

Diagnosis and Management of Thyroid Nodules and Cancer Focus on Thyroglobulin November 16, 2012

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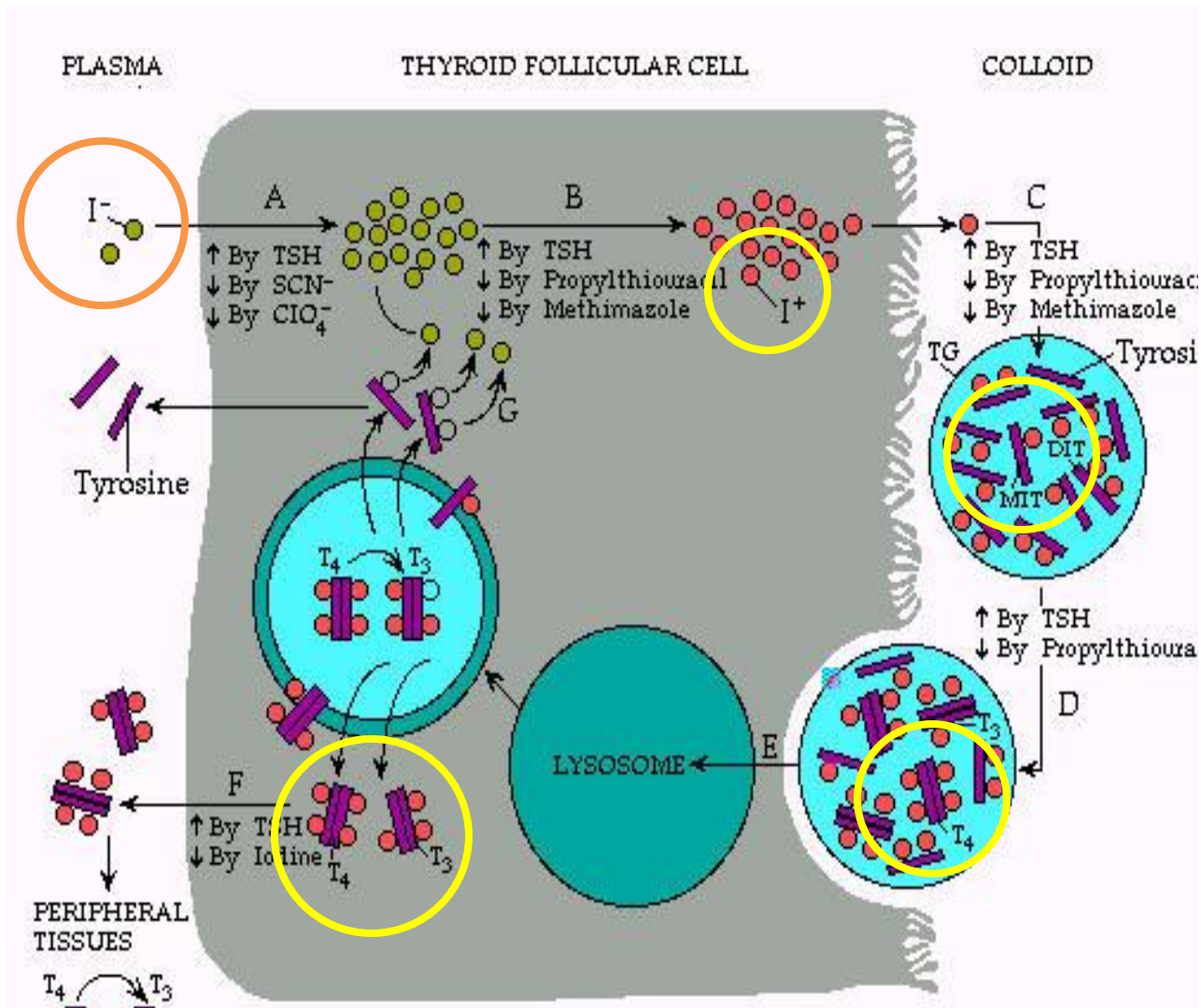
55 Year-Old women with Thyroid Cancer

- Diagnosed with papillary thyroid cancer 15 years before and was treated with thyroidectomy and radiation therapy.
- Treated with thyroid hormone to keep TSH about 0.05-0.1.
- Tg antibody positive and Tg recovery was 35%
- Chest film yearly negative
- Refused RAI scans
- Tg by LC/MS/MS undetectable.

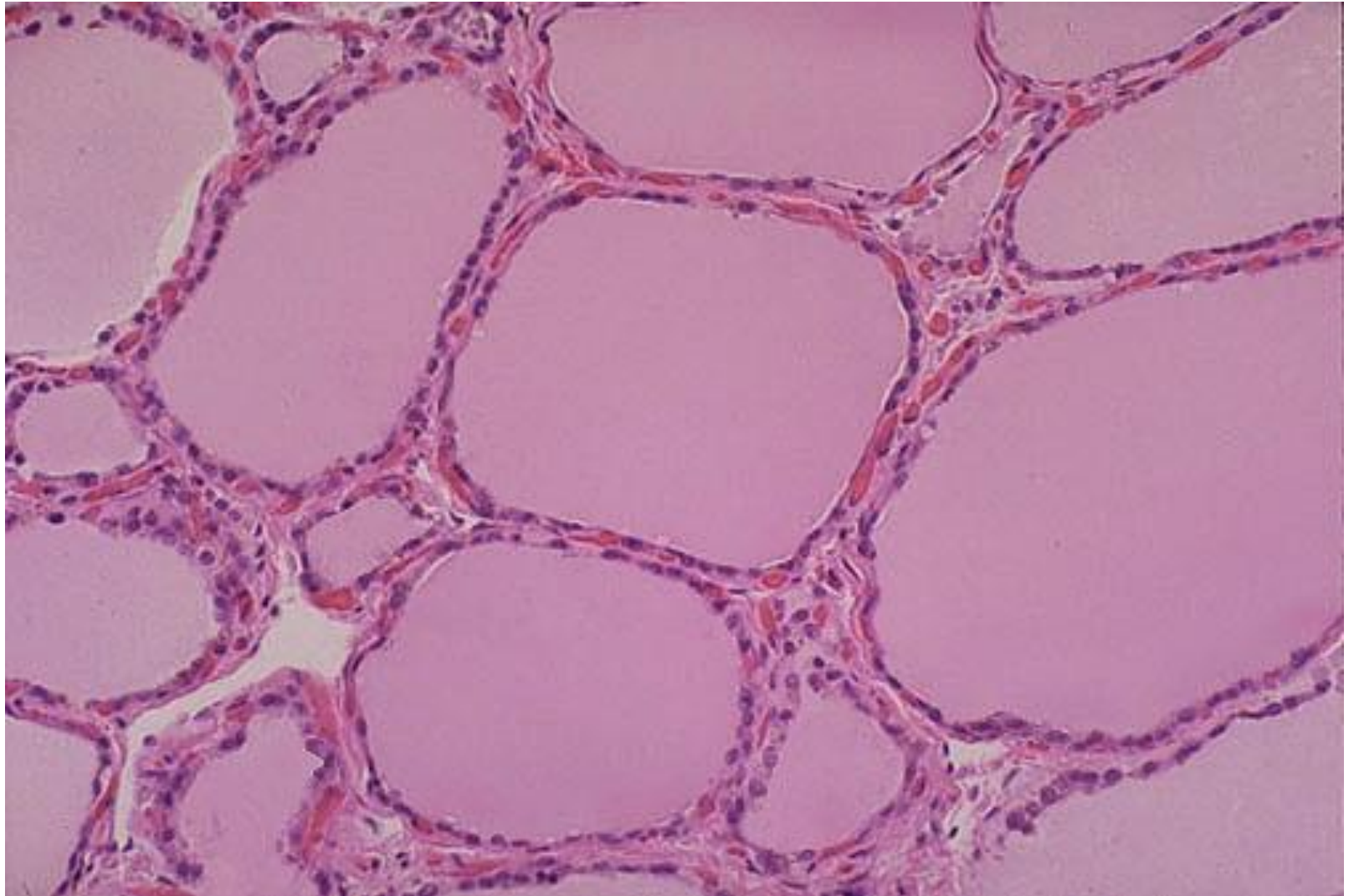
Objectives

- Know how to diagnose thyroid nodules
- Know the main causes of thyroid nodules
- Know risk factors of thyroid cancer
- List your approach to distinguish benign from malignant thyroid nodules.
- What is the role of thyroglobulin measurements in follow up of thyroid cancer?

