How to evaluate molecular testing: How to use it in your practice?

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Disclosures

- No financial disclosures related to this presentation

To reflect back almost 50 years . . .

THE NODULAR THYROID GLAND AND CANCER*

A Practical Approach to the Problem

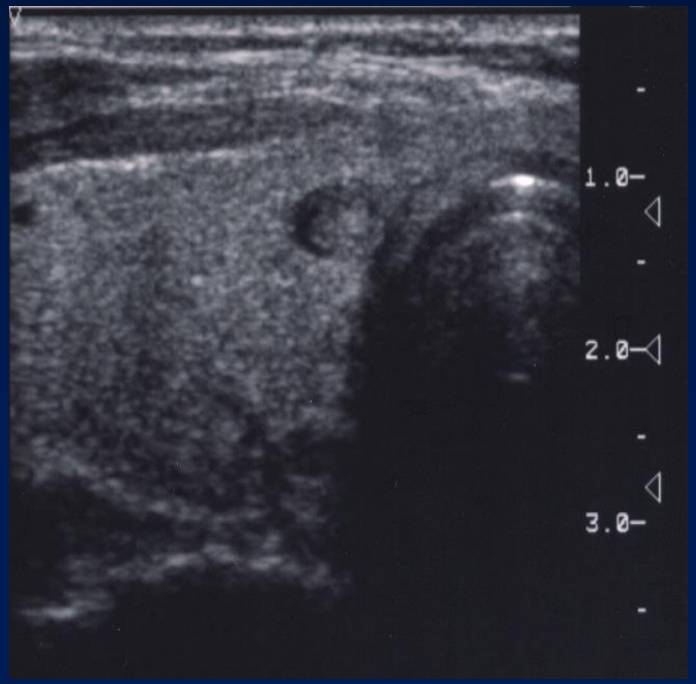
New England Journal of Medicine 1964

How to select that patient with a nodular thyroid gland in whom there is the greatest likelihood of finding a cancer is still the subject of considerable disagreement.

2013
Ultrasound
FNA
Cytology
Molecular analysis

Goal of diagnostic evaluation of thyroid nodules:

- Once we have decided that a nodule has the potential to be clinically relevant
  - FIND CANCER



How good is our DX of cancer?

- 2 expert thyroid surgical pathologists

Appendix Table 4. Interrater Concordance of Thyroid Histopathologic Diagnosis Between the 2 Central HP Members* **765 thyroid tumors**

HP Member 1	HP Member 2		
	Benign	Cancer	Total
Benign	455	51	~~506~~
Cancer	23	~~236~~	259
Total	478	287	~~765~~

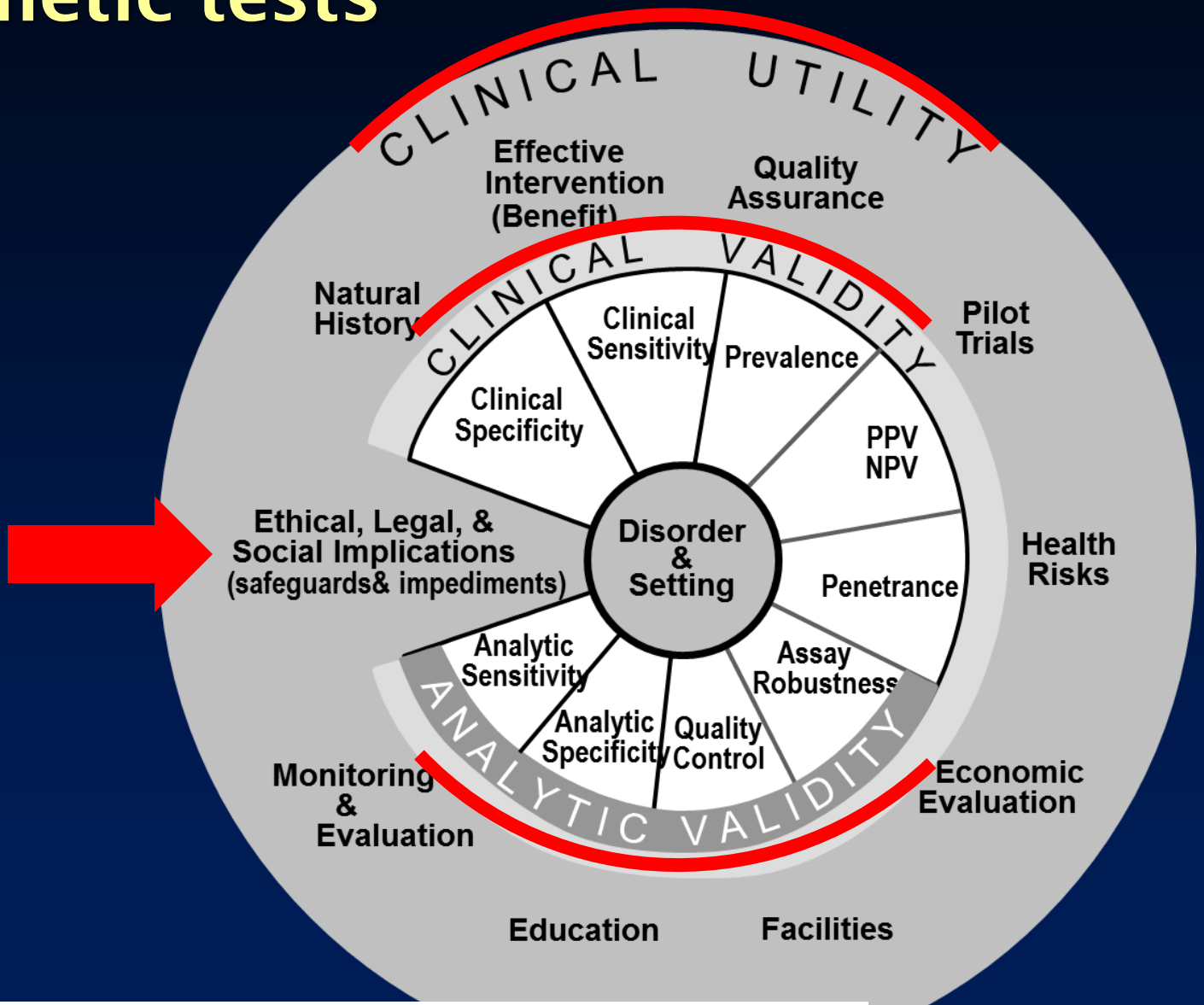
- 90.3% agreement for initial diagnosis
- After conferral 98.5% agreement

