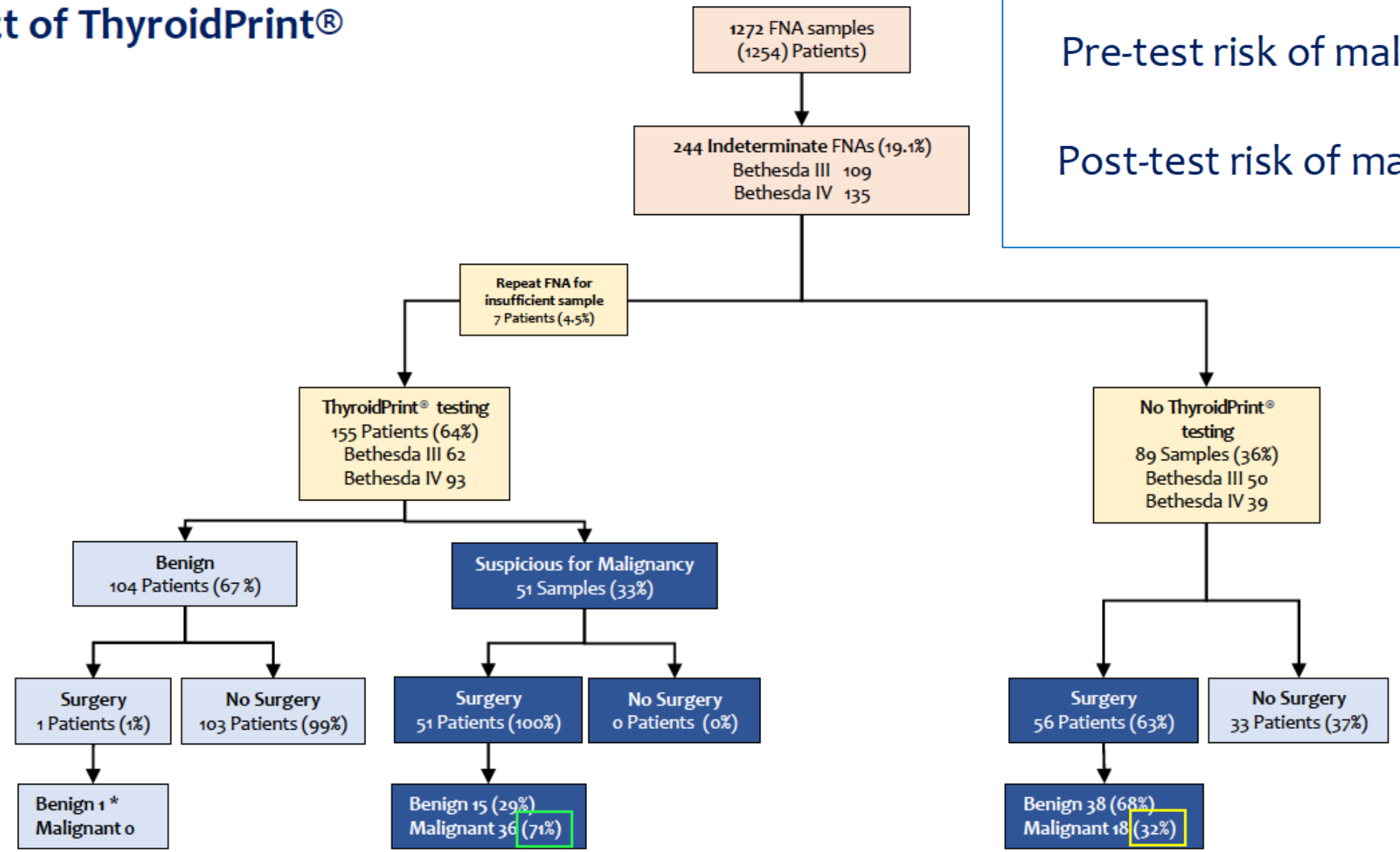
RESEARCH

ThyroidPrint®: clinical utility for indeterminate thyroid cytology

Roberto Olmos[1], José Miguel Domínguez[1], Sergio Vargas-Salas[2], Lorena Mosso[1], Carlos E Fardella[1], Gilberto González[1], René Baudrand[1], Francisco Guarda[1], Felipe Valenzuela[1], Eugenio Arteaga[1], Pablo Forenzano[1], Flavia Nilo[1], Nicole Lustig[1], Alejandra Martínez[1], José M López[1], Francisco Cruz[3], Soledad Loyola[3], Augusto Leon[2], Nicolás Droppelmann[2], Pablo Montero[2], Francisco Domínguez[2], Mauricio Camus[2], Antonieta Solar[4], Pablo Zoroquiain[4], Juan Carlos Roa[4], Estefanía Muñoz[2], Elsa Bruce[2], Rossio Gajardo[2], Giovanna Miranda[2], Francisco Riquelme[2], Natalia Mena[2] and Hernán E González[2]