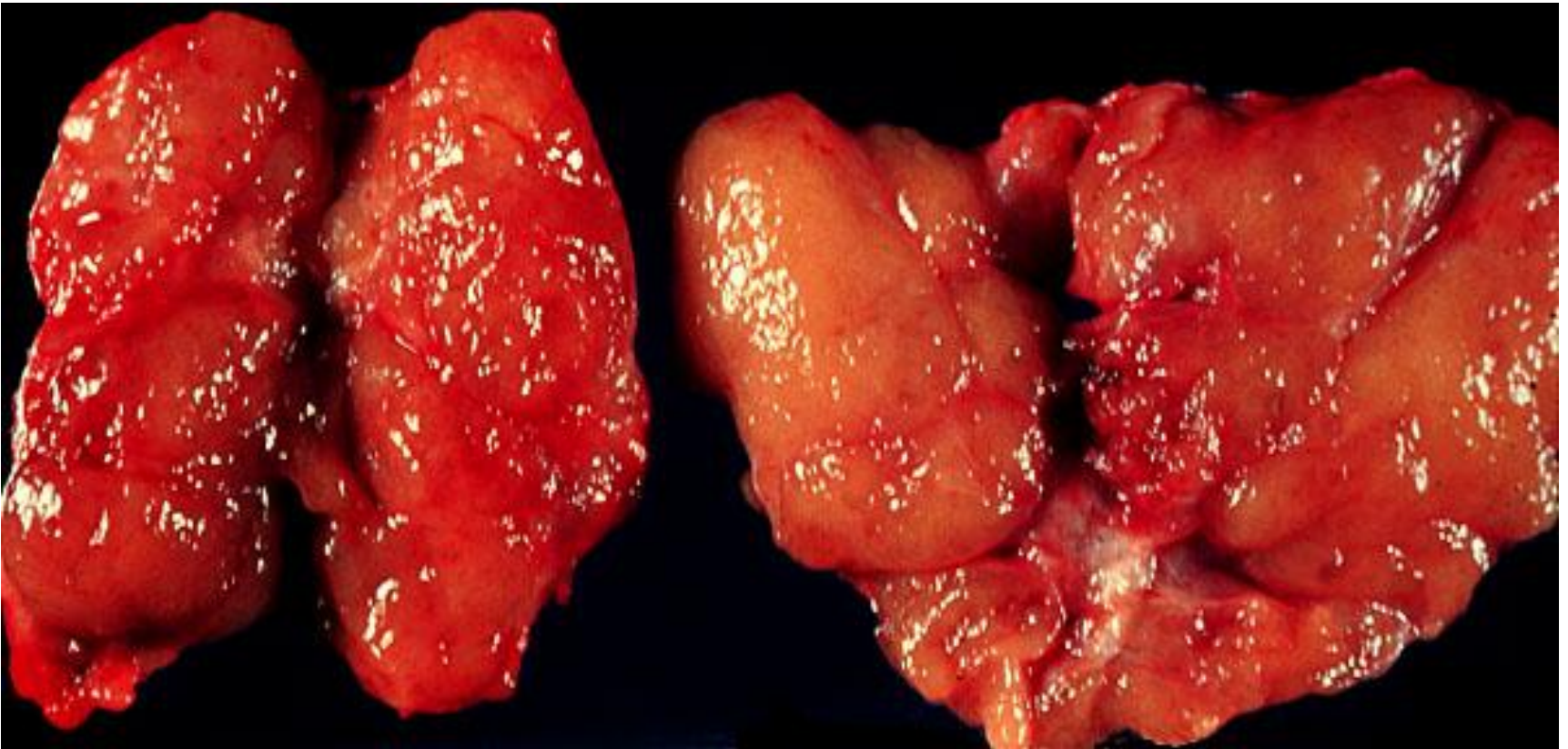
Thyroid Hormone Biosynthesis



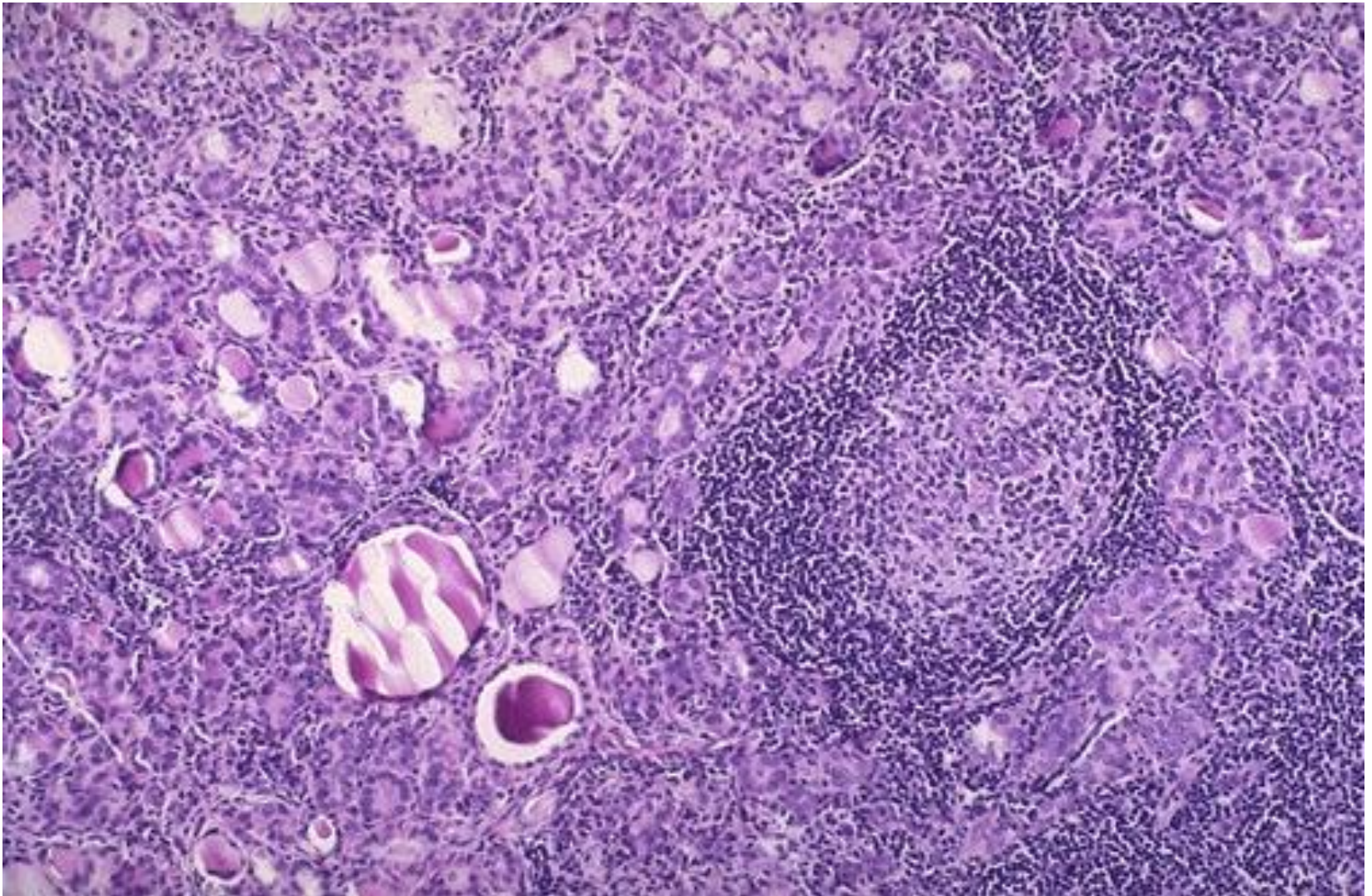
Thyroid



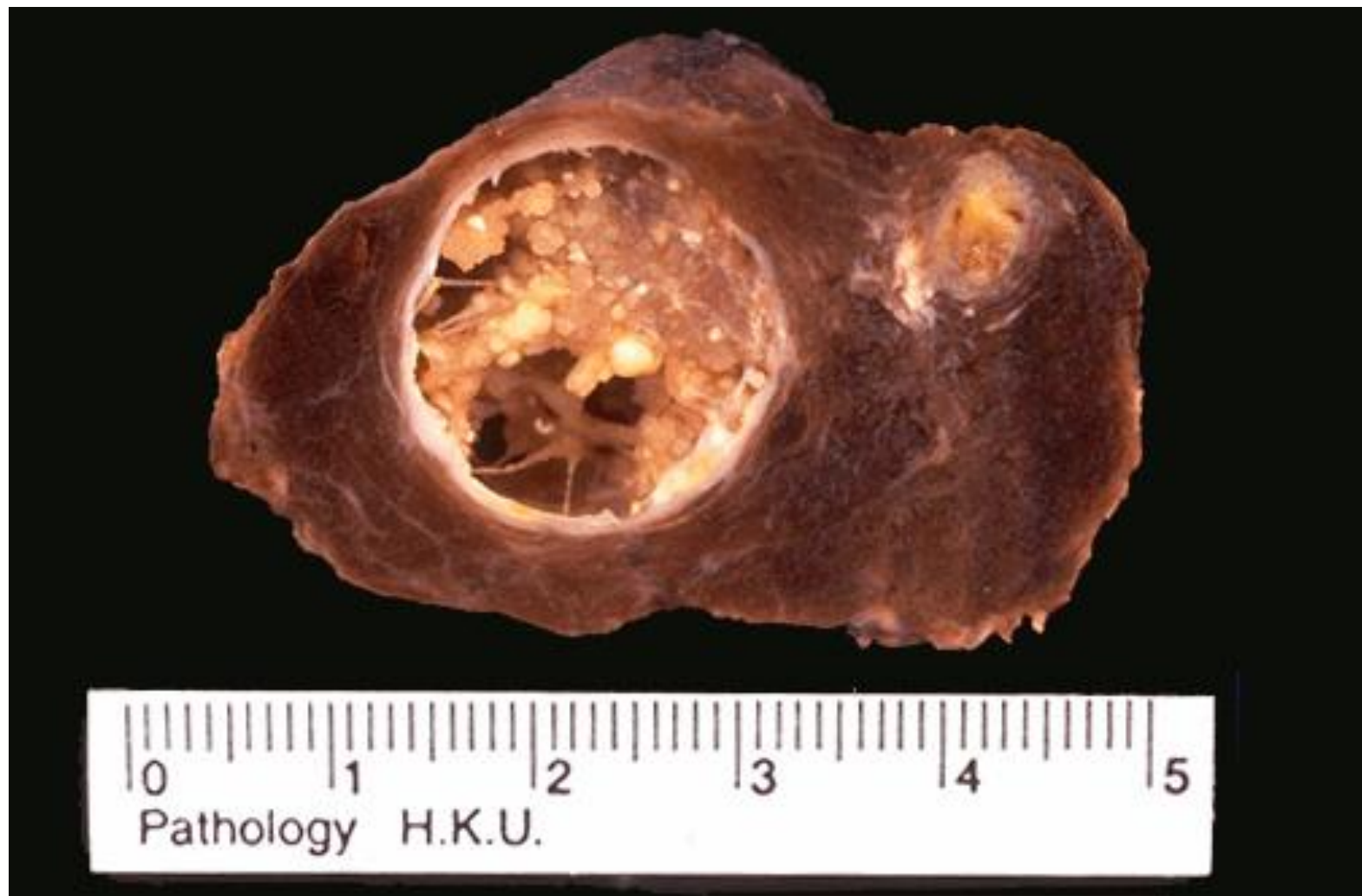
Goiter Thyroiditis



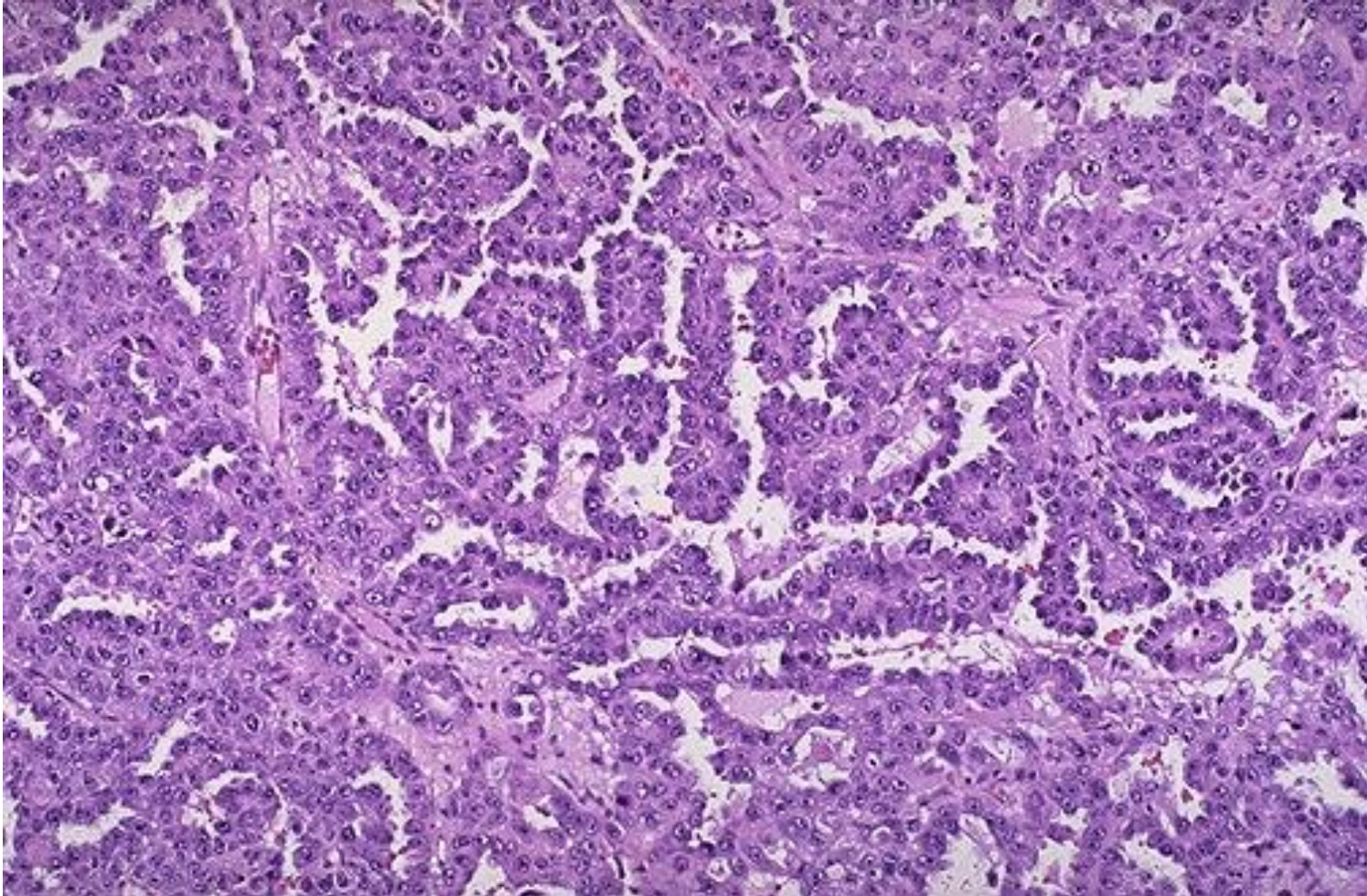
Hashimoto's Thyroiditis



Thyroid Cancer



Papillary Thyroid Cancer



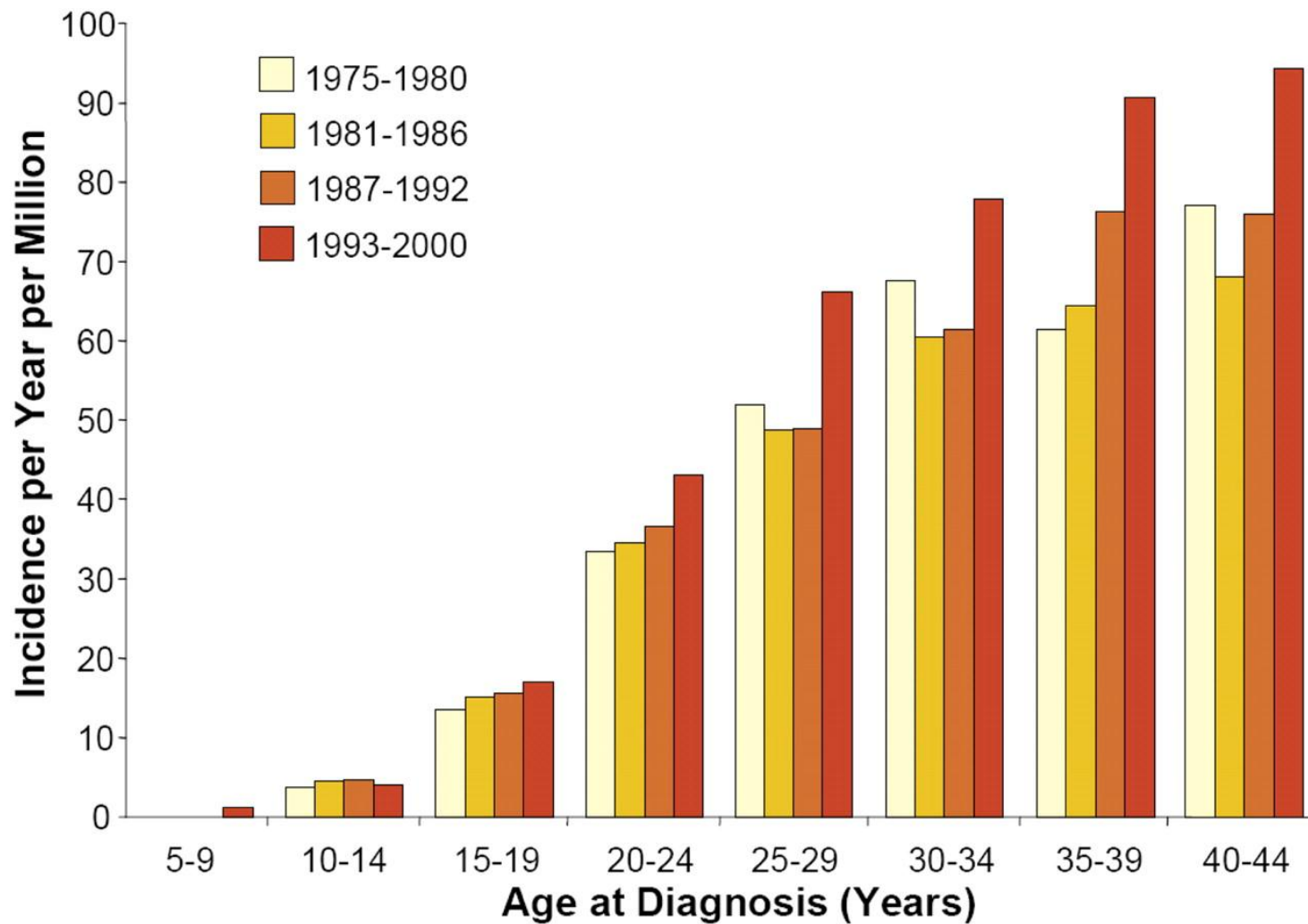
Thyroid Nodules and Cancer

- Epidemiology
- Risk Factors
- Diagnostic Procedures
- Staging
- Treatment
- Follow-up
- Survival

Thyroid Cancer and Nodules

- Thyroid nodules
 - Incidence, 5-8%
 - Thyroid cancer in 5-6.5% of nodules
- Thyroid cancer
 - 0.4% of all cancer deaths

The changing incidence of thyroid cancer as related to age. [Derived from (4).]



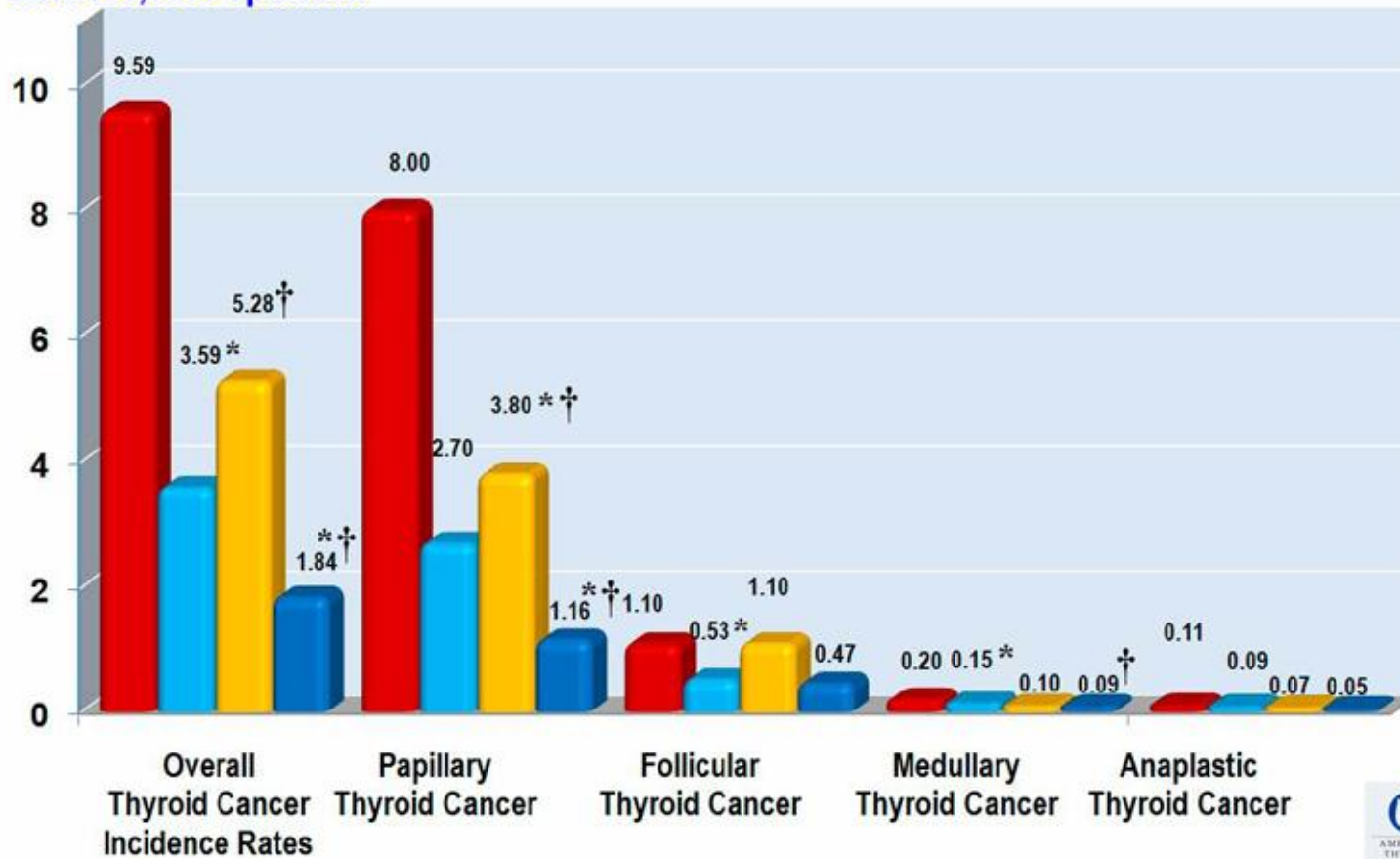
Rivkees S A et al. Endocrine Reviews 2011;32:798-826

ENDOCRINE
REVIEWS

Incidence Rates of Thyroid Cancer in the United States, 1980–2005

■ White Women ■ White Men ■ Black Women ■ Black Men

Rate/100,000 Population



Clinical Thyroidology Volume 21 Issue 4 2009



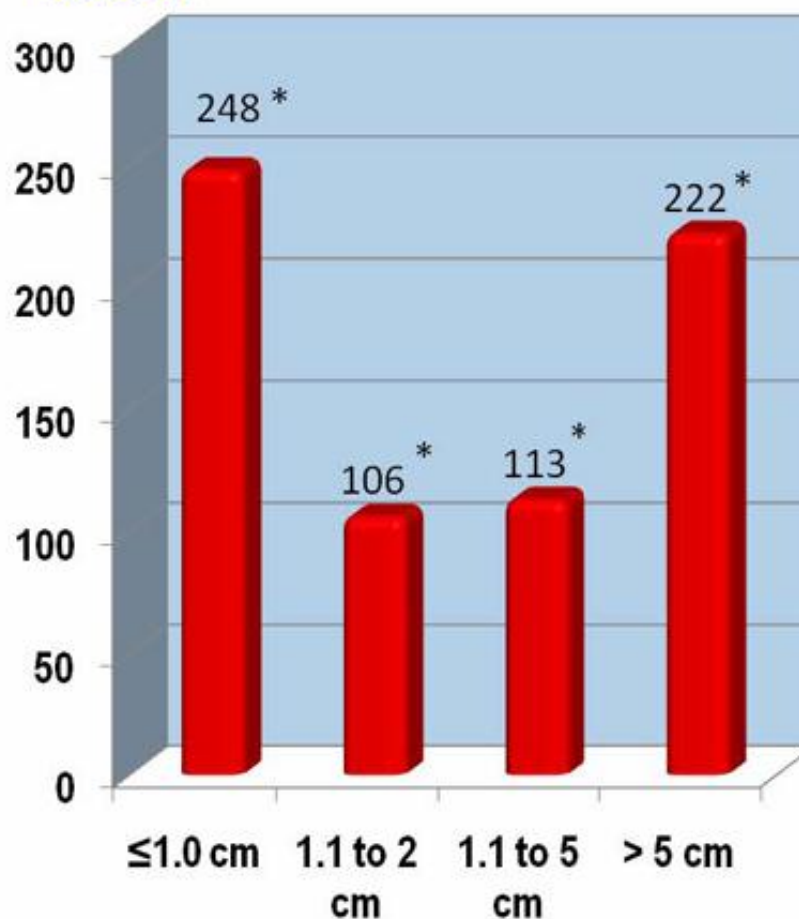
Incidence Rates According to Tumor Size Since 1992-1995

■ Tumor ≤ 1.0 cm ■ 1.1-2.0 cm ■ >2 cm



Increased Incidence According to Tumor Size since 1988–1991 in White Women

Percent

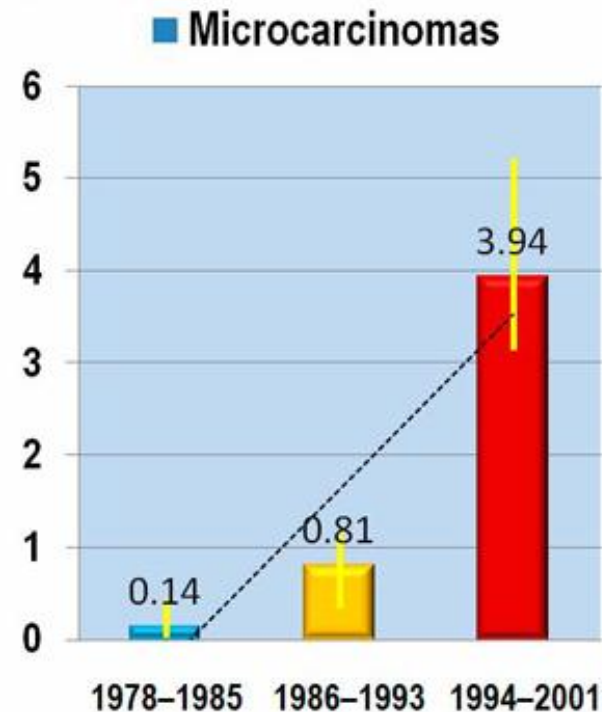
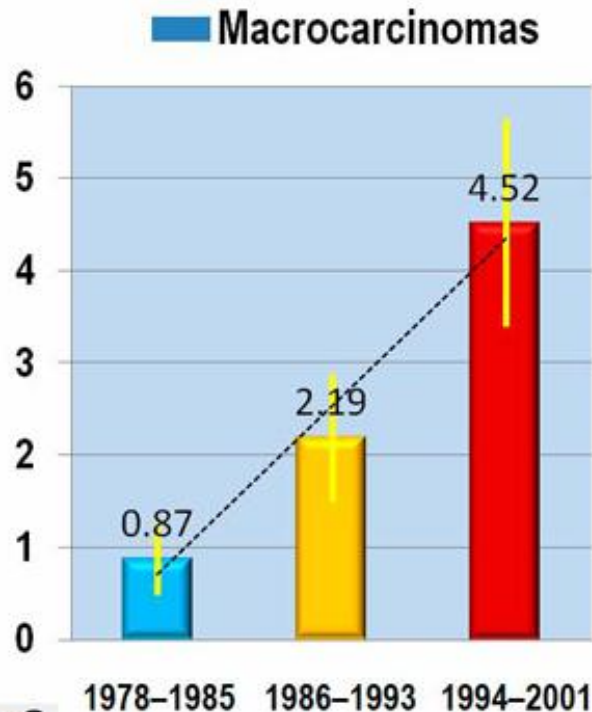


Clinical Thyroidology Volume 21 Issue 3 2009

Enewold et al Clin Thyroidology 21:6-9, 2009

Papillary Thyroid Cancer Incidence Rates of Tumors Larger than 1 cm and Papillary Microcarcinomas in Women

Rates per 100,000 in Women



Clinical Thyroidology Volume 21 Issue 5 2009

Rego-Iraeta et al Clin Thyroidology 21:16-18, 2009

Risks of Thyroid Cancer

- Age
- Children
- Adults: 20-60 years of age

Hereditary

- Medullary
 - Ret oncogene
- Papillary

Radiation Exposure Increases Thyroid Cancer Risk

- External irradiation of head and neck
- Radioactive fallout
- Children particularly at risk
- Screening of exposed persons is recommended vs. routine detection
- Screening includes scintiscans or ultrasound
- FNA Biopsy nodules $>1\text{cm}$ or $<1\text{ cm}$ if changes suggest cancer

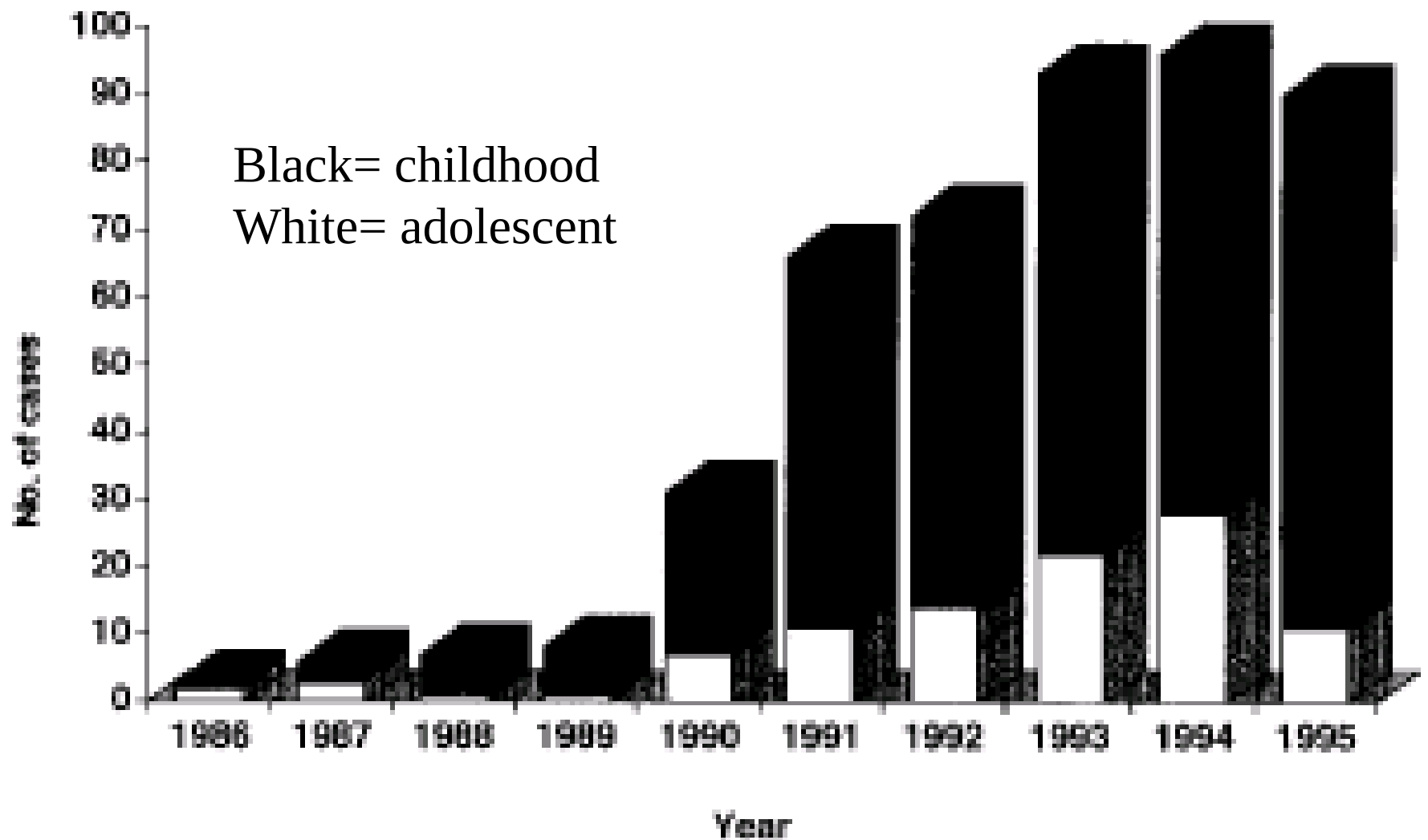


FIG. 2. New cases of childhood (■) and adolescent (□) thyroid carcinoma in Belarus, registered yearly from 1986 to 1995.