Cibas Ann Intern Med 2013:159:325

When should molecular testing be considered in patients with thyroid nodules?

- Never
  - if there is another gold standard dx test
- Always
  - if molecular testing always provides the correct answer
- Sometimes

The ACCE Model System for evaluating genetic tests



http://www.cdc.gov/genomics/gtesting/ACCE/

Analytical Validity:
Accuracy and reliability to measure genotype of interest

- How often is it positive when a mutation is present?
- What range of patient specimens have been tested?
- Quality control program
- Robustness- concordance of results in multiple labs

http://www.cdc.gov/genomics/gtesting/EGAPP/recommend/method.htm

BRAF V600E mutation testing the impact of detection sensitivity

- Percentage of detectable mutant DNA in the admixture of normal DNA

	Detection sensitivity	False positive rate
Sanger sequencing	20%	0%
Dual priming oligonucelotide-PCR	2%	1.4%
MEMO sequencing	0.1%	0.08%

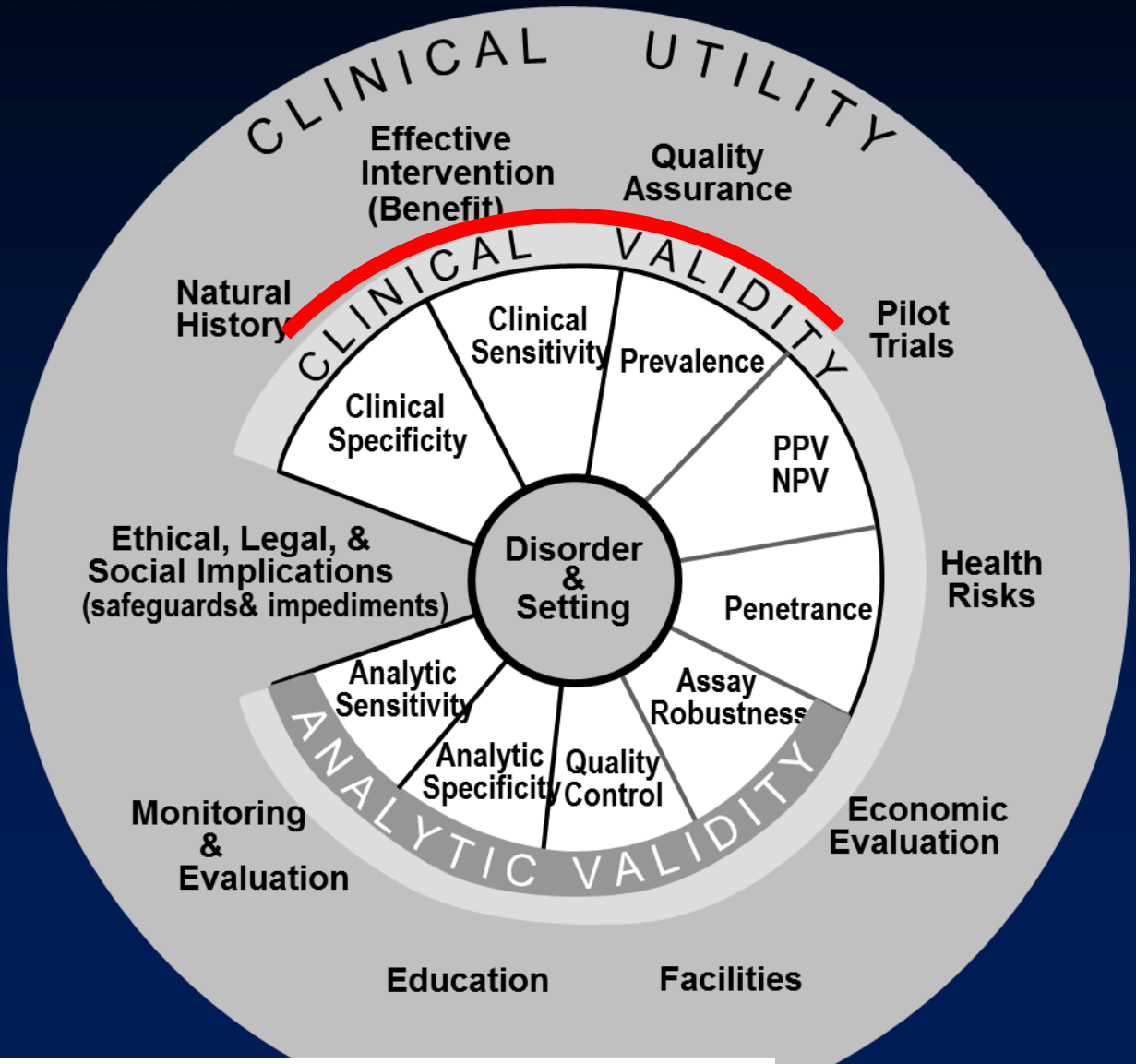
Lee 2012 J Clin Endocrinol Metab 97:2299