Reduction of unnecessary surgery

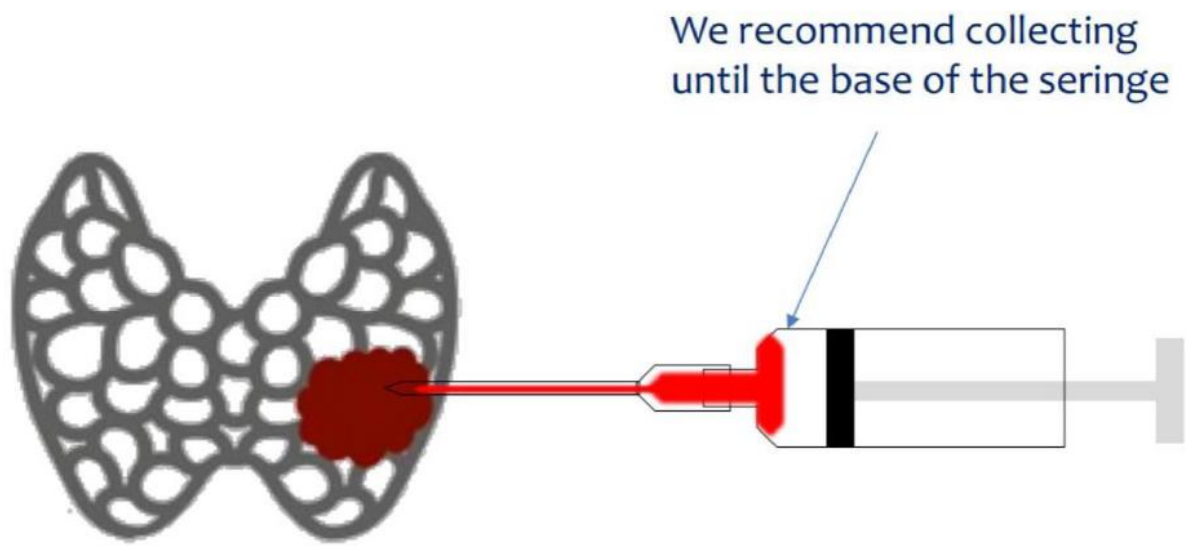
Impact of ThyroidPrint®

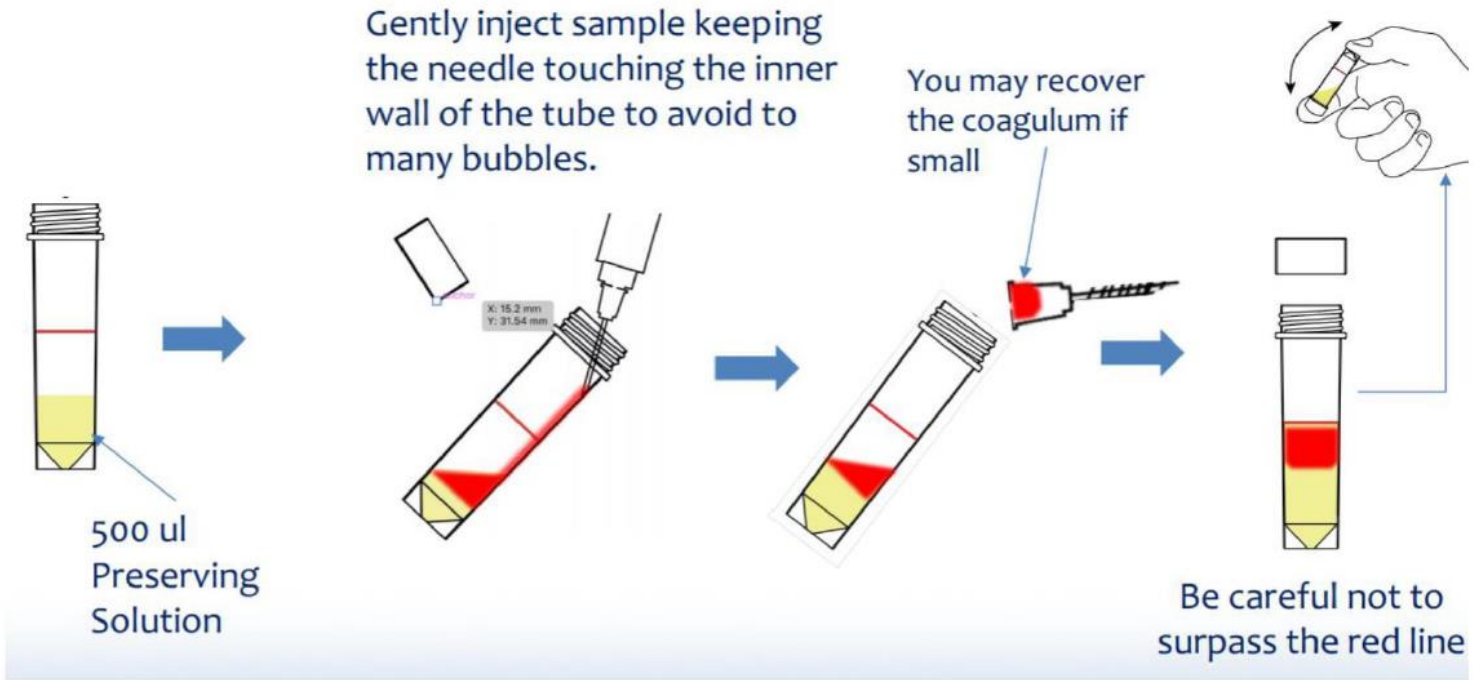


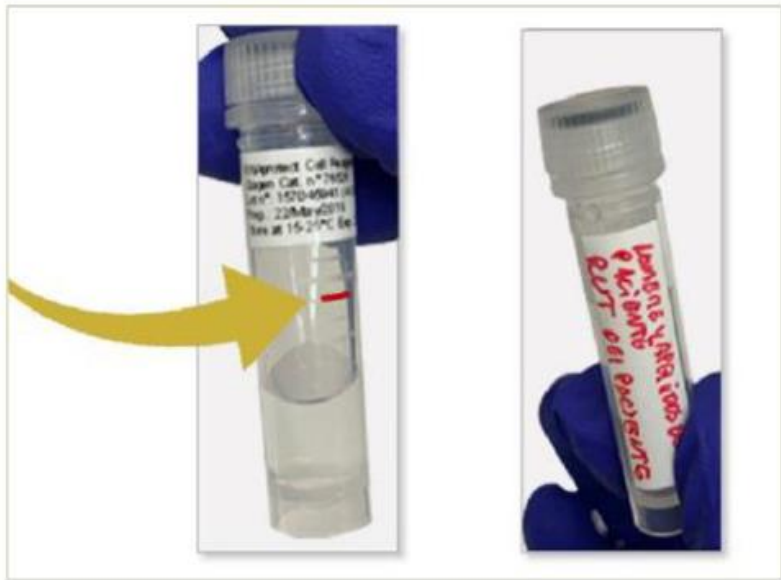
Pre-test risk of malignancy: 32%

Post-test risk of malignancy: 71%

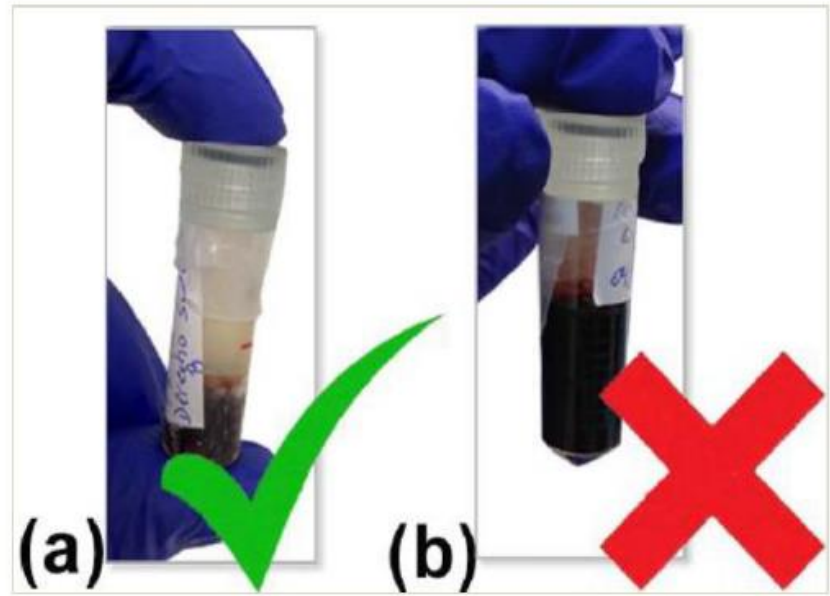
Toma de muestra




Gently inject sample keeping the needle touching the inner wall of the tube to avoid to many bubbles.
You may recover the coagulum if small