Age Distribution Thyroid Cancer Belarus Children

Spitzweg et al,
JCEM 86: 3327-
35, 2001

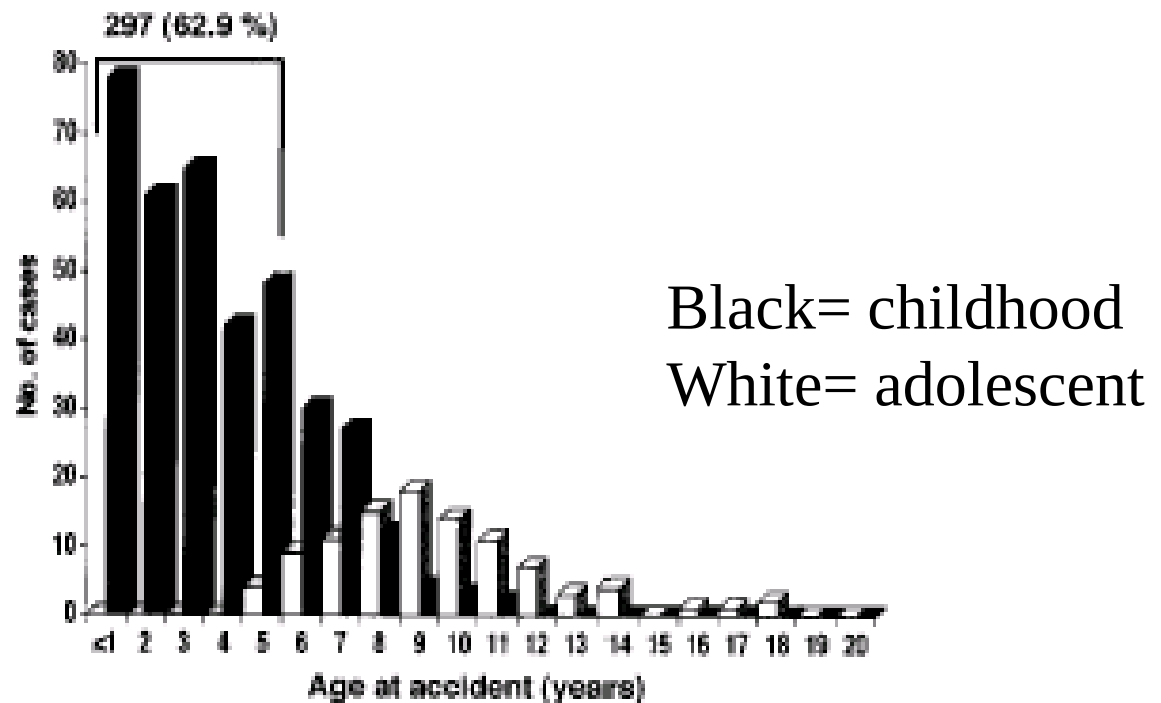


FIG. 3. Age distribution of Belarus thyroid cancer patients at the time of the accident (1986). The *black* column (■) and the *white* column (□) represent patients diagnosed during childhood and during adolescence, respectively. The first column (<1 yr) includes nine patients who were in uterus at the time of the accident. The *square bracket* indicates the total number and percentage of thyroid cancer patients who were 5 yr old or less at the time of the accident.

Oncogenes and Tumor Suppressor Genes in Thyroid Tumors

Neoplasm	Contributory genetic abnormalities	Genetic abnormalities of uncertain importance
Autonomously functioning thyroid nodule	TSH receptor-activating mutation; Gs-alpha mutation decreasing GTPase activity	
Nodular goiter (colloid nodules)	Many nodules are monoclonal, but precise gene abnormalities are unknown	
Follicular adenoma	RAS mutations	c-myc and c-fos overexpressed; PTEN abnormalities
Papillary thyroid carcinoma	RET rearrangements (RET/PTC); NTRK1 rearrangements (TRK)	
Follicular thyroid carcinoma	PAX8-PPARgamma1 fusion	
Follicular thyroid carcinoma	RAS mutations	Frequent loss of heterozygosity in genome of tumor
Anaplastic thyroid carcinoma	P53 mutations	
Medullary thyroid carcinoma	RET activating mutations	

Diagnostic Procedures

- Fine needle aspiration biopsy (FNA)
- Ultrasound
 - Improves FNA diagnostic accuracy, particularly in technically difficult lesions or cystic-solid lesions
- Scintiscan
 - Useful for patients who are hyperthyroid

Detection of Thyroid Nodules: Palpation vs. Ultrasound

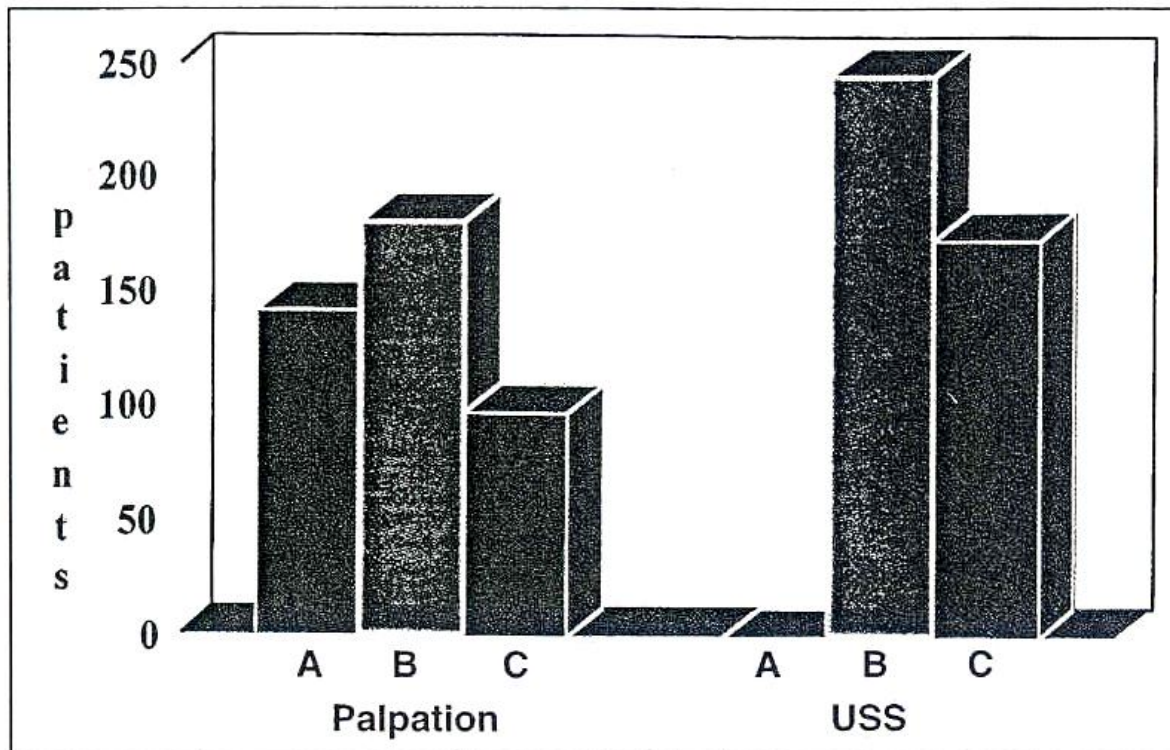


Fig. 1. Comparison between results of clinical palpation and ultrasound scanning (USS) in detection of thyroid nodules in 420 study patients. Patients were subclassified into three categories: with no thyroid nodule (group A), with a single thyroid nodule (group B), or with a multinodular goiter (group C).

Thyroid Incidentalomas

- 1-1.5 cm nodule and risk factors-biopsy
- Cancer detected in 7 of 119 >1 cm
- 5% of 450 nonpalpable nodules have cancer
- Solid, hypoechoic nodules increase likelihood of cancer
- Microcalcification increases risk of cancer to 29%

Thyroid Ultrasound (US)

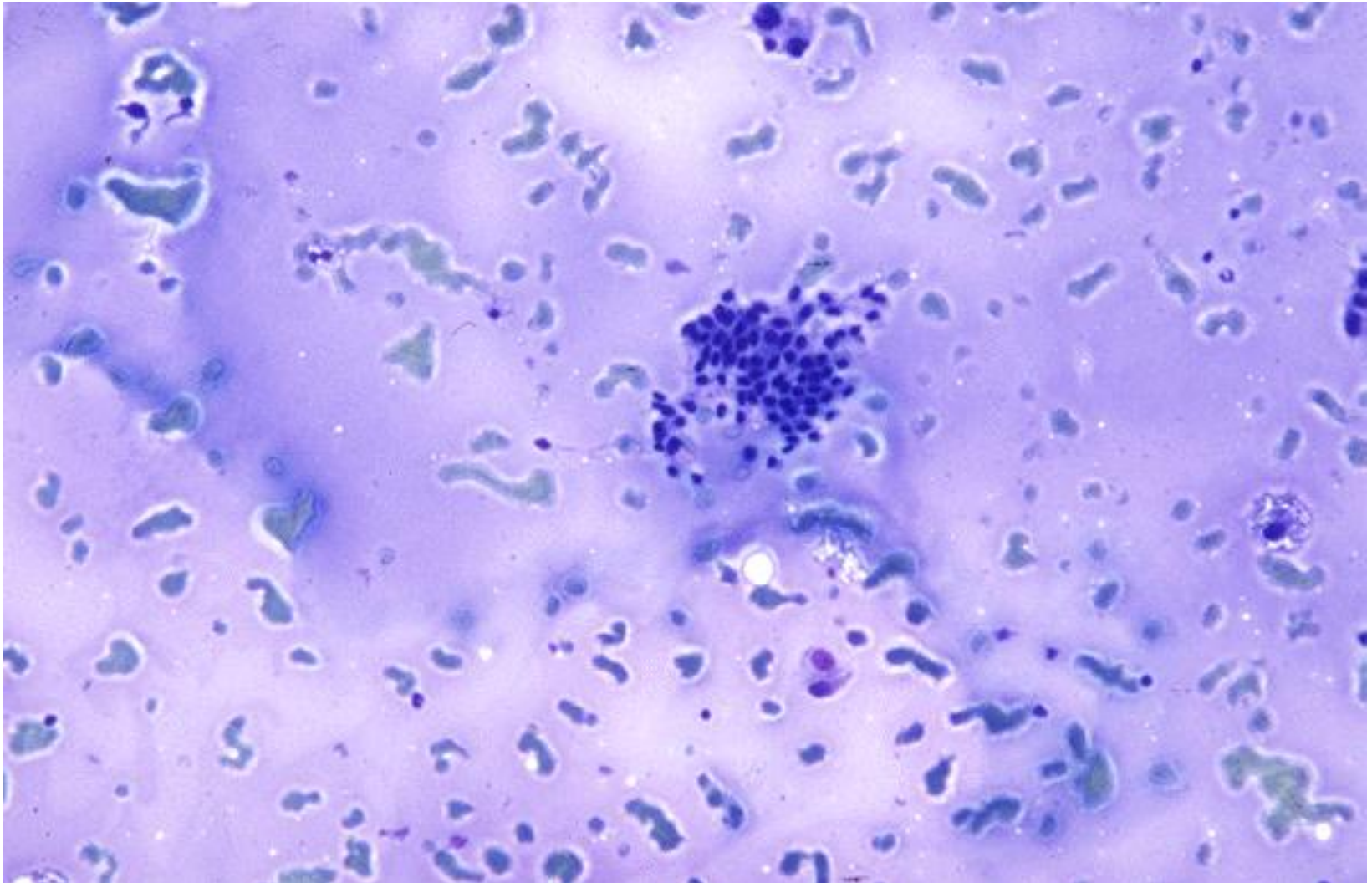
- Sensitivity, 2-3 mm
- Palpable nodule, 20-40% have additional nodules detected by US
- 20% have a nodules <1cm, not recommended for FNA unless hypoechoic or microcalcification is observed
- Nodules <1cm, 30% have non-diagnostic cytology

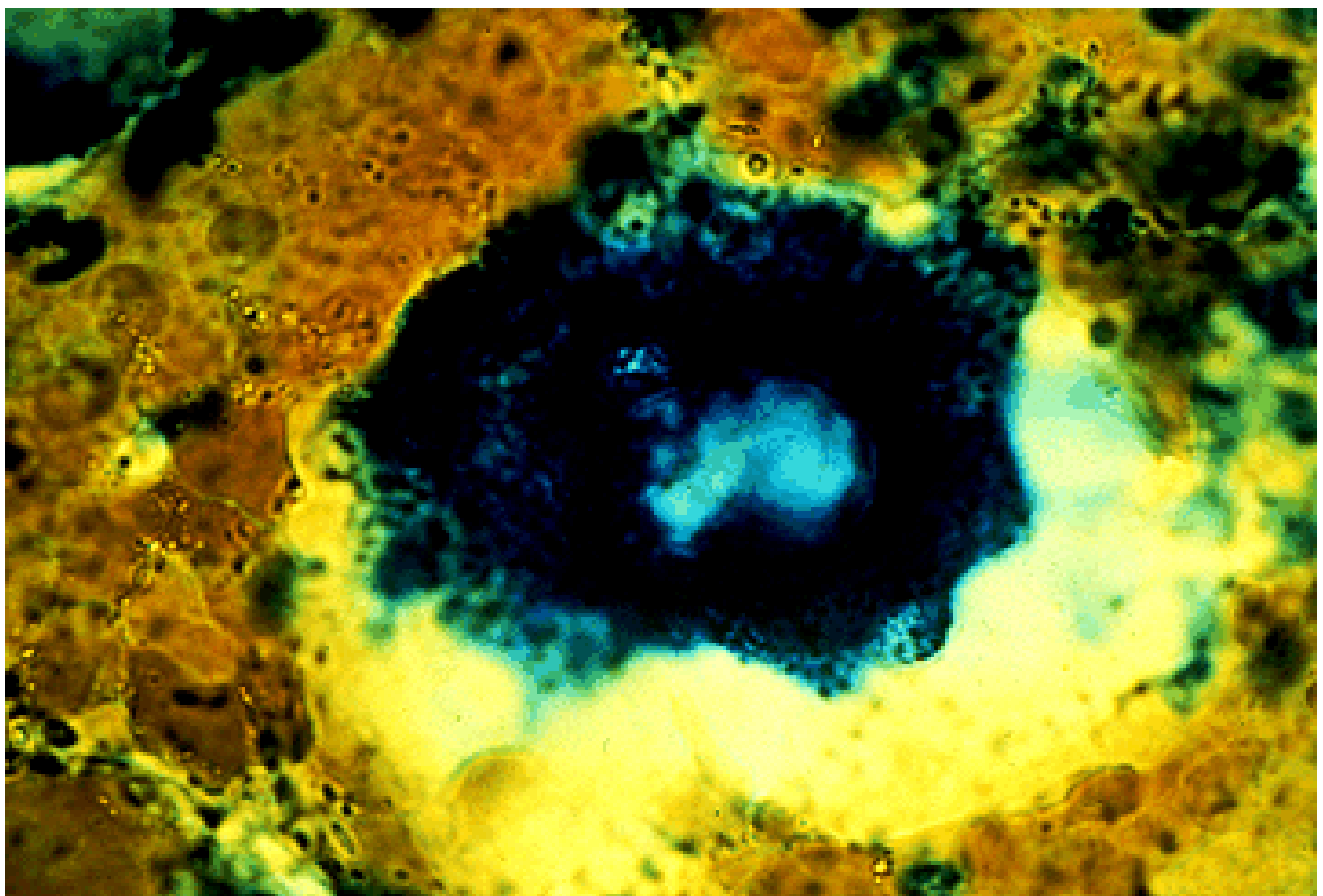
Thyroid Ultrasound's Detection of Nonpalpable Thyroid Nodules



Nonpalpable recurrent papillary thyroid carcinoma Sonogram of the right thyroid lobe in the longitudinal plane from a patient who had had a left lobectomy for papillary thyroid carcinoma. Although the physical examination was normal, the sonogram shows a 8.6 mm hypoechoic nodule (arrow) that represented tumor. L = thyroid lobe. Courtesy of Manfred Blum, MD.

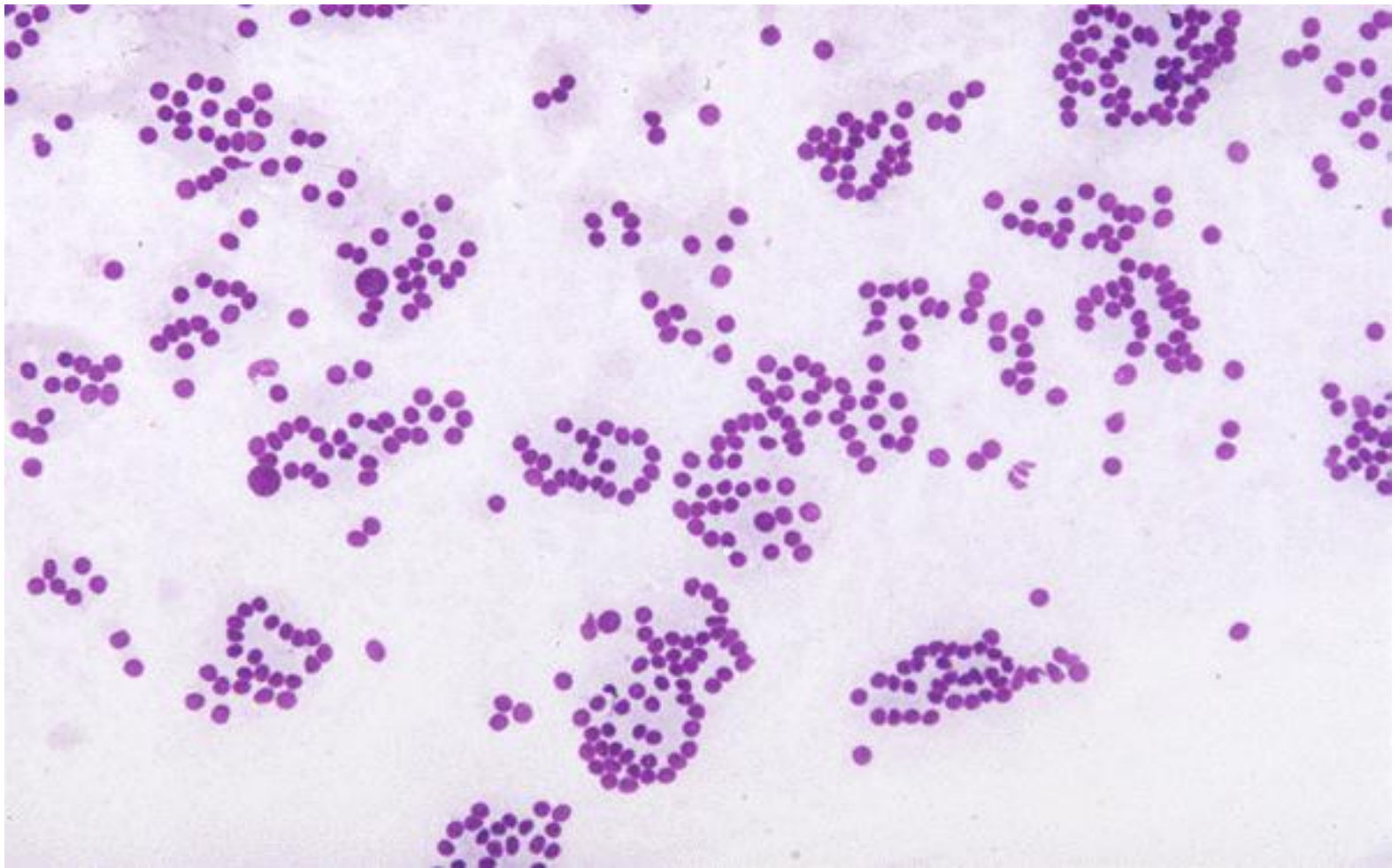
Benign Thyroid Epithelium





Psammoma body Fine needle aspirate of a papillary carcinoma of the thyroid showing a psammoma body. The laminations can best be appreciated under the microscope by moving the depth of focus.