And this IMPACTS clinical practice. .

- 87 yo woman with 1.7cm nodule with FLUS cytology
- BRAF V600E mutation present by QUEST assay run in duplicate with detection sensitivity of 0.1%
- Total thyrx and pathology benign
- Local hospital performed Sanger sequencing (detection sensitivity 10-15%)→ **no BRAF V600E mutation detected**

DiLorenzo Endocr Practi Epub 2013 EP13294.CR

The ACCE Model System for evaluating genetic tests



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Clinical Validity:
Accuracy with which a test predicts the disorder of interest

- the sensitivity, specificity, and predictive values of a test in relation to a particular disorder
- evaluation of testing in a similar population to which test is targeted
- longitudinal cohort studies

http://www.cdc.gov/genomics/gtesting/EGAPP/recommend/method.htm

	Cancer	Benign
Test Pos	TP True Positive	FP False Positive
Test Neg	FN False Negative	TN True Negative
	Total cancer= TP +FN	Total benign= FP + TN

	Cancer	Benign
Test Pos	TP True Positive	FP False Positive
Test Neg	FN False Negative	TN True Negative
	Total cancer= TP +FN	Total benign= FP + TN

- **Sensitivity-- ability of test to identify disease, so the fraction of those with disease who test positive**

$$\frac{TP}{TP + FN}$$

- **Specificity-- ability of test to identify those without disease, so the fraction of those without disease who will test negative**

$$\frac{TN}{TN + FP}$$

Sensitivity and specificity are characteristics of the test. The population does not affect the results.

- The relevant questions for the clinician and patient--
- What is the chance that a person with a positive test truly has the disease?
- What is the chance that a person with a negative test result is disease free?

Positive and negative predictive values are influenced by the prevalence of disease in the population being tested.