500 ul Preserving Solution
Be careful not to surpass the red line



Marca de nivel máximo de llenado y etiquetado del tubo de recolección con al menos dos identificadores del paciente

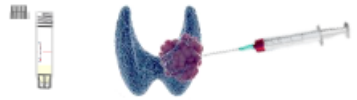


Se señala el correcto sellado y nivel de llenado del tubo de recolección

Fine Needle Aspiration (FNA) Collection and Handling Protocol for Idylla™ ThyroidPrint® Assay RUO

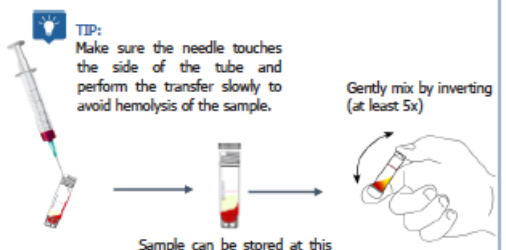
1 Fine Needle Aspiration

Collect FNA sample according to routine procedures.
Specific recommendations on FNA procedure for ThyroidPrint® can be found at www.thyroidprint.com/fnacollection/



2 Specimen collection

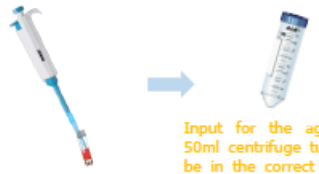
TRANSFER FNA sample (max 300 µl) to a 2.0 ml RNAse-free cryovial pre-filled with 500 µl RNAprotect® Cell Reagent (BC-Cat. Nr xxxx).
If not used immediately, STORE FNA samples in RNAprotect® Cell Reagent at 2-8°C for up to seven days.