Follicular Epithelial Pattern, Adenoma



FNA Cytologic Results

- Nondiagnostic
 - Benign
 - Macrofollicular
 - Colloid adenomas
 - Thyroiditis
 - Suspicious or indeterminate
 - Microfollicular or follicular neoplasms
 - Malignant
- Repeat with US guidance
 - Follow
 - Surgical excision
 - Total thyroidectomy, RAI, T4
 - Follow thyroglobulin

Original Article

Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

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Edmund S. Cibas, M.D., Darya Chudova, Ph.D., James Diggans, Ph.D., Lyssa
Friedman, R.N., M.P.A., Richard T. Kloos, M.D., Virginia A. LiVolsi, M.D., Susan J.
Mandel, M.D., M.P.H., Stephen S. Raab, M.D., Juan Rosai, M.D., David L.
Steward, M.D., P. Sean Walsh, M.P.H., Jonathan I. Wilde, Ph.D., Martha A.
Zeiger, M.D., Richard B. Lanman, M.D., and Bryan R. Haugen, M.D.

N Engl J Med
Volume 367(8):705-715
August 23, 2012



The NEW ENGLAND
JOURNAL of MEDICINE

Study Overview

- A significant fraction of fine-needle aspirates obtained from thyroid nodules are read as indeterminate.
- A new molecular test accurately predicts whether a cytologically indeterminate nodule is benign 93% of the time, permitting a conservative approach to management.



Indeterminate Cytology (Veracyte)

- 15-30% FNAs indeterminate
- Expression 167 genes
- 85/265 indeterminate malignant
- 78/85 gene-expression classifier correctly identified malignancy
- Sensitivity 92% (CL 84-97%)
- Negative predictive value, 85-95%

Conclusions

- These data suggest consideration of a more conservative approach for most patients with thyroid nodules that are cytologically indeterminate on fine-needle aspiration and benign according to gene-expression classifier results.



TABLE 1. Cytological and molecular results of ultrasonographic guided FNAB for the patients with thyroid nodules

Cytological diagnoses	BRAF^{V600E} (+)	BRAF^{V600E} (–)	Total
Benign	0 (0%)	504 (100%)	504 (58.5%)
ACUS	45 (31.9%)	96 (68.1%)	141 (16.3%)
Suspicious for FN	1 (10%)	9 (90%)	10 (1.2%)
Suspicious for malignancy	46 (85.2%)	8 (14.8%)	54 (6.2%)
Malignant	129 (92.1%)	11 (7.9%)	140 (16.2%)
Nondiagnostic	NA	NA	16 (1.8%)
Total	221 (25.5%)	644 (74.5%)	865 (100%)

FN. Follicular neoplasm: NA. not available.

ACUS=atypical cells of undetermined significance

Kim et al JCEM 96: 658-64, 2011

TABLE 2. Comparison of two diagnostic tests for detecting thyroid malignancy in thyroid nodules

	Cytology	BRAF^{V600E} mutation	Cytology and BRAF^{V600E} mutation
Sensitivity	100	89.6	89.6
Specificity	36.4	95.5	95.5
PPV	92.9	99.4	99.4
NPV	100	52.5	52.5
Accuracy	93.3	90.2	90.2
κ value	0.51 $\pm$ 0.11	0.63 $\pm$ 0.07	0.63 $\pm$ 0.07

The permanent pathological diagnosis was used as a reference test to calculate each parameter.

Kim et al JCEM 96: 658-64, 2011

Approximately 5% of Siblings of patients are also Diagnosed with Thyroid Cancer

TABLE 2. STAGE OF THE TUMORS DISCOVERED BY ULTRASONOGRAPHIC SCREENING
OF SIBLINGS OF PATIENTS WITH PAPILLARY THYROID CANCER

	<i>T1a</i> (<i>tumor</i> ≤ 1 cm)	<i>T1b</i> (<i>tumor</i> > 1 cm and ≤ 2 cm)	<i>T3</i> (<i>tumor</i> with extrathyroid invasion)	<i>Total</i>
Nx (apparently no lymph nodes affected) ^a	21	6	6	33
N1a (central cervical metastases)	4	2	3	9
N1b (lateral cervical metastases)	0	1	0	1
Total	25	9	9	43

^aLymph node dissection was only performed in the case of a pre- or perioperative suspicion of metastasis, not prophylactically.

Rosario et al Thyroid 22: 805-8, 2012

Thyroid Cancer Staging

TNM Classification System for Differentiated Thyroid Carcinoma

Definition

T1	Tumor diameter <1 cm
T2	Primary tumor diameter 1–4 cm
T3	Primary tumor diameter >4 cm
T4	Primary tumor invasion beyond the thyroid gland capsule
TX	Primary tumor size unknown, but without extrathyroidal invasion
N0	No metastatic nodes
N1a	Ipsilateral cervical node metastases
N2b	Bilateral, midline, contralateral, or mediastinal node metastases
NX	Nodes not assessed at surgery
M0	No distant metastases
M1	Distant metastases
MX	Distant metastases not assessed

Stages

	Patient age <45 years	Patient age 45 years or older
Stage I	Any T, any N, M0	T1, N0, M0
Stage II	Any T, any N, M1	T2, N0, M0 T3, N0, M0
Stage III		T4, N0, M0 Any T, N1, M0
Stage IV		Any T, any N, M1

Screening compared to Routine Detection of Thyroid Cancer

- High risk populations
 - Radiation fallout
 - Head and neck irradiation
 - Family History

Prognosis: Recurrence & Cancer Death

Treatment and Management of Thyroid Cancer

- Treatment
 - Surgery
 - Radioiodine
 - TSH-suppressive thyroid hormone therapy
- Monitoring
 - Serum thyroglobulin measurement
 - Whole-body ^{131}I scanning

Fuchshuber et al: *Oncology*. 1998;12:99–106.
Schlumberger et al: *N Engl J Med*. 1998;338:297–306.

Mazzaferri: *Am J Med*. 1994;97:418–428.

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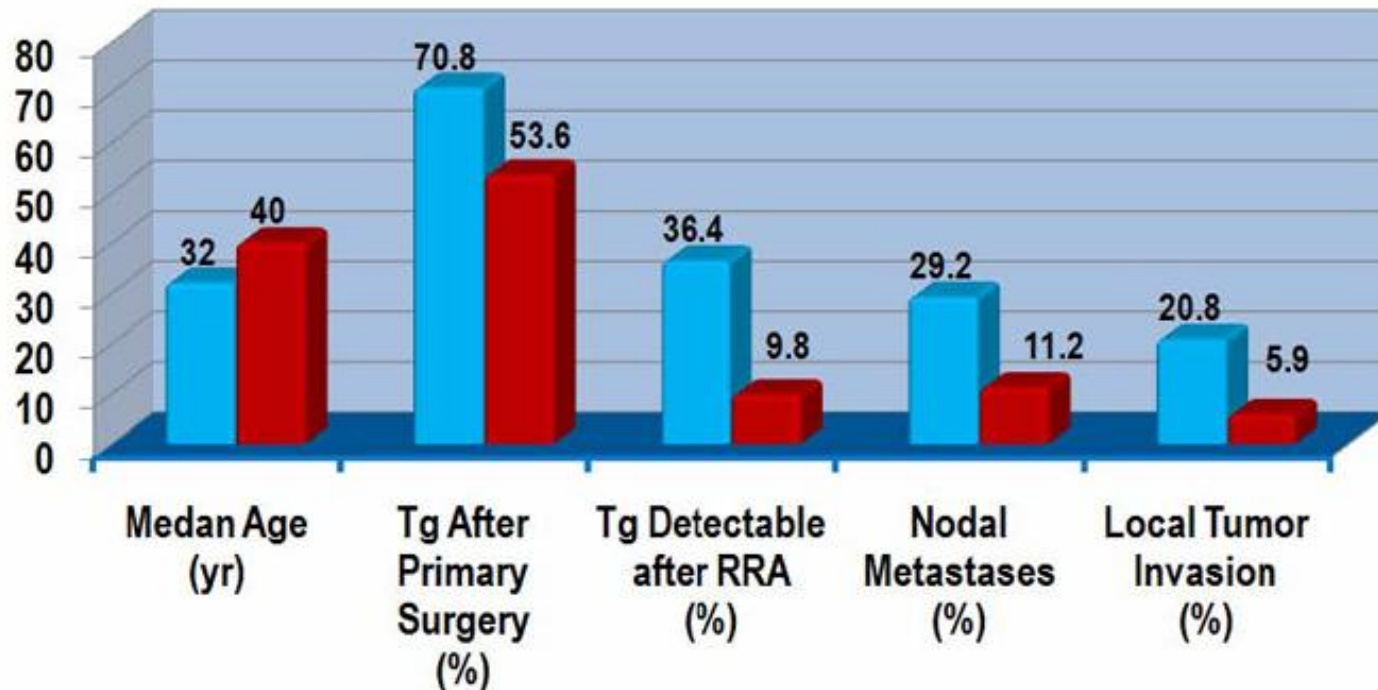
Initial American Thyroid Association risk of recurrence classification

Low risk	Intermediate risk	High risk
All of the following are present:	Any of the following is present:	Any of the following is present:
No local or distant metastases	Microscopic invasion into the perithyroidal soft tissues	Macroscopic tumor invasion
All macroscopic tumor has been resected	Cervical lymph node metastases or ¹³¹ I uptake outside the thyroid bed on the post-treatment scan done after thyroid remnant ablation	Incomplete tumor resection with gross residual disease
No invasion of locoregional tissues	Tumor with aggressive histology or vascular invasion (eg, tall cell, insular, columnar cell carcinoma, Hurthle cell carcinoma, follicular thyroid cancer)	Distant metastases
Tumor does not have aggressive histology (eg, tall cell, insular, columnar cell carcinoma, Hurthle cell carcinoma, follicular thyroid cancer)		
No vascular invasion		
No ¹³¹ I uptake outside the thyroid bed on the post-treatment scan, if done		

Reproduced with permission from: Tuttle RM, Tala H, Shah J, et al. Estimating risk of recurrence in differentiated thyroid cancer after total thyroidectomy and radioactive iodine remnant ablation: using response to therapy variables to modify the initial risk estimates predicted by the new American Thyroid Association staging system. *Thyroid* 2010; 20:1341. Copyright © 2010 Mary Ann Liebert, Inc.

Logistic Analysis of Predictive Factors for Disease Recurrence

■ With Recurrence (N = 48) ■ Without Recurrence (N = 420)

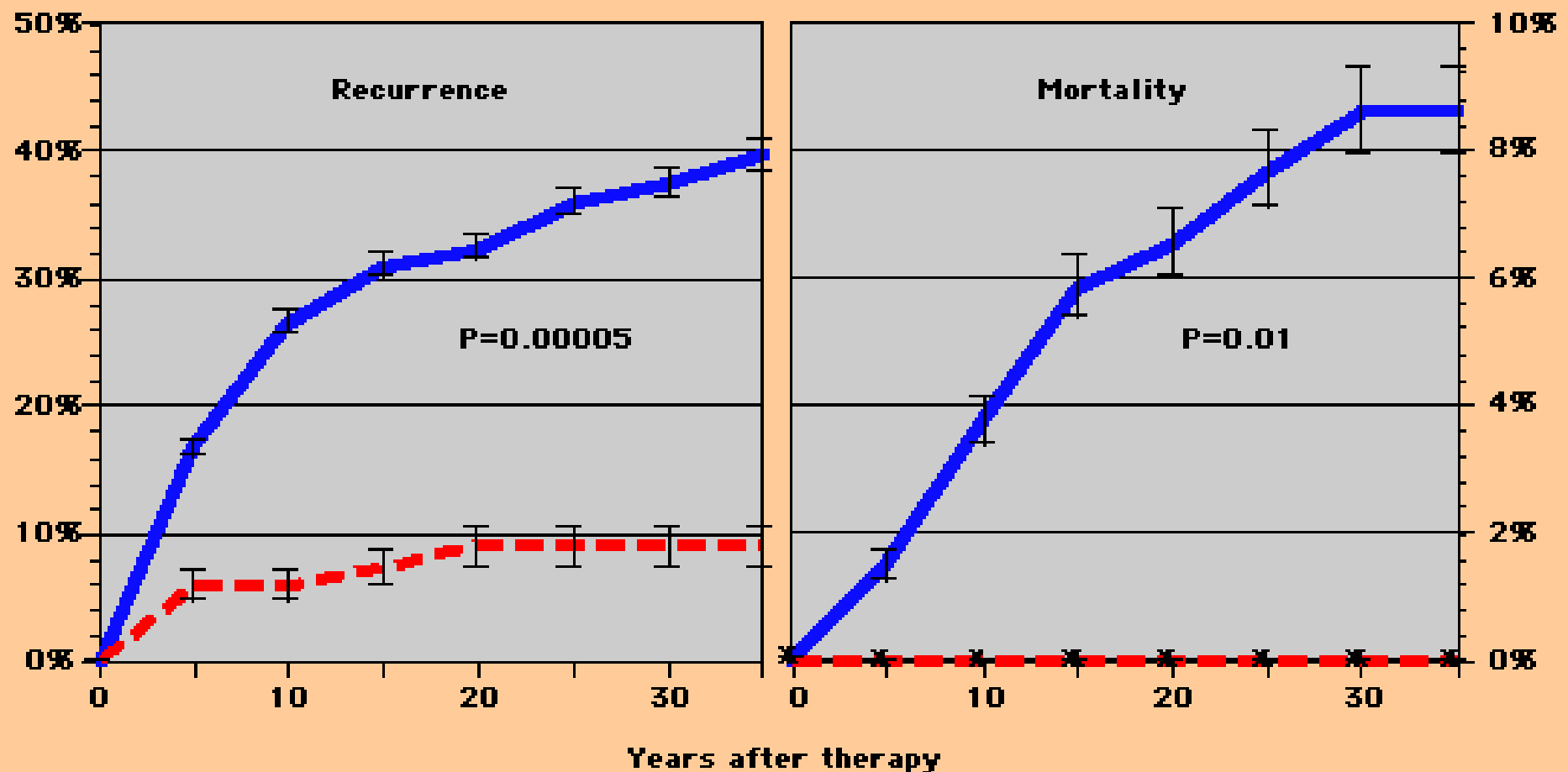


Simple Logistic Analysis of Predictive Factors for Disease Recurrence



Clinical Thyroidology Volume 22 Issue 10 2010

Peltari et al Clin Thyroidology 21:11-13, 2009



Radioiodine ablation reduces recurrence and mortality in stage II and stage III thyroid cancer Long-term development of recurrent disease (left panel) or death (right panel) from thyroid cancer in patients without distant metastases at presentation, who received either ^{131}I ablation (red dashed lines) or no ablation (blue solid lines). (Data from Mazzaferri, EL, Jhiang, SM, Am J Med 1994; 97:418.)

Clinicopathologic Factors Correlated with TgAb Levels at 6 to 12 Mo

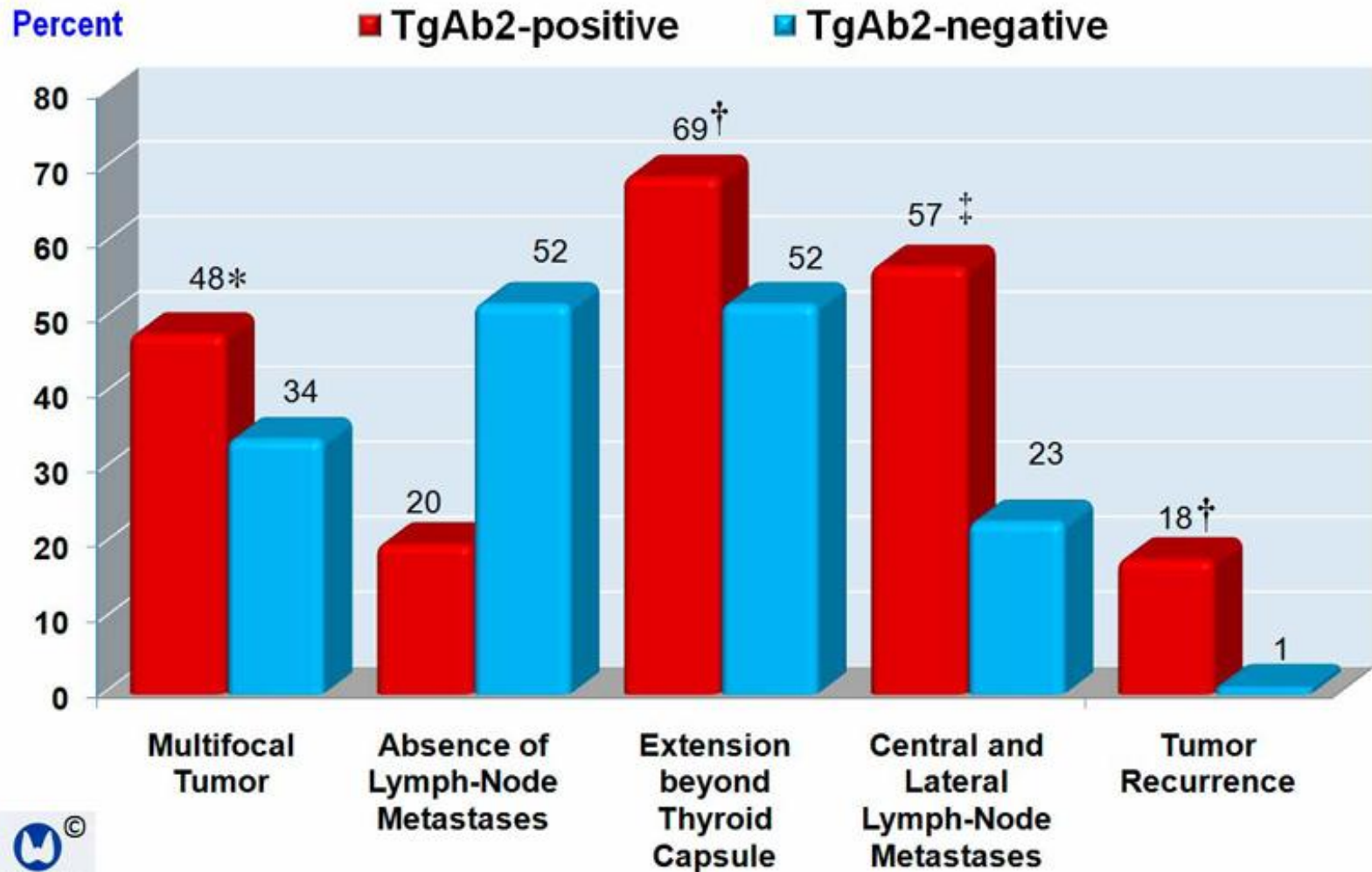


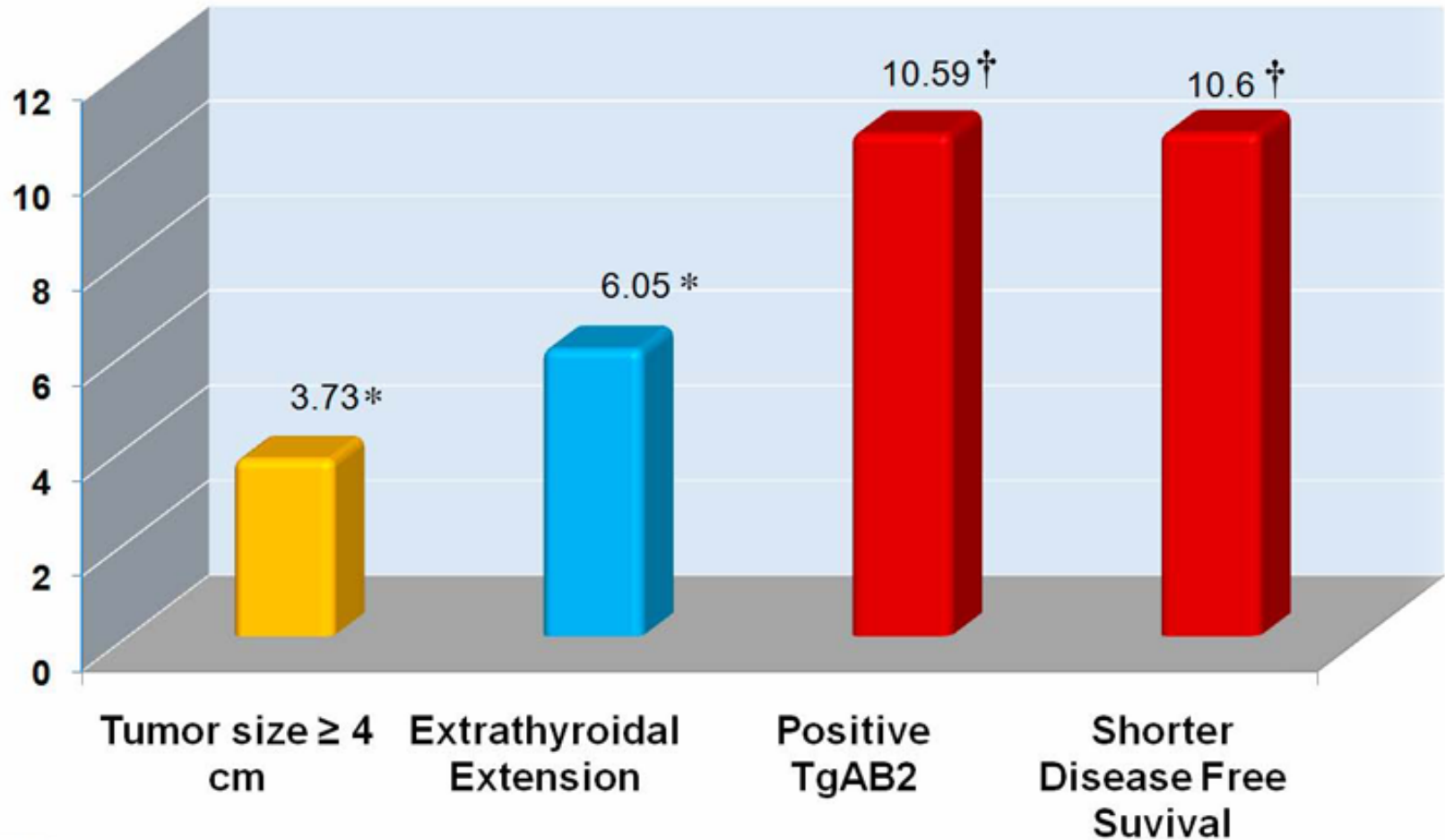
Fig. 1



Clinical Thyroidology Volume 21 Issue 1 2009

Clinopathologic Factors Associated with Recurrent Disease

Hazard Ratio (HR)