Test: 90% sensitivity and 90% specificity

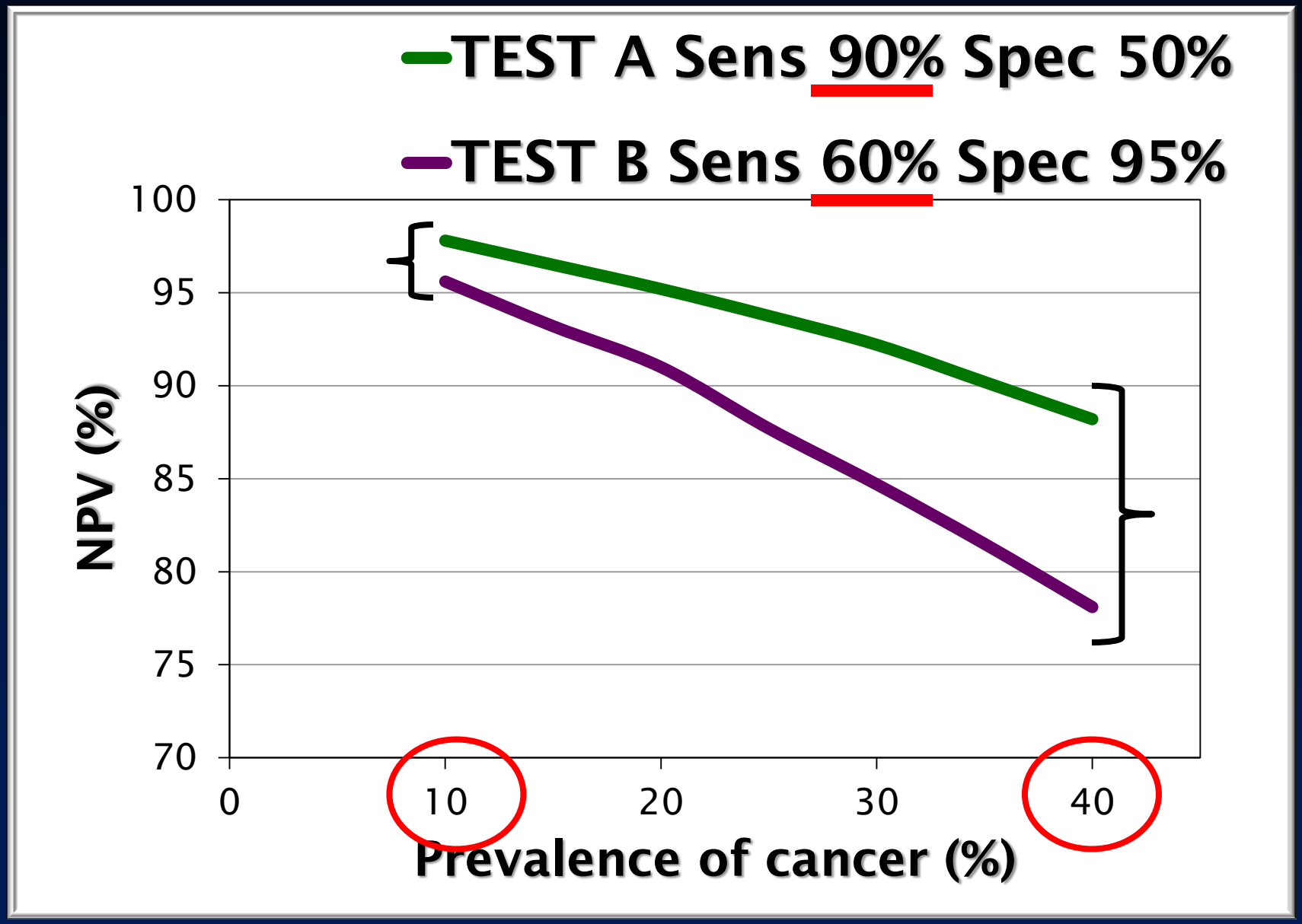
	Cancer	Benign
Test Pos	TP 18	FP 8
Test Neg	FN 2	TN 72
	20	80