CAUTION:
Do not transfer large clots or tissue to the RNAprotect® Cell Reagent. Adding more than 300 µl FNA sample to the cryovial risks generating invalid results.

3 Sample Preparation: Transfer

RECORD the FNA sample volume to check the volume on the graduated cryovial and verify using a p1000 micropipette. Transfer the entire sample volume (max 800 µl) to a 50 ml centrifuge tube using a p1000 micropipette.



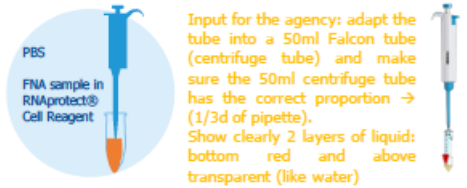
Input for the agency: the 50ml centrifuge tube should be in the correct proportion → (1/3d of pipette).

CAUTION:
Do not transfer tissue or clots that do not pass through the tip of the micropipette.

4 Sample Preparation: Dilution

Calculate: 3200 µl – FNA sample volume = PBS 1X volume

Add the PBS 1X to the FNA sample in the 50 ml centrifuge tube, using a p1000 micropipette, to the total volume of 3200 µl.



Input for the agency: adapt the tube into a 50ml Falcon tube (centrifuge tube) and make sure the 50ml centrifuge tube has the correct proportion → (1/3d of pipette).
Show clearly 2 layers of liquid: bottom red and above transparent (like water)

5 Sample Preparation: Mixing

Mix thoroughly the sample using a p1000 micropipette by pipetting up and down 10 times. Use the pipetting motion to breakdown any tissue/clots that may be present in the sample.

Input for the agency: Add drawing of micropipette with movement arrow up and down 10x.

TIP:
For ease of storage divide the diluted sample into 800 µl aliquots using 1.5 ml tubes.

CAUTION:
Tissue/clots in the sample may clog the extraction membrane in the cartridge and lead to run failure.

6 Testing or Storage

Immediately RUN Idylla™ ThyroidPrint® Assay
Or STORE the prepared sample at -15 to -25 °C up to 30 days.

or -80 °C
-112 °F

Input for the agency: temperatures to be adapted in the visual
-15 to -25 °C or 5 °F to -13° F

Idylla™ ThyroidPrint® Assay**

First-in-class cartridge-based Assay for indeterminate thyroid nodules

For Research Use Only, assay currently under development

Idylla™ ThyroidPrint® Assay**

qPCR of 10 genes in a diagnostic kit

Proprietary algorithm analysis

ThyroidPrint Score
Reported as either 'High' or 'Low'

CXCR3, CXCL10, CCR3, CCR7, CXADR — Tumor Inflammatory microenvironment Genes
TIMP1, CLDN1, KRT19 — Tumor Epithelial Genes
AFAP1L2, HMOX1 — Stabilizing Genes

Unique sample-to-insight seamless workflow

Scan
Sample & Cartridge

Insert Sample
in the Cartridge

Insert Cartridge in the Idylla™ Platform and obtain the result within 3 hours

(1) Haugen et al, 2015 American Thyroid Association Management guidelines for adult patients. Thyroid, 2016
(2) Gonzalez et al, A 10-Gene Classifier for Indeterminate Thyroid Nodules: Development and Multicenter Accuracy Study. Thyroid, 2017
(3) Zafereo et al, A Thyroid Genetic Classifier Correctly Predicts Benign Nodules with Indeterminate Cytology: Two Independent, Multicenter, Prospective Validation Trials. Thyroid, 2020
(4) Olmos et al., ThyroidPrint®: clinical utility for indeterminate thyroid cytology. End Rel Cancer, 2023