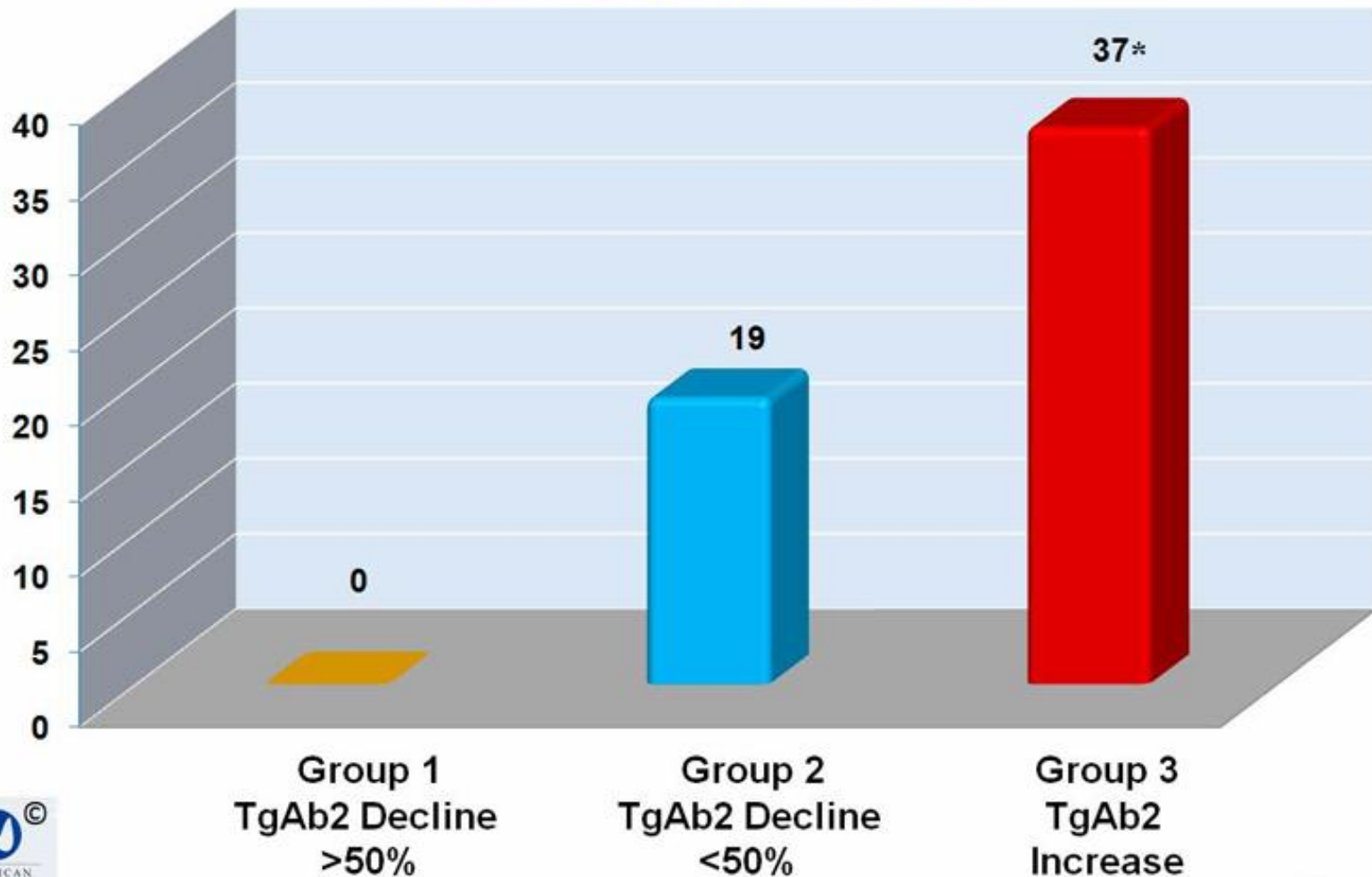


Clinical Thyroidology Volume 21 Issue 1 2009

Fig. 3

The Association of Recurrent/Persistent Disease with Changing TgAb2 Values

Percent with persistent disease



Clinical Thyroidology Volume 21 Issue 1 2009

Fig. 4

Recombinant Human TSH Use in Treatment of Thyroid Cancer

- rh-TSH can be used in patients on thyroid hormone
- Tg response can be measured
- Radioiodine therapy can be administered following its stimulation of thyroid tissue
- Are the results as good as with withdrawal of thyroid hormone and RAI therapy?

Thyroglobulin (Tg) as a Tumor Marker

- Post thyroidectomy and radioiodine therapy serum Tg should be unmeasurable
- Thyroid hormone therapy will reduce levels in hormone responsive tissue, benign or cancerous
- Less well differentiated thyroid cancer may not produce Tg
- Antithyroglobulin antibodies interfere with Tg measurements by IA and results must be interpreted with caution and may be unreliable

Main Points of Thyroid Cancer Treatment

- Early treatment is critical to outcome
- Main location of disease: neck and lung
- Best detected by Tg under TSH stimulation
 - T4 withdrawal Tg > 10 ng/ml
 - rh-TSH Tg > 2 ng/ml
- Follow up whole body scan less useful
- Post-therapy (high dose) whole body scans are best

Thyroid Hormone's Effect on Serum Thyroglobulin

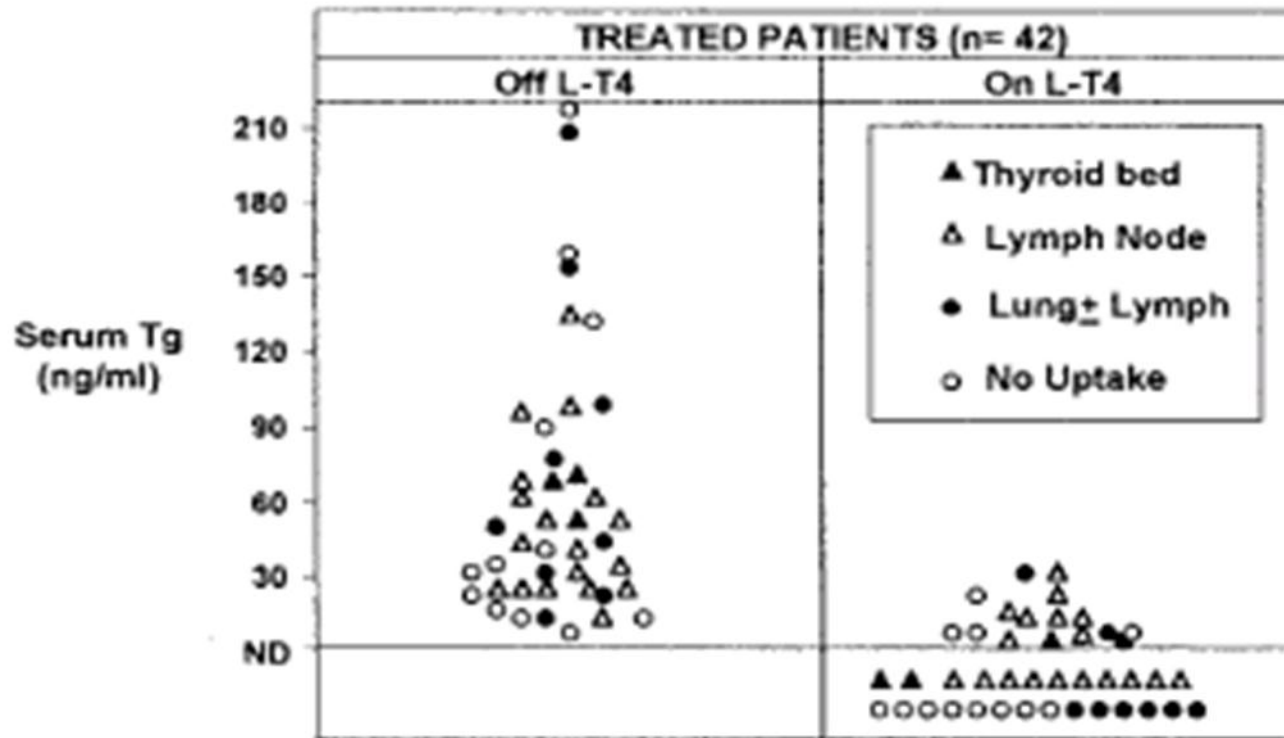


FIG. 2. Relationship between the results of the ^{131}I posttherapy WBS and the individual values of serum Tg both off and on L-T₄ before therapy. N.D., Not detectable.

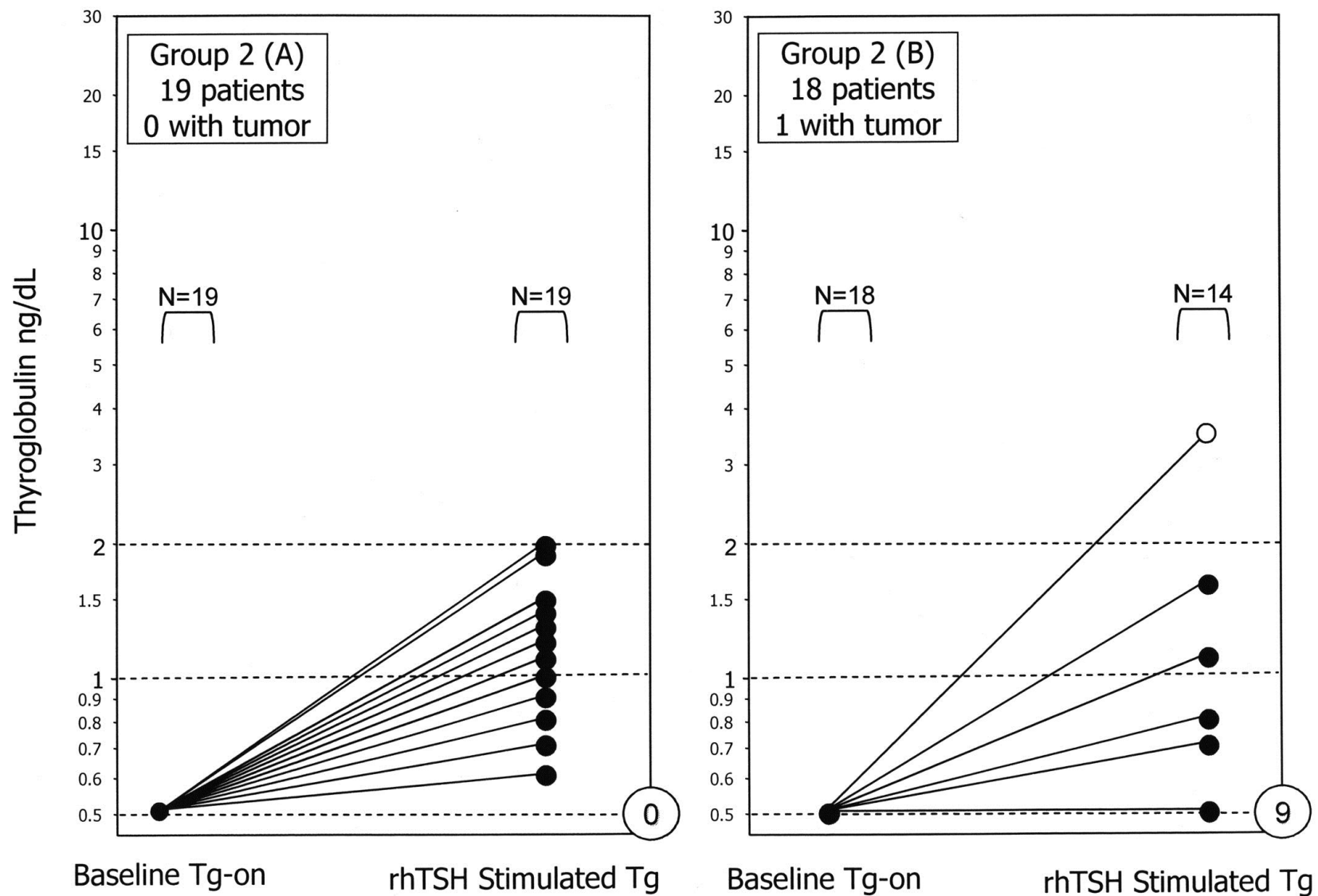


Figure 2

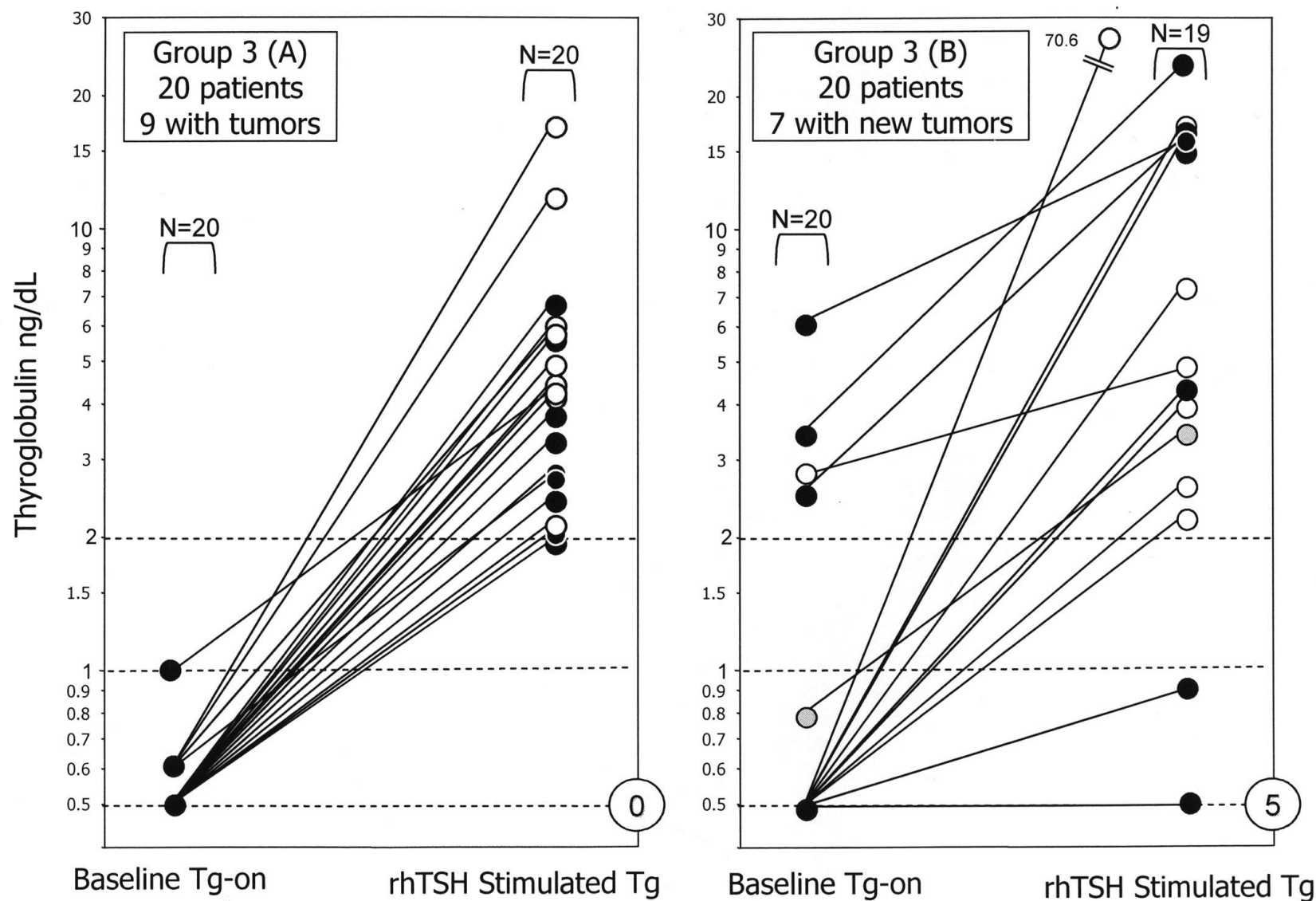


Figure 3

Positive Test rhTSH Tg>2 ng/mL

During First rhTSH Study

	Tumor Positive N=18	Tumor Negative N=89	
Tg>2 N=20	16 TP	4 FP	PPV 80%
Tg≤2 N=87	2† FN	85 TN	NPV 98%
	Sensitivity Specificity		
	89% 96%		

Positive Test LT4 Tg>0.5 ng/mL

During First rhTSH Study

	Tumor Positive N=18	Tumor Negative N=89	
Tg>0.5 N=5	4 TP	1 FP	PPV 80%
Tg≤0.5 N=102	14† FN	88 TN	NPV 86%
	Sensitivity Specificity		
	22% 99%		

Figure 4

Kloos et al JCEM 90:5047-57, 2005

Summary

- Thyroid nodules are common
 - About 10% > 1 cm are malignant
- Thyroid cancer is more common in women
- Thyroid FNA and Ultrasound are most useful in diagnosis
- Staging correlates with prognosis
- Early treatment reduces risk of recurrence and death

Summary (continued)

- Tg measurement is highly useful in follow up for recurrence, particularly after rh-TSH or withdrawal from thyroid hormone
- Triple therapy
 - Includes near total thyroidectomy, RAI and TSH suppression with T4
 - greatly reduces recurrence rates and death.