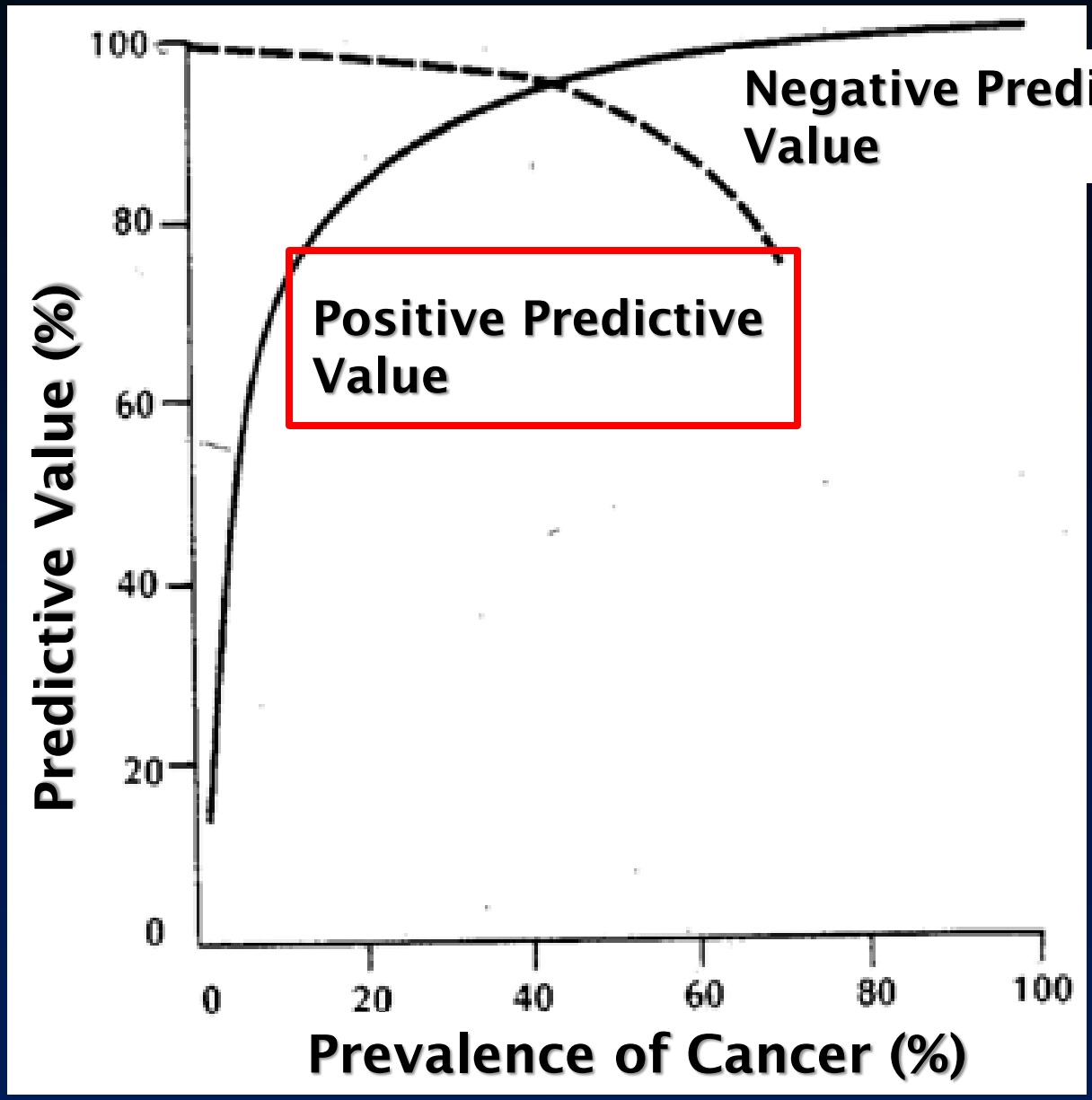
Example 1:
20% of population has cancer
PPV TP/all positive results= 18/26 = 69%
NPV TN/all negative results=72/74 = 97%

	Cancer	Benign
Test Pos	TP 63	FP 3
Test Neg	FN 7	TN 27
	70	30

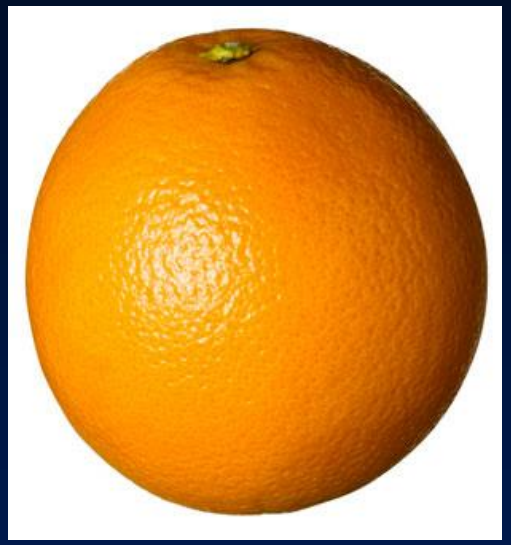
Example 2:
70% of population has cancer
PPV TP/all positive results= 63/66 = 95%
NPV TN/all negative results= 27/34 = 79%

NPV Decreases as Cancer Prevalence Rises





TEST: 95% sensitivity
95% specificity



Evaluating molecular tests to apply to your patients:

- How robust is the definition of the studied population?
- Is the cancer risk similar?
- What is the distribution of cancer histologies associated with that cytology classification?

How robust is the definition of the population?

Indeterminate subclassifications not reliably reproducible

	Bethesda 6 category classification	
FNA diagnosis	*Kappa (95% CI)*	*Agreement**
Unsatisfactory/nondiagnostic	0.8 (0.66–0.97)	strong
Benign	0.6 (0.43–0.78)	fair
FLUS	0.3 (0.18–0.45)	poor
Follicular neoplasm	0.5 (0.34–0.65)	fair
Suspicious for malignancy	0.2 (0.06–0.37)	poor
Malignant	0.6 (0.46–0.79)	fair

INTRAobserver concordance: 68%
INTERobserver concordance: 25%

Walts Diagn Cytopathol 2012 40:E62068

Is the cancer risk similar?