DEPARTAMENT DE SALUT DE VALÈNCIA
CLÍNIC-LA MALVA-ROSA

RESULTADOS DEL USO DEL TEST MOLECULAR THYROIDPRINT® EN NÓDULOS TIROIDEOS CON CITOLOGÍA INDETERMINADA

MATERIAL Y MÉTODOS

- Estudio prospectivo observacional
- Nódulos tiroideos con citología indeterminada: Bethesda III ó IV
- 55 determinaciones en 52 pacientes
  - 20 secuenciales (PAAF para citología → 45 días → PAAF para molecular)
  - 35 simultáneas (PAAF para citología y molecular si procede)

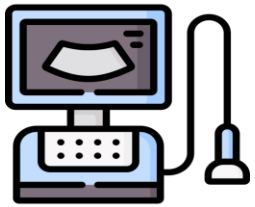
MATERIAL Y MÉTODOS: Variación

- Cuando hacemos PAAF y no sale claramente coloide (y no vamos a operar por otras razones la paciente), cogemos muestra para los cristales y el tubo (laminillas y después el resto)
- Si nos parece muestra escasa, lavamos la aguja
- Guardamos en nevera (puede estar 1 mes)
- Si Bethesda III ó IV (10-14 días): hacemos Test molecular
- Citas al paciente a las 3 semanas con todo hecho

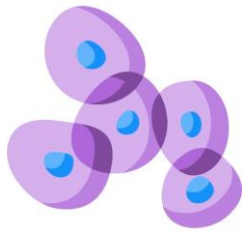
RESULTADOS

- 28 de Febrero de 2024
- 26 de Noviembre de 2024

- 52 pacientes
- 82% mujeres
- Edad media 59 años (30-86)



- 40% TIRADS-3
- 50% TIRADS-4
- 10% TIRADS-5



- 82% Bethesda IV
- Solo 3 nódulos Bethesda III (2 de ellos x2, 1 era la segunda PAAF)

RESULTADOS

ThyroidPrint®

LOW	→	**59,6 %** 31 pacientes
HIGH	→	**19 %** 10 pacientes
Inválido (*)	→	**15 %** 8 pacientes
Indeterminado	→	3 casos

(*) Un total de 8 muestras insuficientes, 3 obtenidas de forma secuencial y 5 simultáneas.

RESULTADOS

Solo **35%** de los pacientes fueron remitidos a cirugía

LOW

4 pacientes

→ **BENIGNOS**

HIGH

7 pacientes operados de 10

- **Carcinoma papilar** → 2 pacientes
- **Carcinoma folicular** → 1 paciente
- **NIFPT** → 1 paciente
- **Adenoma folicular** → 2 pacientes
- **Hiperplasia nodular** → 1 paciente

CONCLUSIONES

- El test molecular ThyroidPrint® tiene un **alto valor predictivo negativo**.
- Reduce la necesidad de intervención quirúrgica en pacientes con nódulos de citología indeterminada.

  En nuestra muestra de pacientes:

  ✓ Se redujo un 60% la necesidad de cirugía

  ✓ Se evitaron 34 cirugías (18 versus 52)

  ✓ Se ahorraron 51.399,28 euros

Coste hemitiroidectomía → 3452,92 euros
Coste ThyroidPrint® → 1200 euros

- La obtención simultánea de la muestra para citología y test molecular es una aproximación válida.

Mensajes para casa

- Los nódulos tiroideos son un motivo de consulta cada vez más frecuente
- La ecografía es el mejor método para decidir cuales precisan PAAF, acompañado de la clínica del paciente, y TIRADS-ACR es el sistema más eficaz
- Los nódulos tiroideos de citología indeterminada Bethesda III y IV representan un 25% de las PAAF, y la mayoría son benignos
- Los test moleculares comerciales pueden ayudar a evitar cirugías innecesarias y en algunos casos a dar información pronóstica (y probablemente a cambiar la extensión de la cirugía) en nódulos con mutaciones de alto riesgo
- El test molecular Thyroidprint® nos pueden ayudar a no operar pacientes de bajo riesgo de malignidad, y es coste-eficaz en nuestro medio

GRACIAS POR LA ATENCIÓN