Triple Antibody Tg Assay

- Antisera to three epitopes was produced
- Rationale binding to epitope site not used by AntiTgAb
- Assay compared well with other Tg assays
- Did not overcome the AntiTgAb interference

Quantitative Real Time RT-PCR Assay for the Detection of Thyroid Specific mRNAs

Cindy Meadows
Supervisor Molecular Hematopathology & Genetics
ARUP Laboratories

Thyroglobulin and thyroid cancer: part 2 of presentation: analytical method and performance

Alan L. Rockwood, Mark M. Kushnir, A.
Wayne Meikle

Pathology Grand Rounds

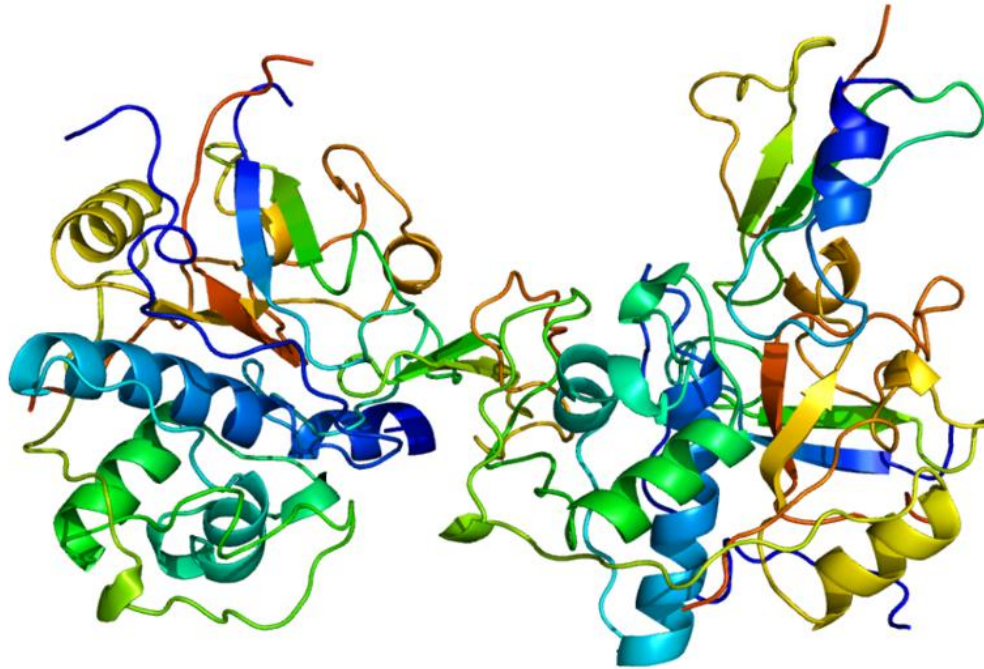
University of Utah

16 November 2012

Learning objectives for part 2

- Relevant concepts of tandem mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS)
- Use of peptide generated by tryptic digestion of thyroglobulin as surrogate for Thyroglobulin analysis (SISCAPA technique)
- Application of these principles to analysis of thyroglobulin

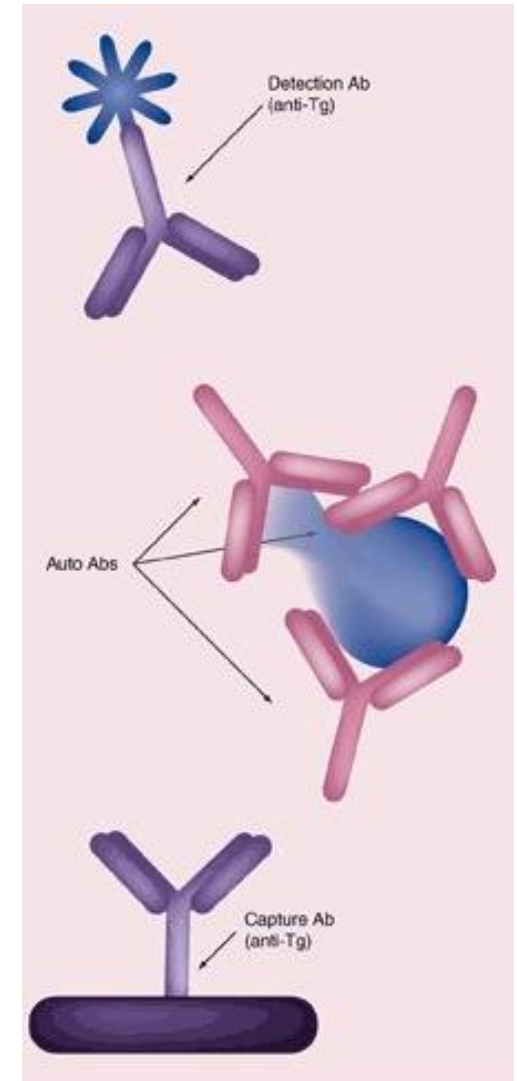
Thyroglobulin



- Thyroglobulin is dimer-protein
- Molecular weight: 660,000
- Thyroglobulin synthesized only in thyroid
- 19 epitopes for antibody binding

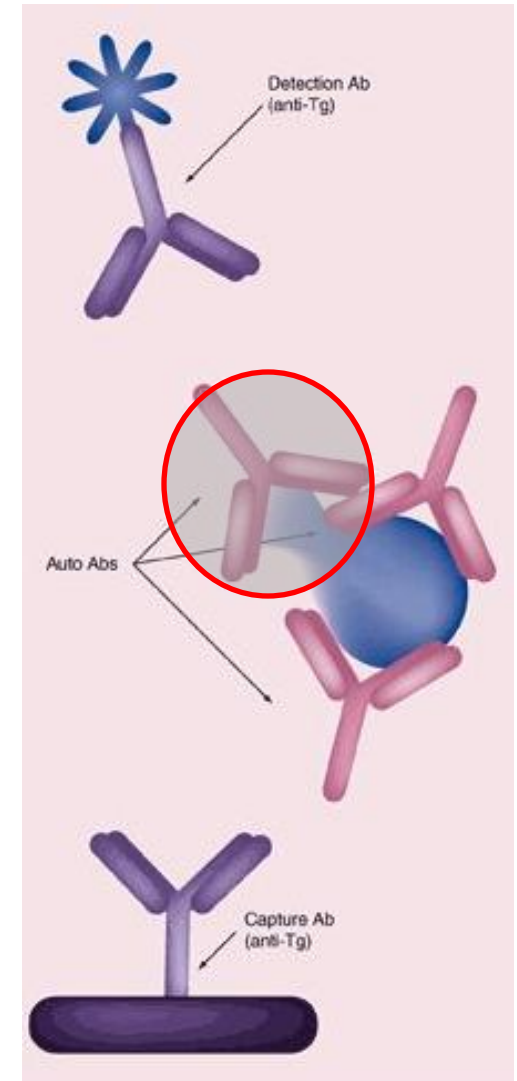
Thyroglobulin Autoantibodies (Tg-AAb) Interfere with Measurement of Tg

- Currently immunoassays (IAs) are the only methodology for Tg testing
- Immune based diagnostic tests for Tg use capture and detection antibodies
- IAs work for samples from patients who don't have Tg-AAb
- Presence of Tg-AAb causes false-negative results in Tg immunoassays



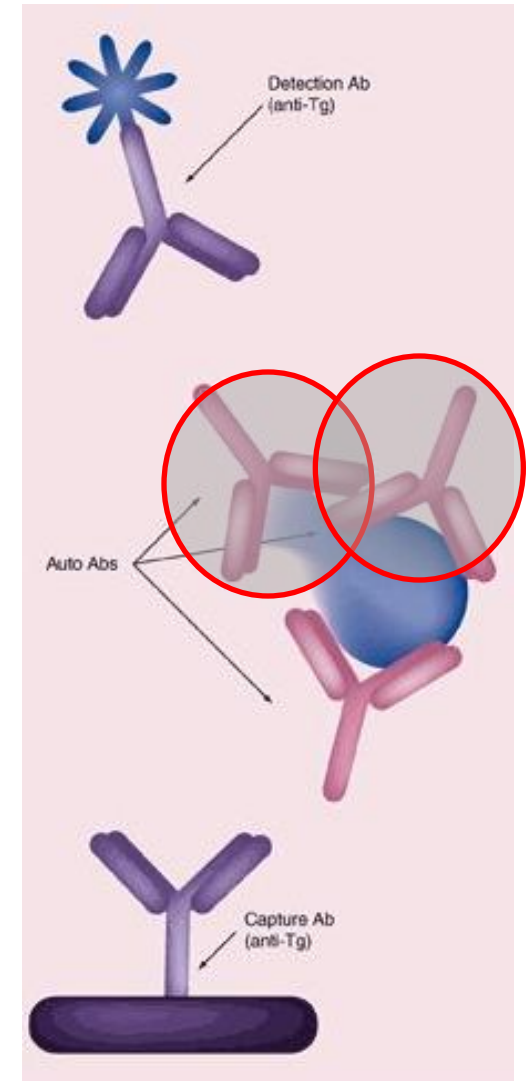
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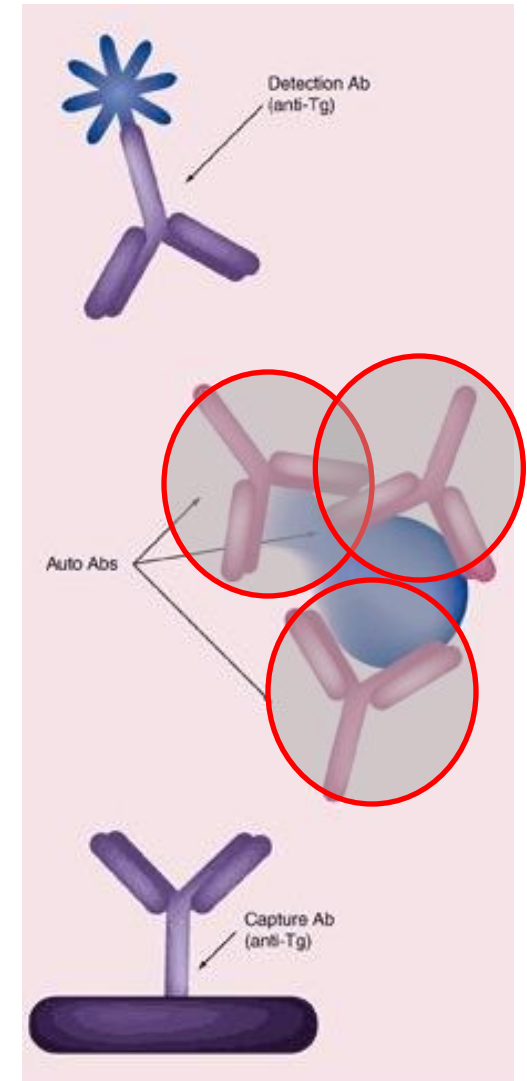
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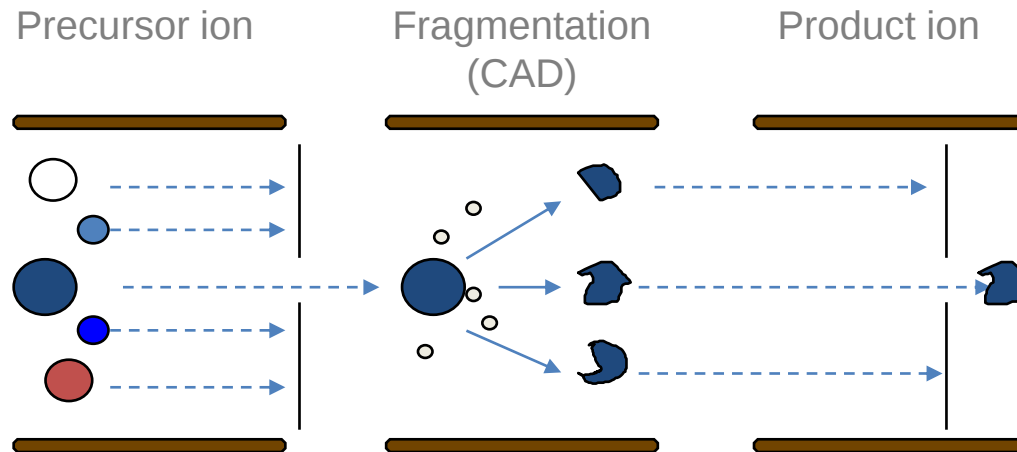
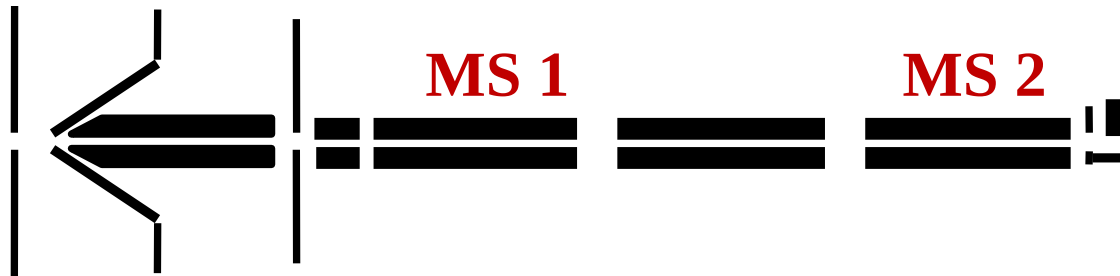


Mass Spec Concepts

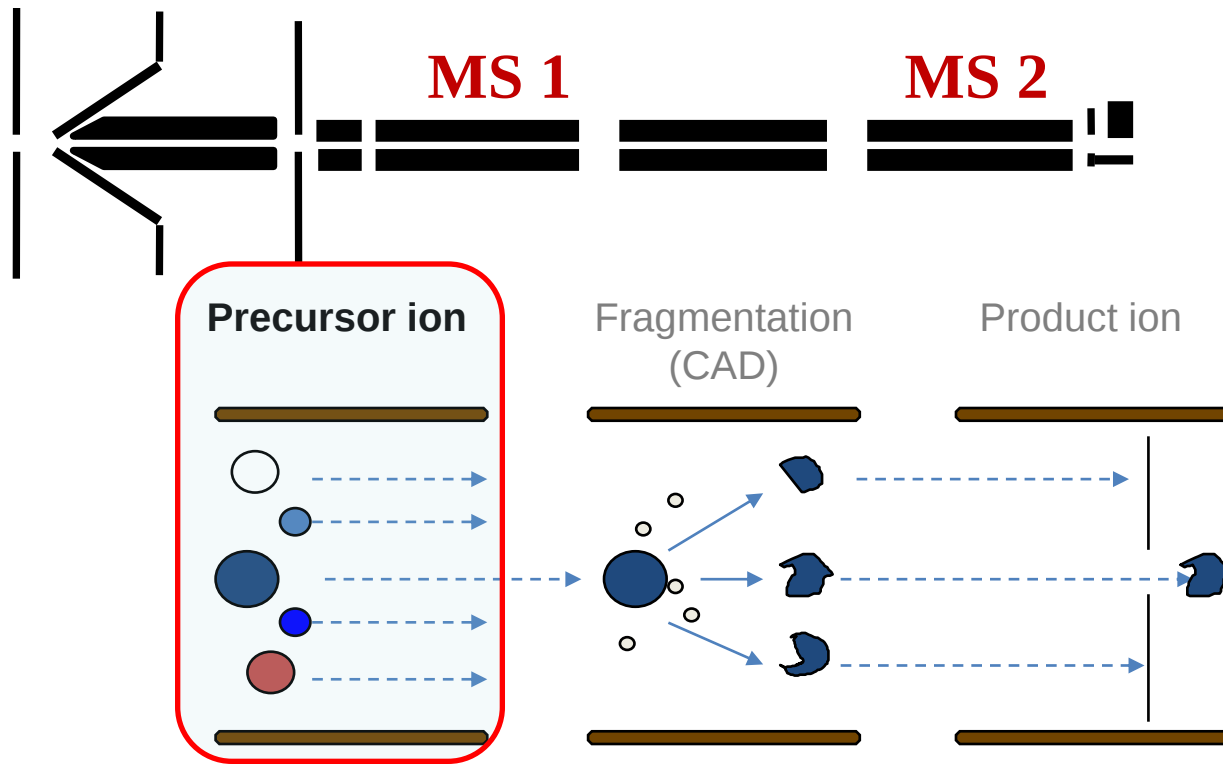
What a Mass Spectrometer Measures

- Mass to charge ratio (m/z), or roughly speaking the molecular weight
 - What is the compound?
- Relative abundance
 - How much is there?