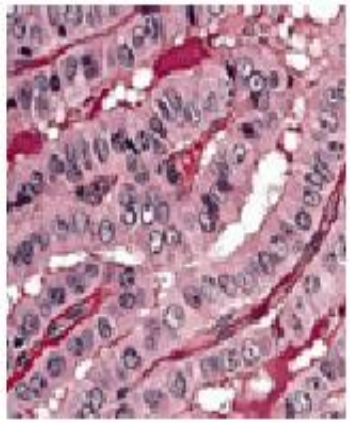
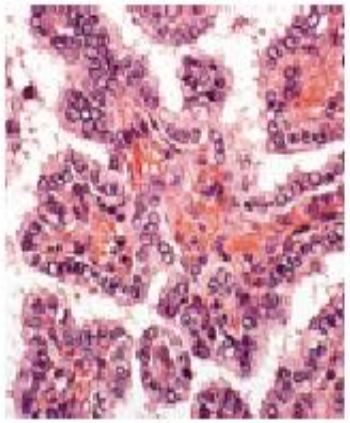
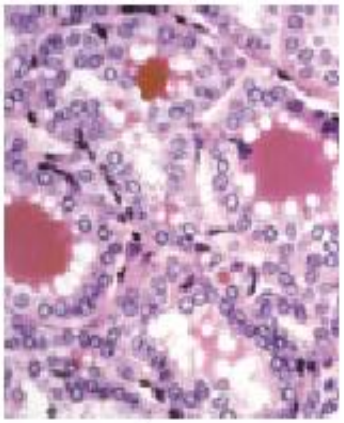
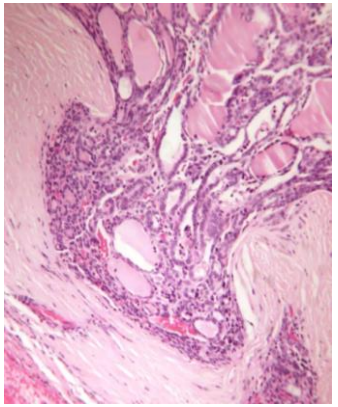
Bethesda Classification for Thyroid Cytology

	Diagnostic Category	BETHESDA Malignancy risk	REPORTED Malignancy risk
I	Nondiagnostic or unsatisfactory	~1-5%	0-32%
II	Benign	2-4%	2-7%
III	Atypia or follicular lesion of unknown significance (AUS/FLUS)	5-15%	6-48%
IV	Follicular neoplasm	15-30%	14-34%
V	Susp for malignancy	60-75%	53-87%
VI	Malignant	97-100%	96-100%

Baloch ZW, Diag Cytopath 36:425, 2008; Wang Thyroid 2011 21:243

What is the distribution of cancer histologies associated with that cytology classification?

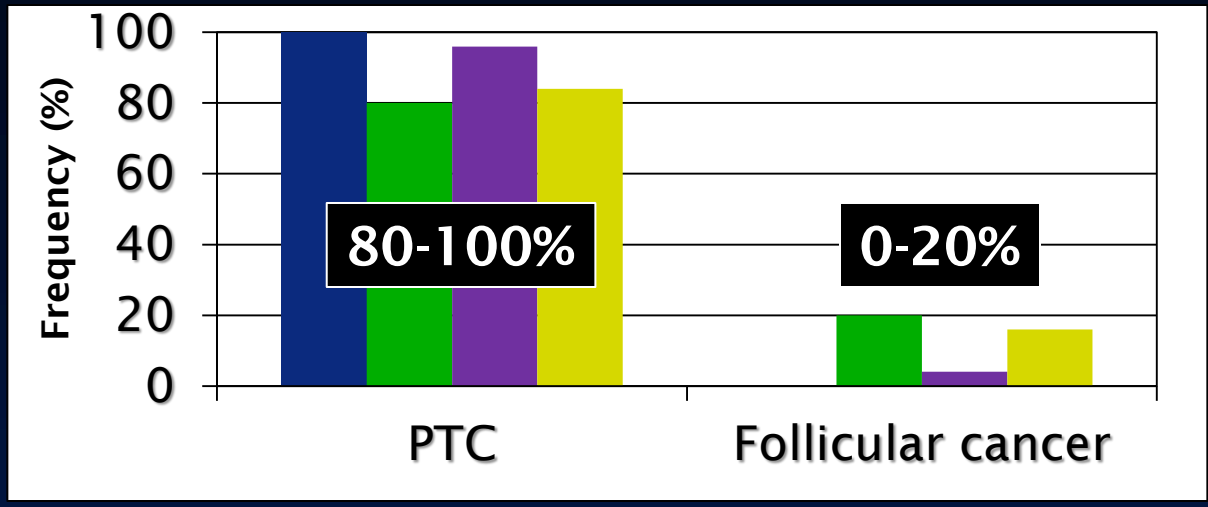
"Cancer" is not one entity

Tall cell	PTC classic	PTC FV	Follicular cancer
			
BRAF 70-80%	BRAF 40%	BRAF 10% NRAS 10%	RAS 30%

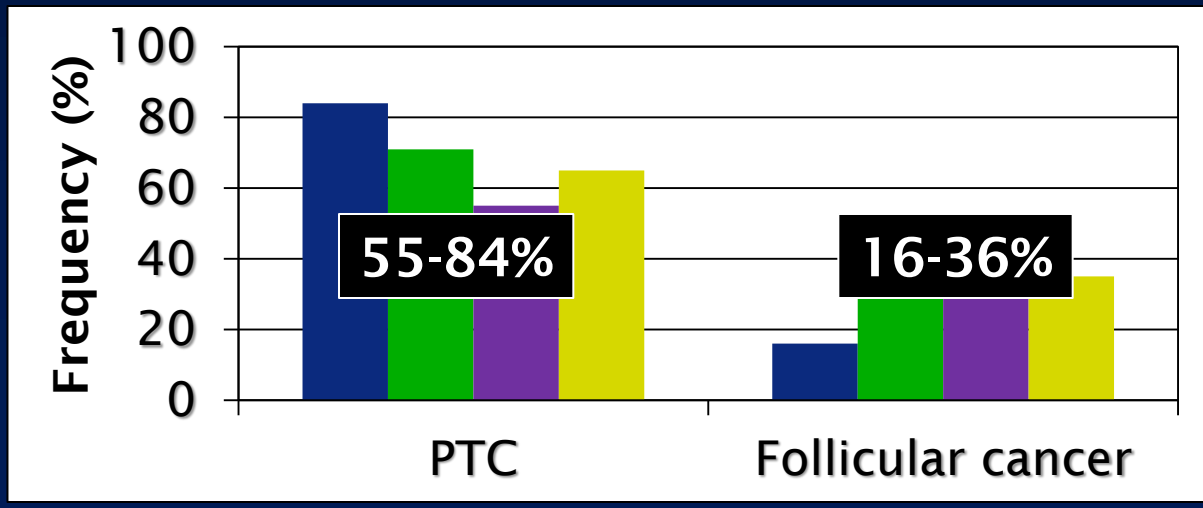
RET/PTC1 30%

Distribution of Malignant Histology

AUS/FLUS →



Follicular neoplasm →



Nikiforov J Clin Endocrinol Metab 2011 96:3390 ; Yang Cancer 2007;11:306; Yassa Cancer. 2007;111:508-16; Theoharis Thyroid 2009;19:1215-23

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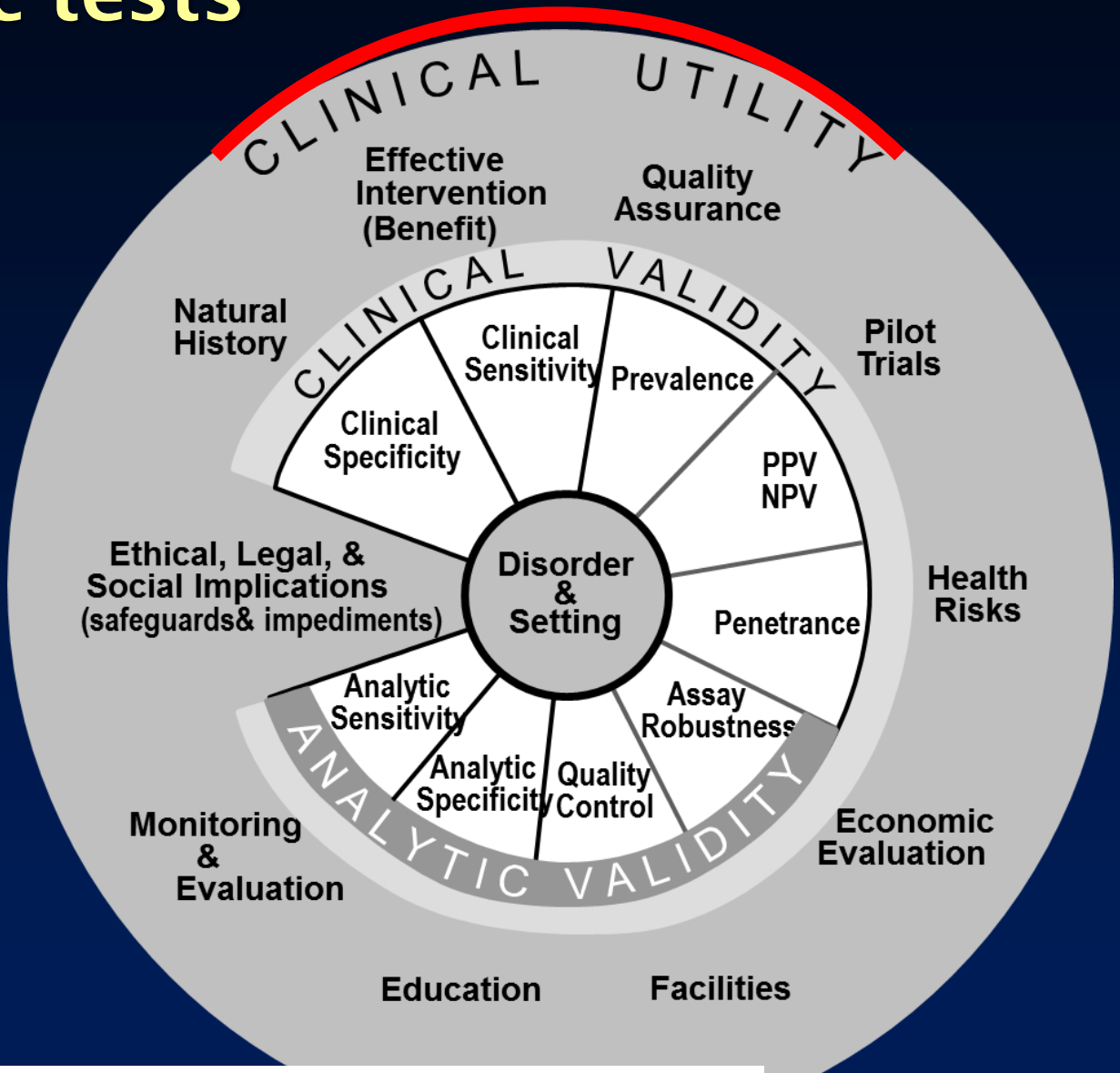