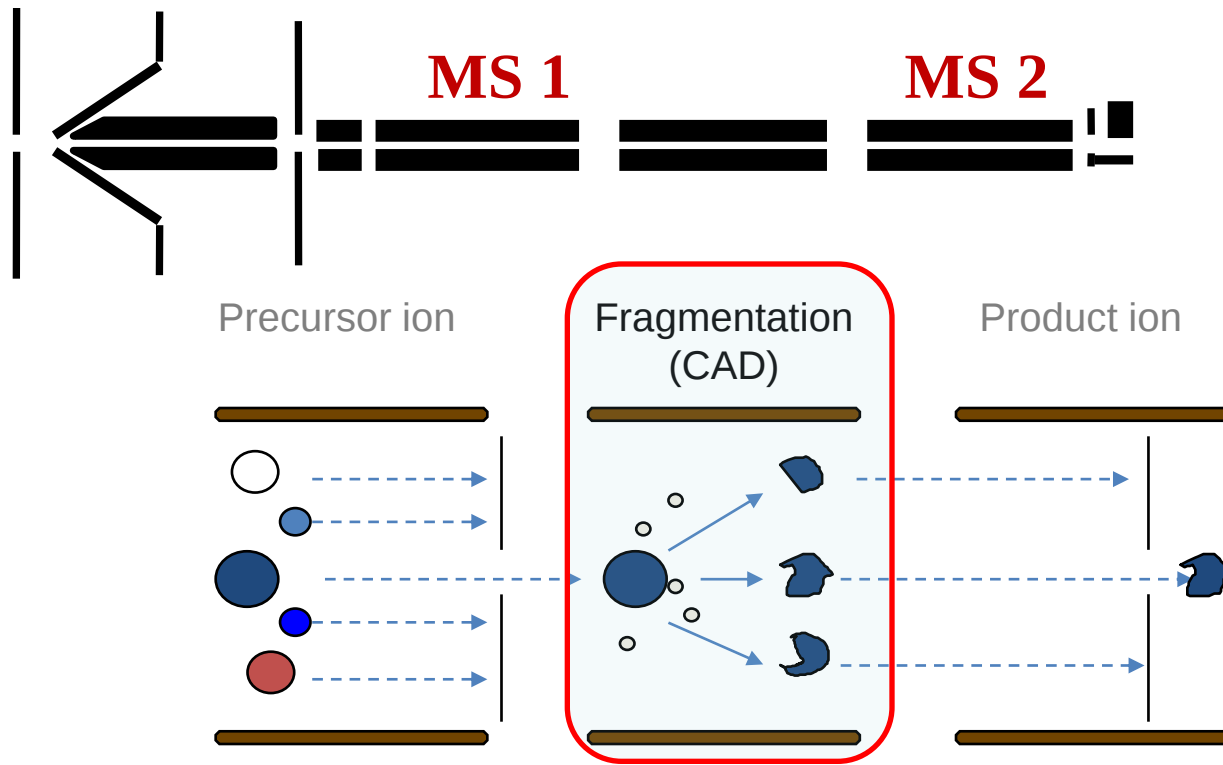
Tandem Mass Spectrometer



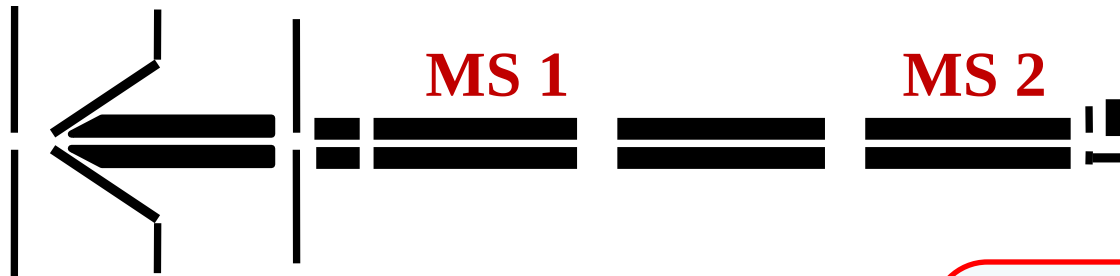
Tandem Mass Spectrometer



Tandem Mass Spectrometer



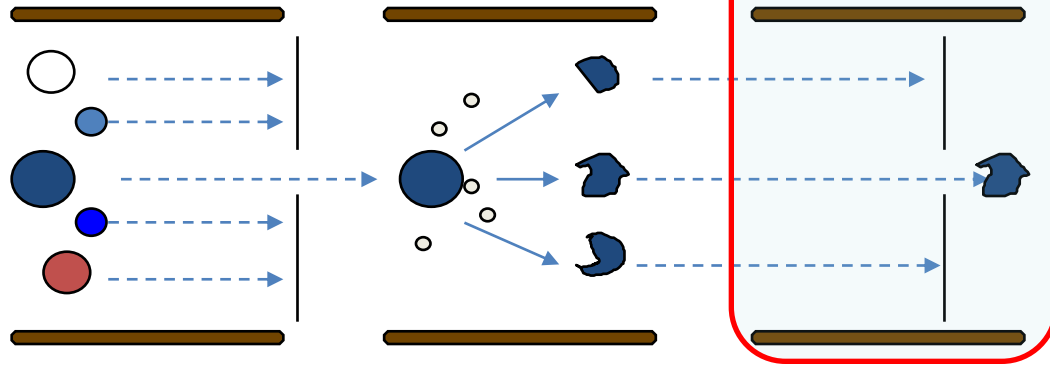
Tandem Mass Spectrometer



Precursor ion

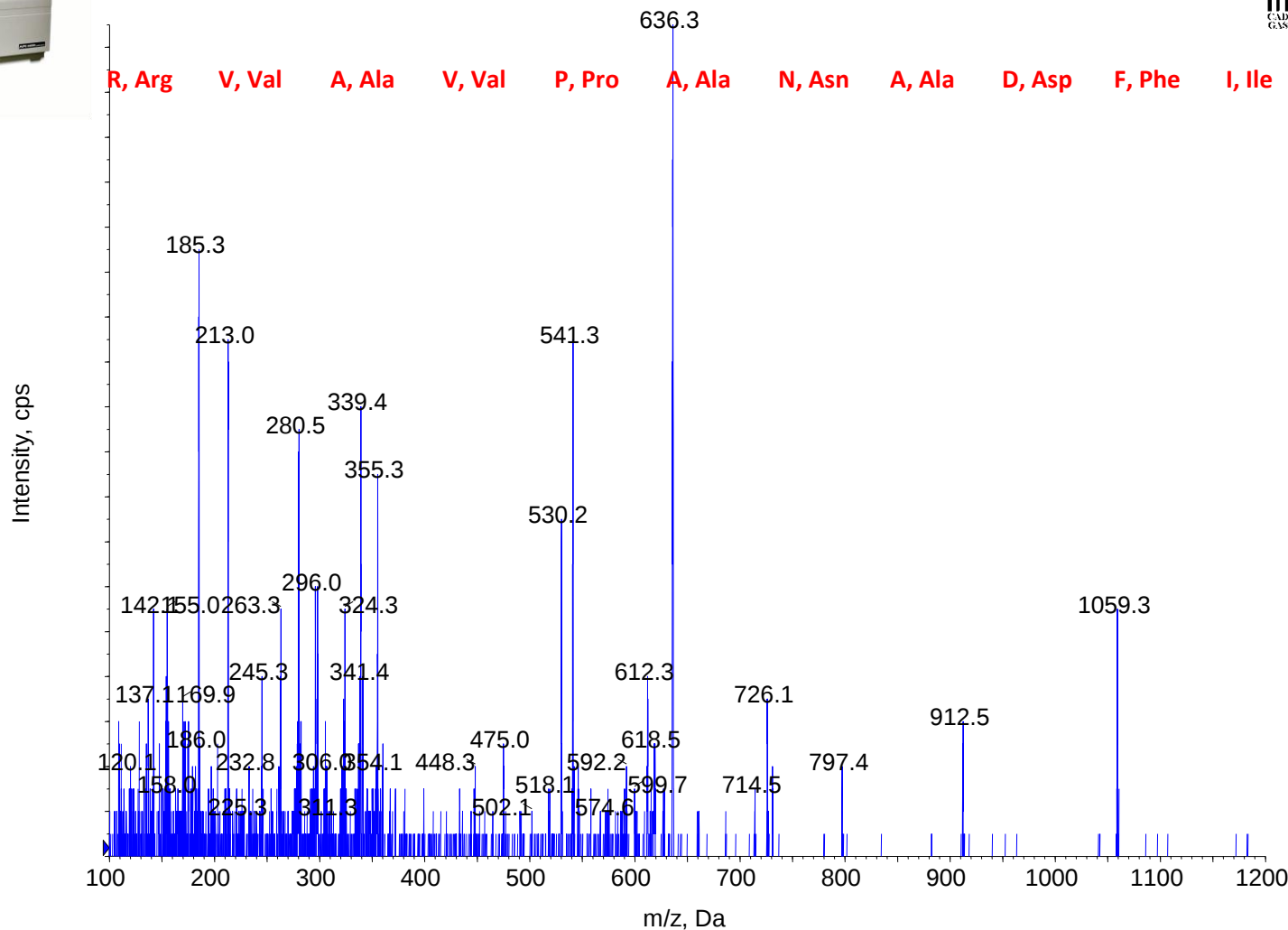
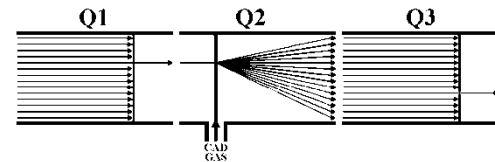
Fragmentation
(CAD)

Product ion



Product Ion Mass Spectrum of Tg-specific Peptide

Precursor ion



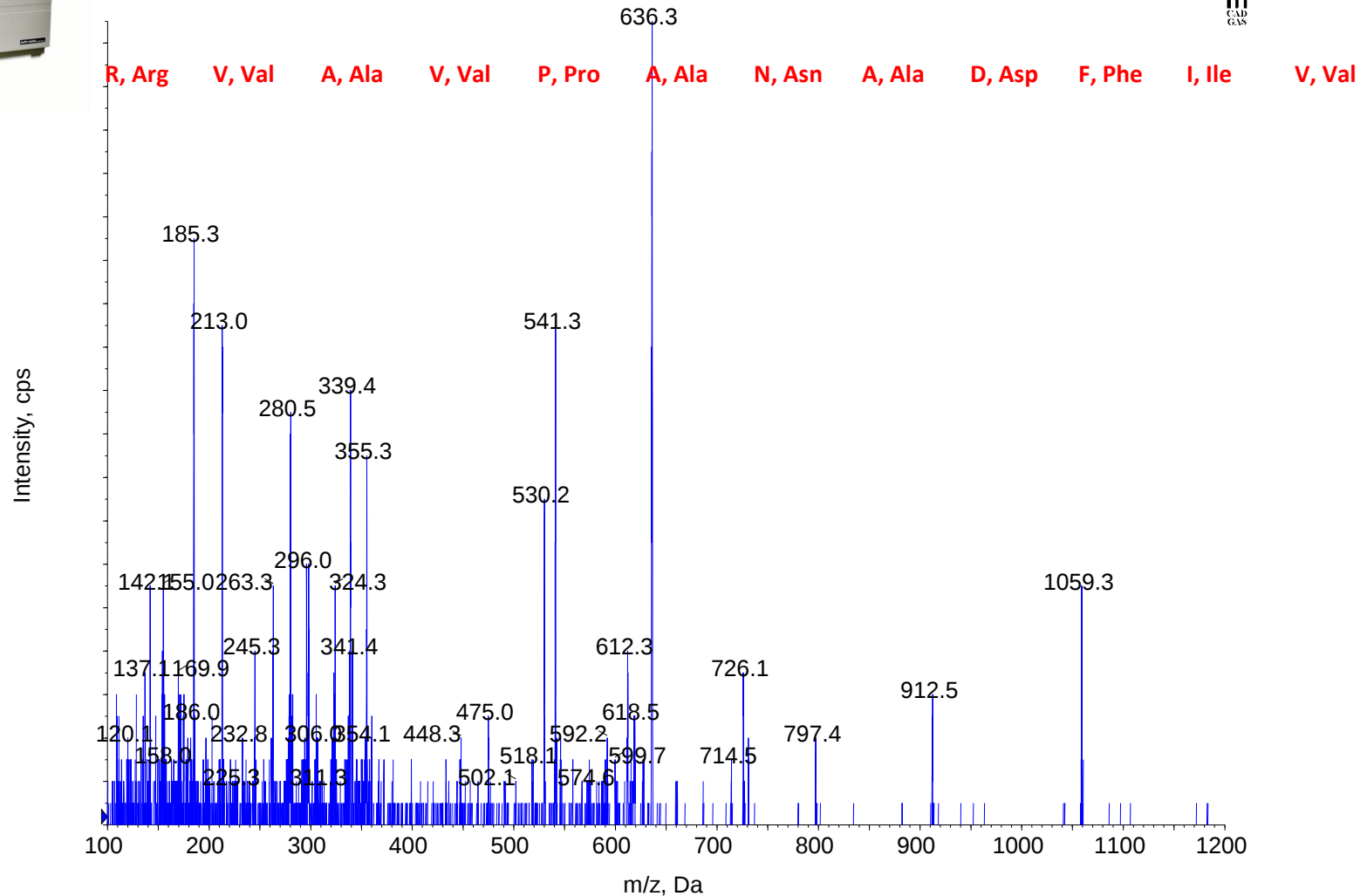
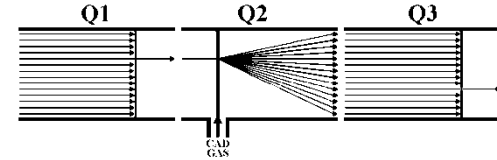
R, Arg V, Val A, Ala V, Val P, Pro A, Ala N, Asn A, Ala D, Asp F, Phe I, Ile V, Val



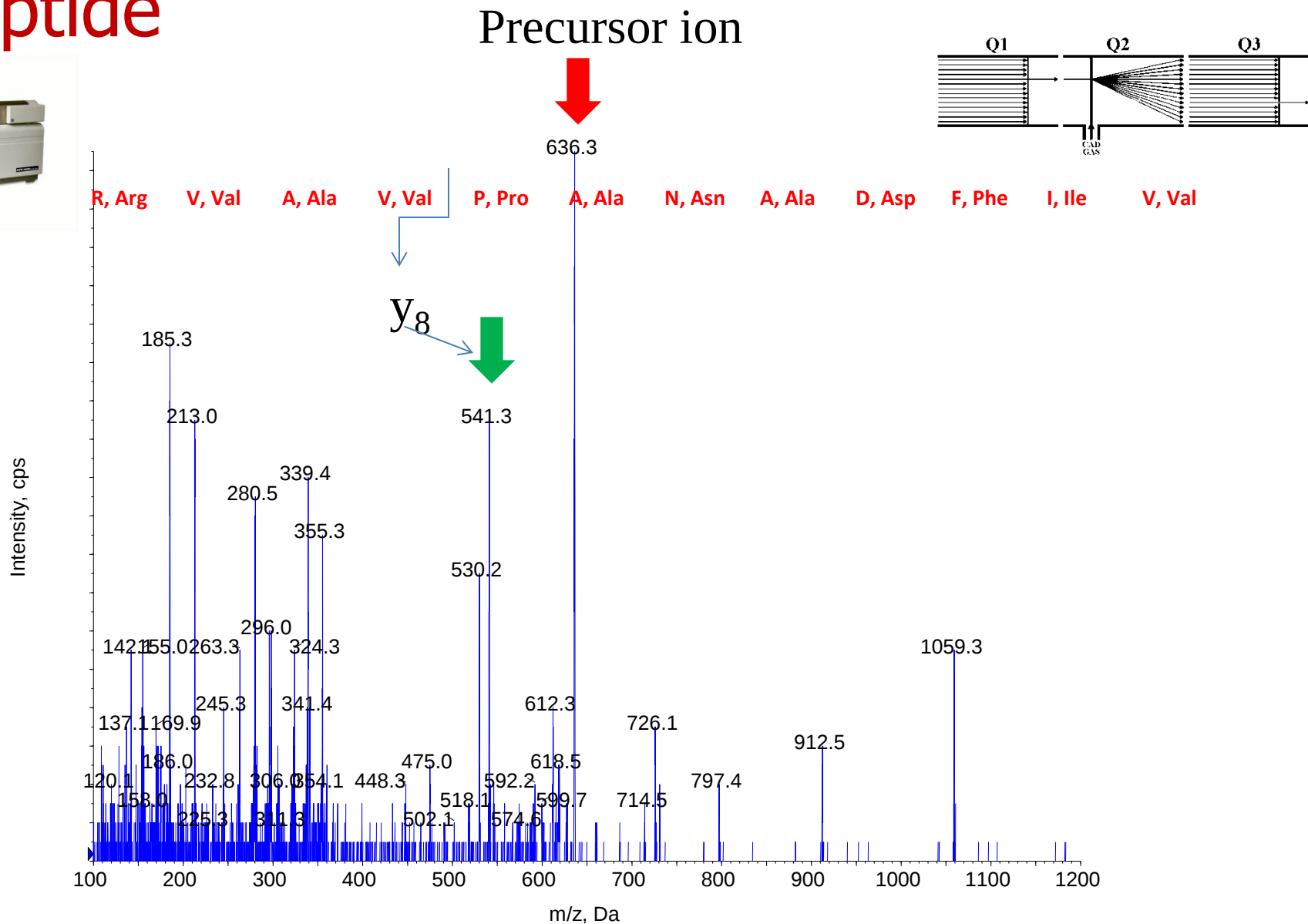
Product Ion Mass Spectrum of Tg-specific Peptide



Precursor ion



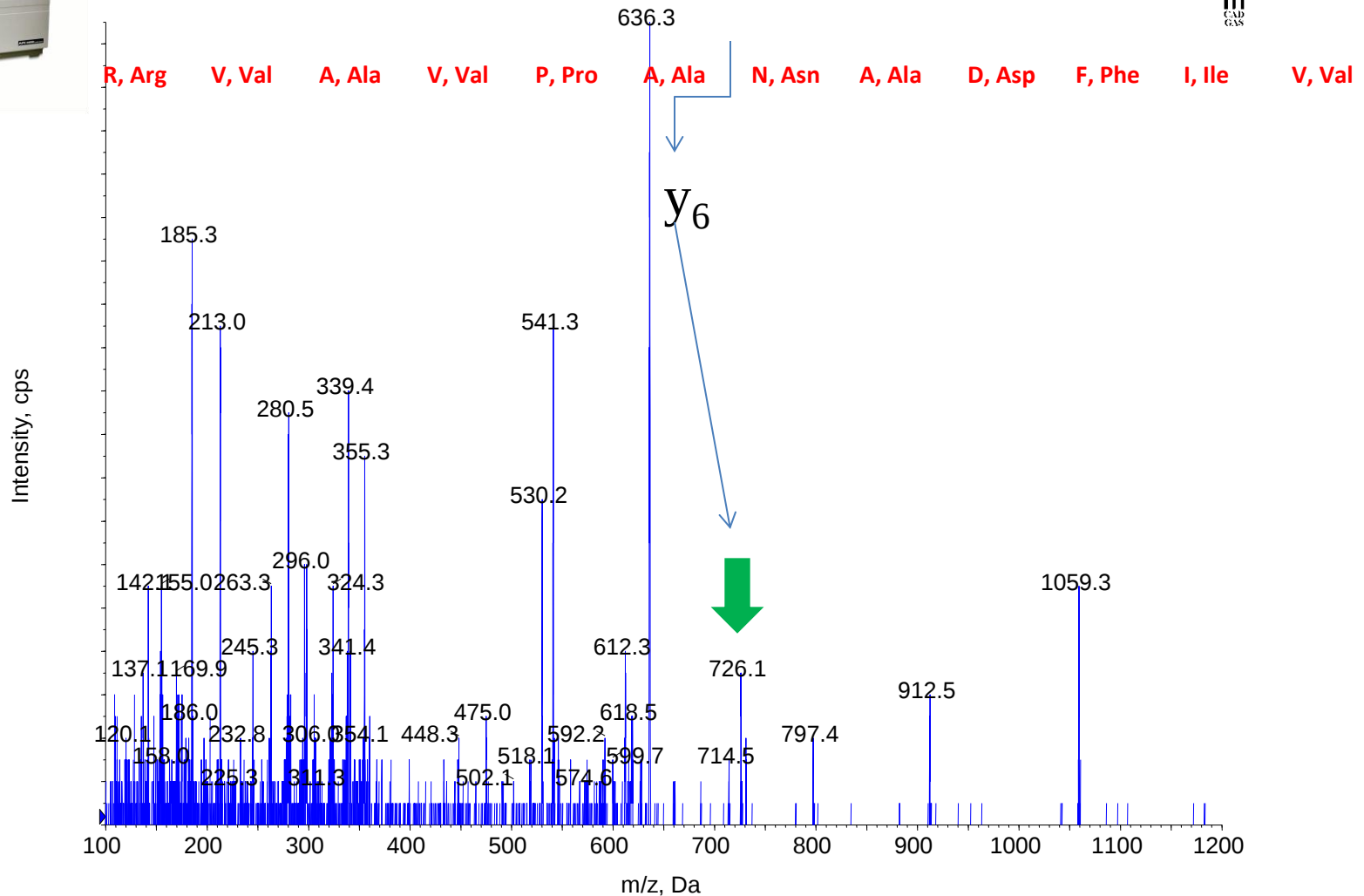
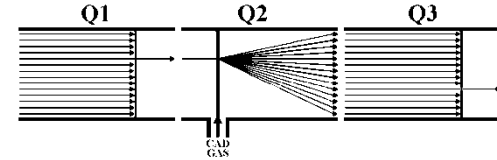
Product Ion Mass Spectrum of Tg-specific Peptide



Product Ion Mass Spectrum of Tg-specific Peptide



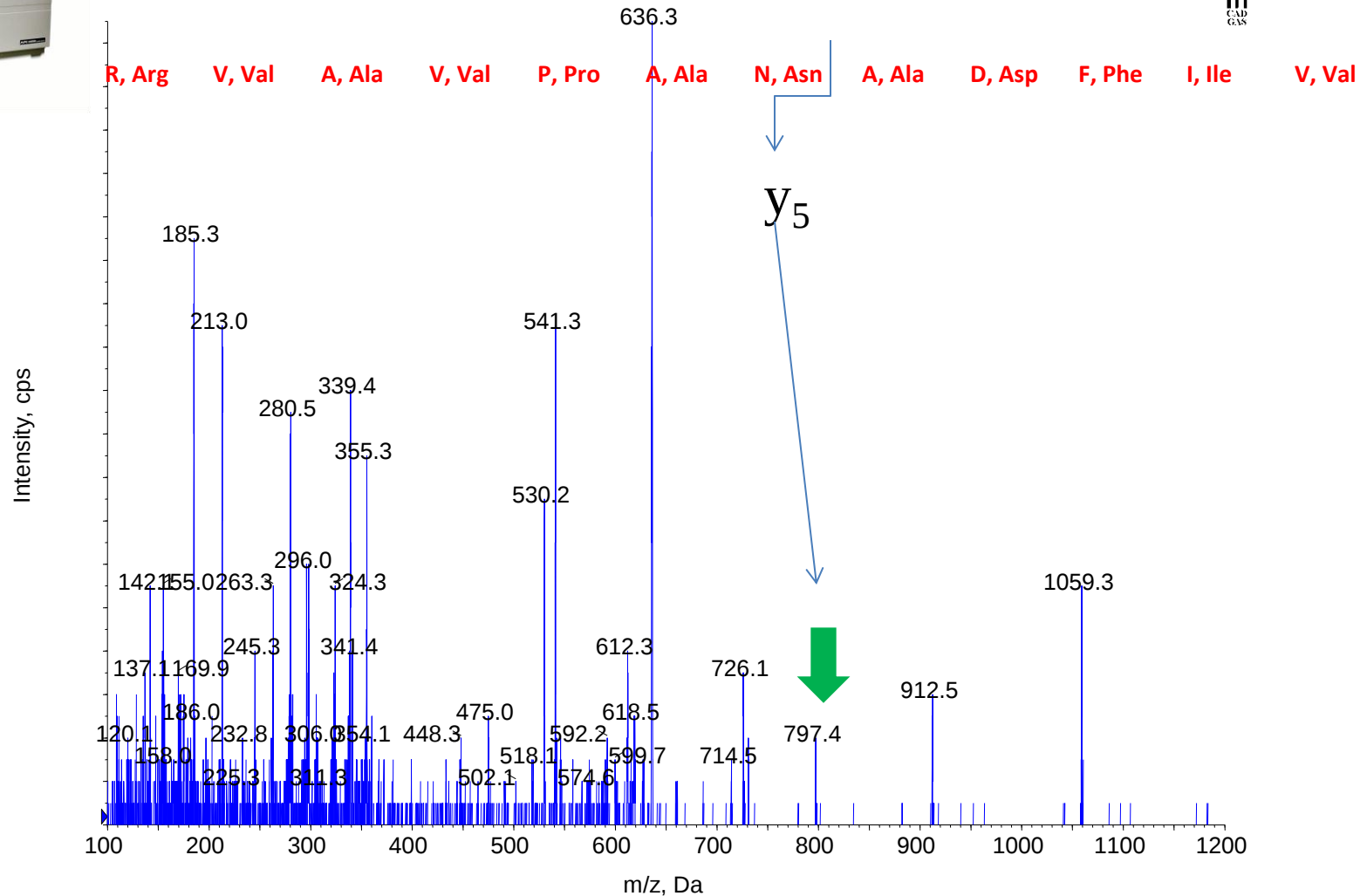
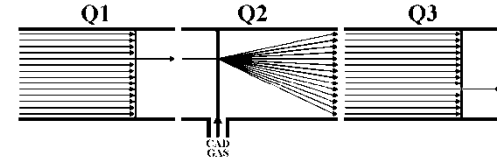
Precursor ion