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Clinical Utility:
Likelihood that using the test to guide management will significantly improve health-related outcomes

- Clinical effectiveness analyses
  - Assumptions of model
  - Robustness of sensitivity analyses
- Real world application-
  - Differences in estimated magnitude of treatment effect between RCTs and observational studies

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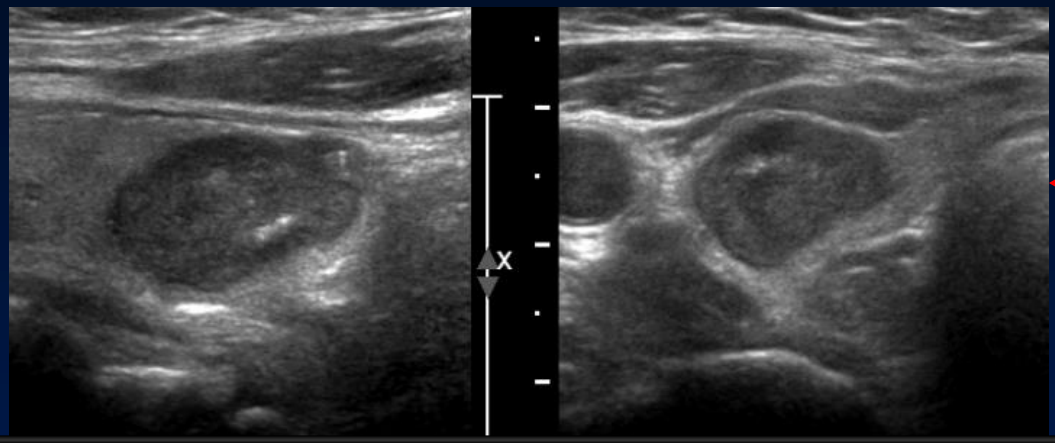
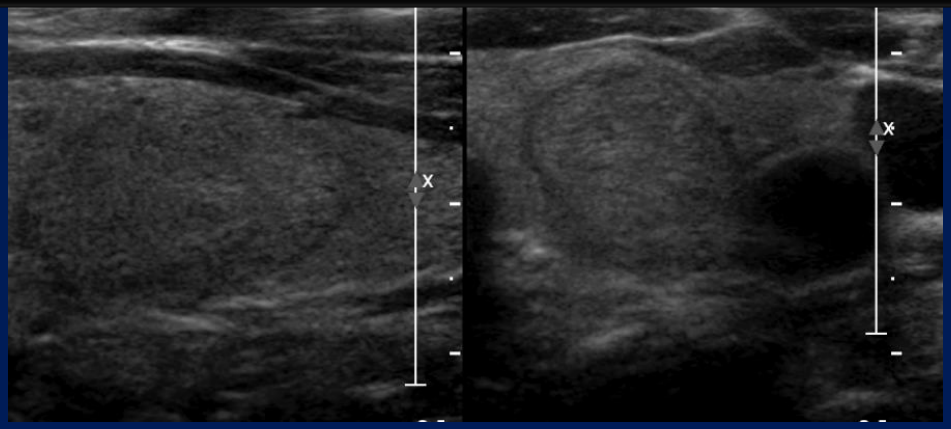
And we didn't talk about ultrasound

Two nodules with FLUS cytology



Based upon US, which nodule has the higher "pre test" cancer risk?



How to evaluate molecular testing: Can I use it in my practice?

- Know the test
- Know the studied population
- Know your patients