Product Ion Mass Spectrum of Tg-specific Peptide



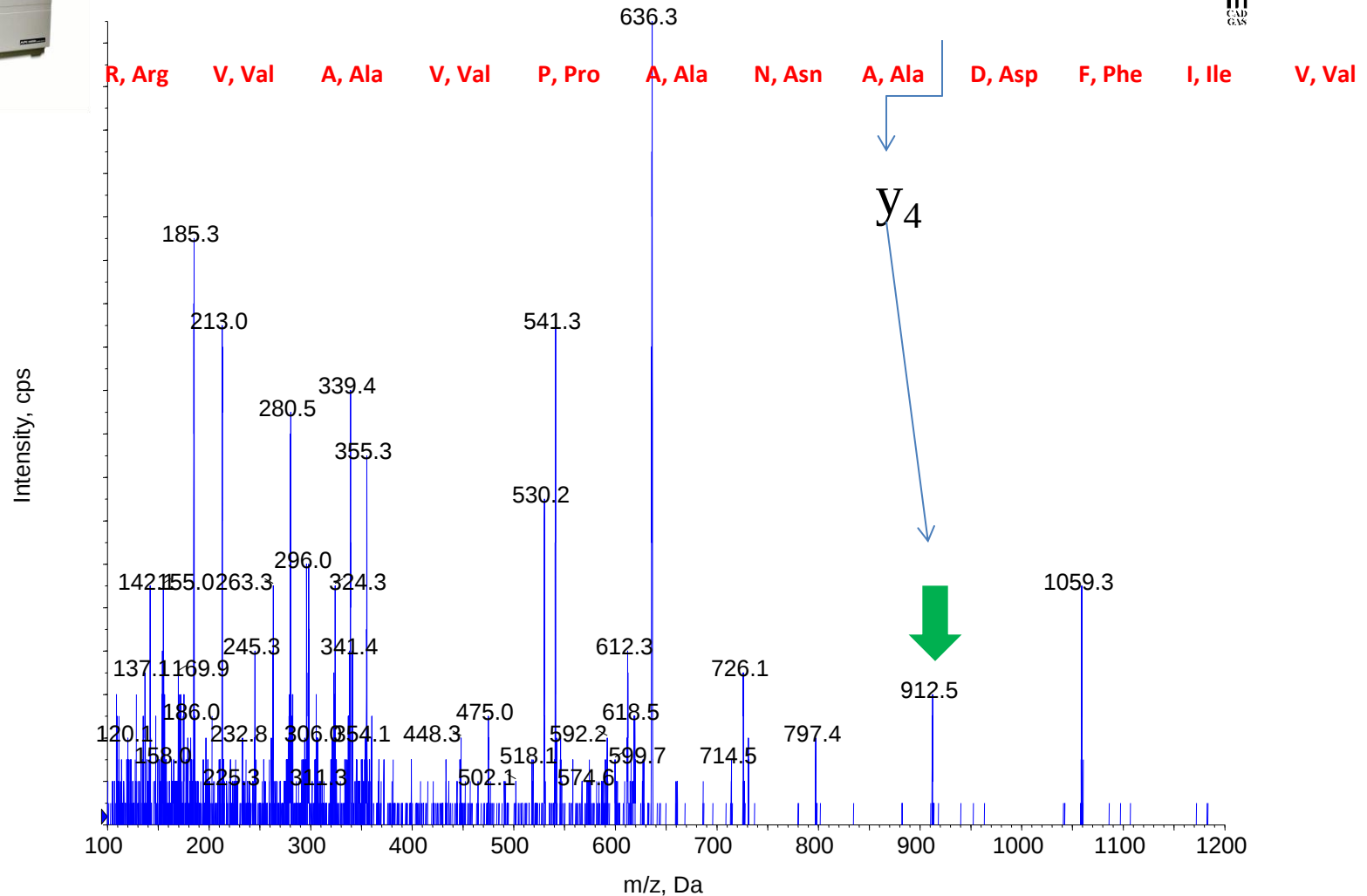
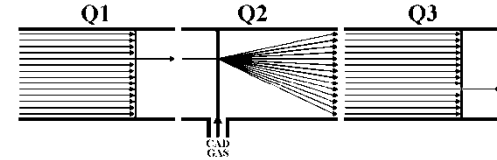
Precursor ion



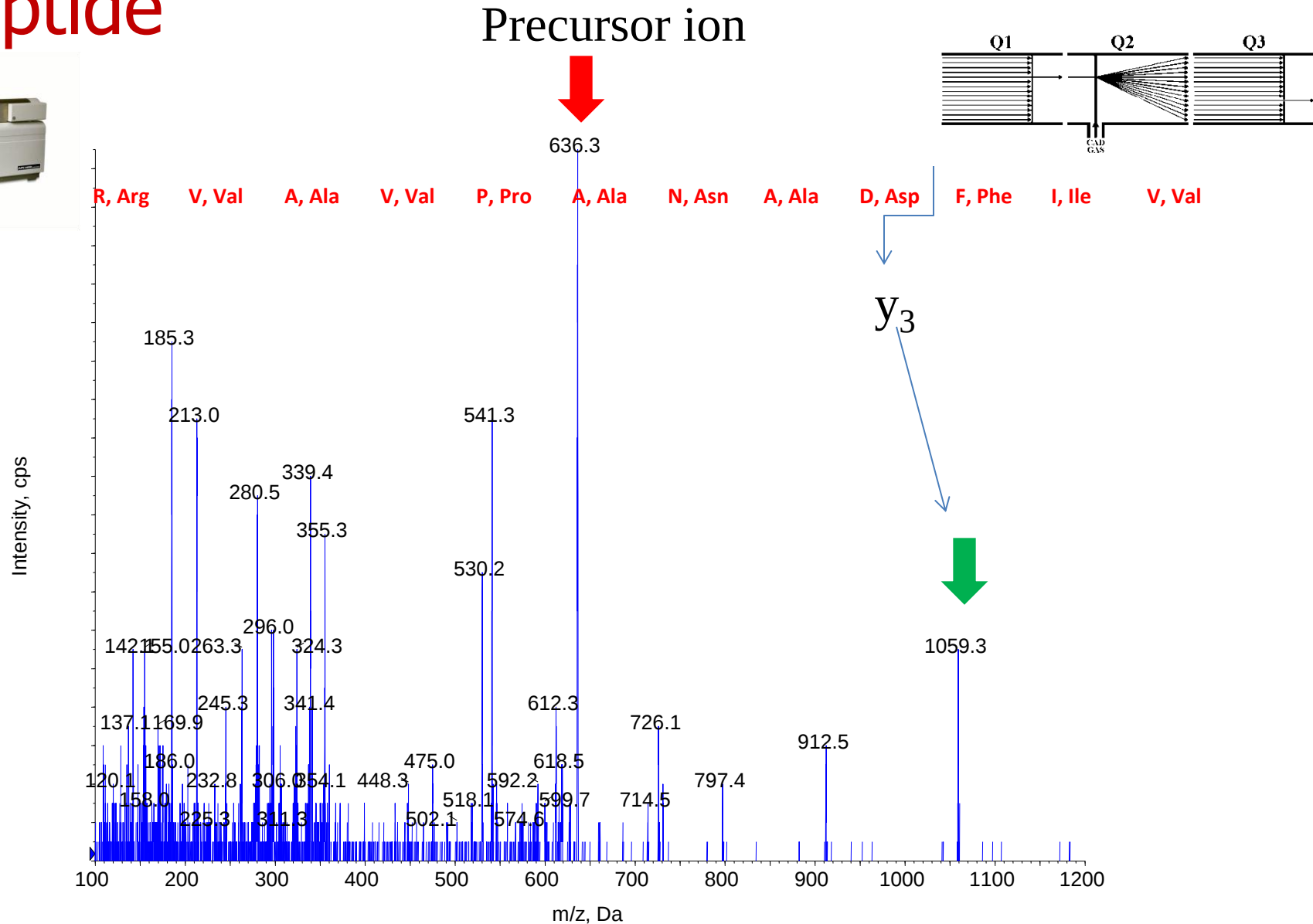
Product Ion Mass Spectrum of Tg-specific Peptide



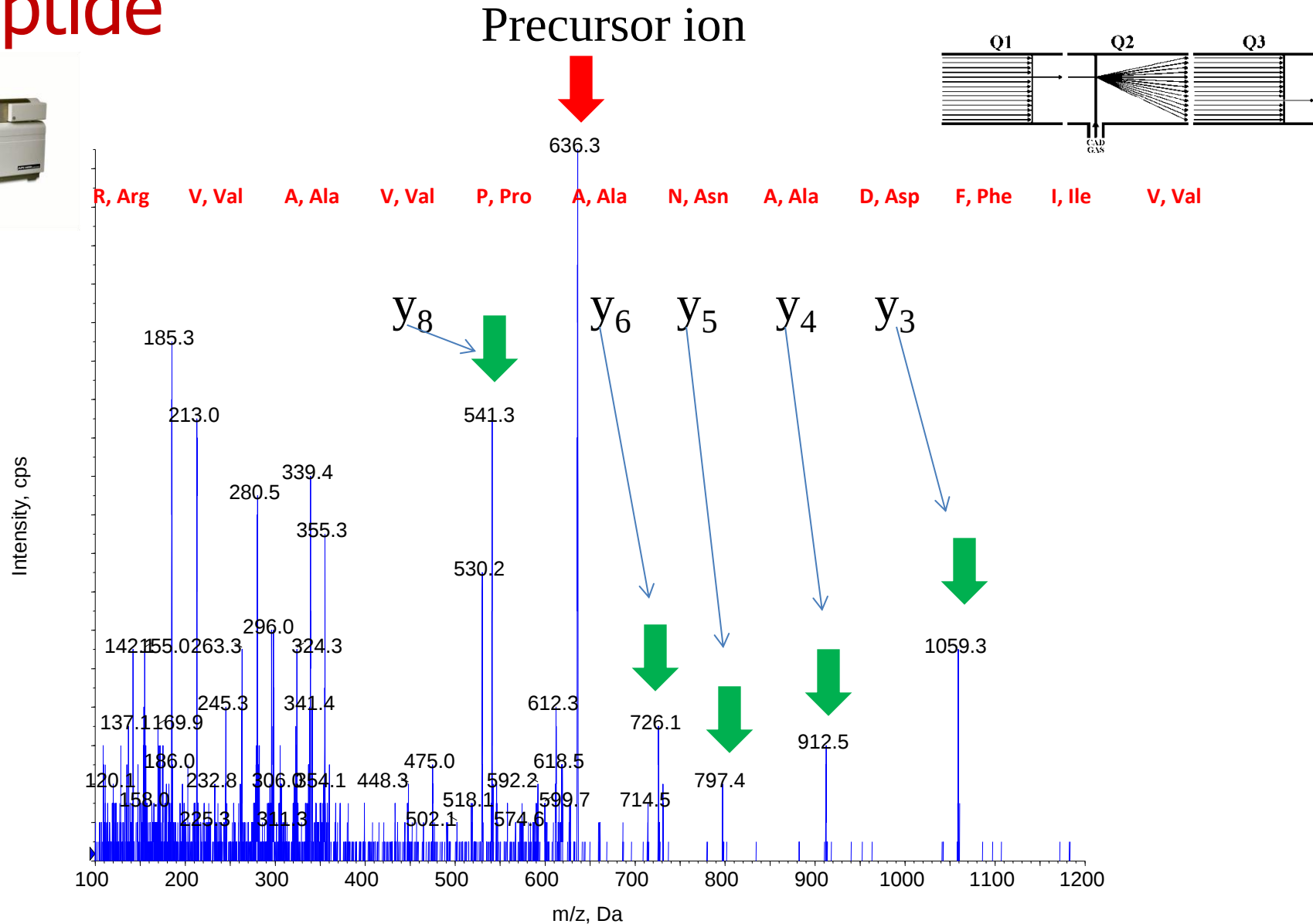
Precursor ion



Product Ion Mass Spectrum of Tg-specific Peptide



Product Ion Mass Spectrum of Tg-specific Peptide



Adding a liquid chromatograph to a tandem mass spectrometer



- Molecules characterized by three physical properties
 - Chromatographic retention time
 - Parent ion mass
 - Daughter ion mass

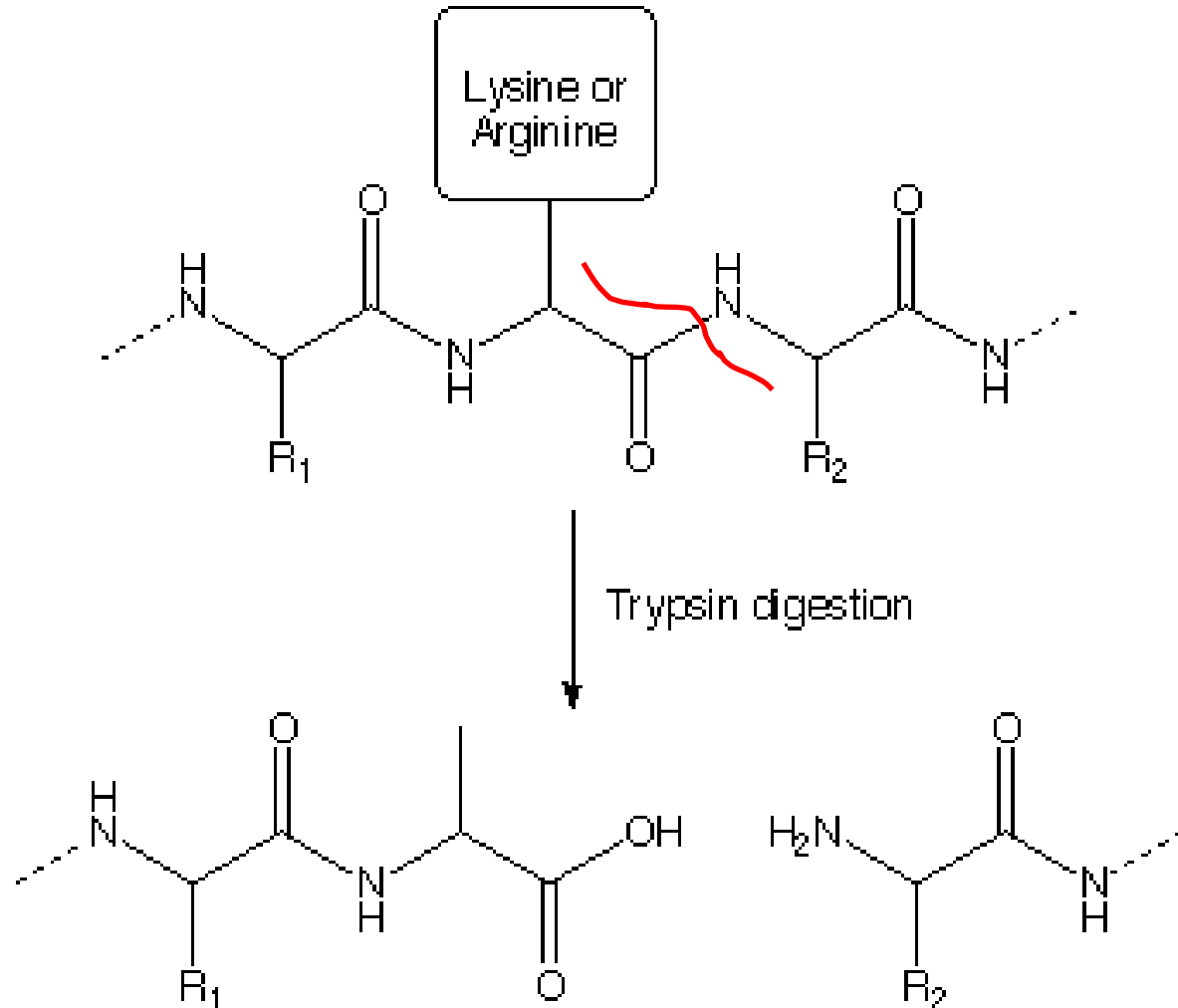
Adding a liquid chromatograph to a tandem mass spectrometer



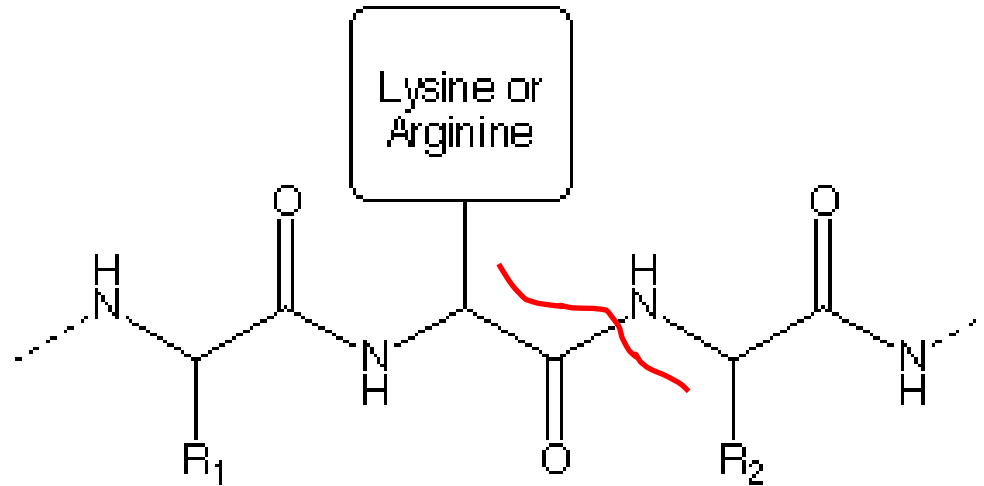
- Molecules characterized by three physical properties
 - Chromatographic retention time
 - Parent ion mass
 - Daughter ion mass
- Even more selective for quantitative analysis than tandem mass spectrometry alone

Digestion by trypsin

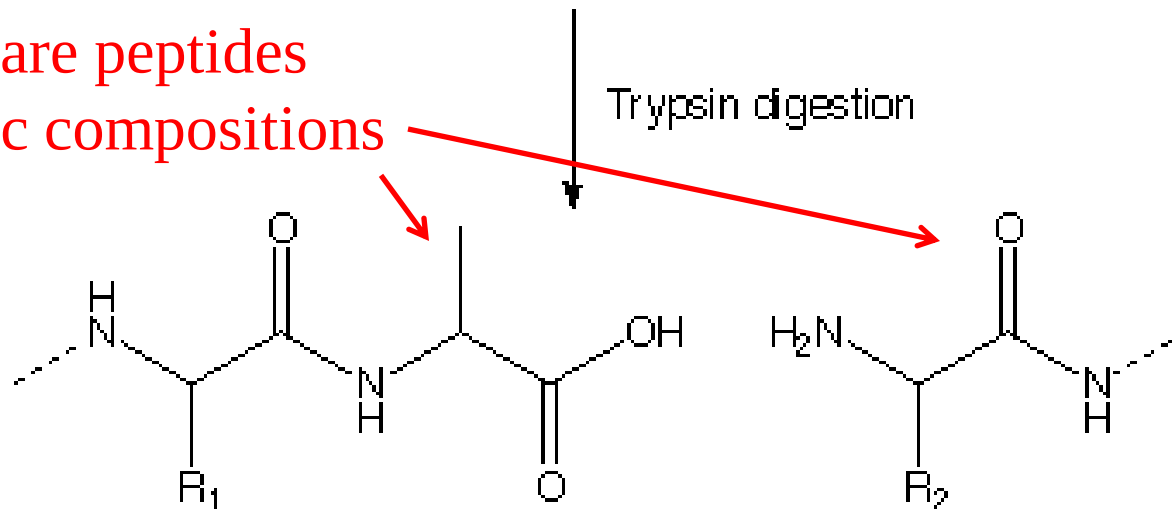
Trypsin cuts proteins at Arg and/or Lys residues



Trypsin cuts proteins at Arg and/or Lys residues



Products are peptides
of specific compositions



Tryptic Peptides from Proteins



A selected tryptic peptide can act as a surrogate marker for the protein

Tryptic Digest of Thyroglobulin

- Tryptic digest of Tg produces more than 400 peptides

```
1 malvleiftl lasicwvsan ifeyqvdaqp lrpcelqret aflkqadyvp qcaedgsfqt
61 vqcqndgrsc wcvgangsev lgsrqgrpv acslfcqlgk qqillsqyin stdtsylpqc
121 qdsgdyapvq cdvqhvcwc vdaegmevyg trqlgrpkrc prsceirnr llhgvqdksp
181 pqcsaagefm pvqckfvntt dmmifdlvhs ynrfpdaftv fssfgrrfpe vsgyhcads
241 qgrelaetgl ellldeiydt ifagldlpst ftettlyril qrrflavqsv isgrfrcptk
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2701 ellpnrqglk kadcsfwsky isslksadg akgggsaese eeeltagsl redllslqep
2761 gsktyek
```

Tryptic Digest of Thyroglobulin

- Tryptic digest of Tg produces more than 400 peptides
- One or more peptides selected and measured as surrogate for Tg

```
1 malvleiftl lasicwvsan ifeyqvdaqp lrpcelqret aflkqadyvp qcaedgsfqt
61 vqcqndgrsc wcvgangsev lgsrqgrpv acslfcqlgk qqillsygin stdtsylpqc
121 qdsgdyapvq cdvqhvgcwc vdaegmevyg trqlgrpkrc prsceirnr llhgvqdksp
181 pqcsaagefm pvqckfvntt dmmifdlvhs ynrfpdaftv fssfgrrfpe vsgyhcads
241 qgrelaetgl ellldeiydt ifagldlpst ftettlyril qrrflavqsv isgrfrcptk
301 ceverftats fghpyvpscr rngdyqavqc qtegpwcwv agqkemhgrt qqgeppscae
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541 dgtmnkptvg sfgeinlqe nqnalkflas llelpeflif lqhaisvped vardlgdvme
601 tvldsqtcq tperlfvpst ttegsyedvq cfsgecwcvn swgkelpgsr vrdgqprcpt
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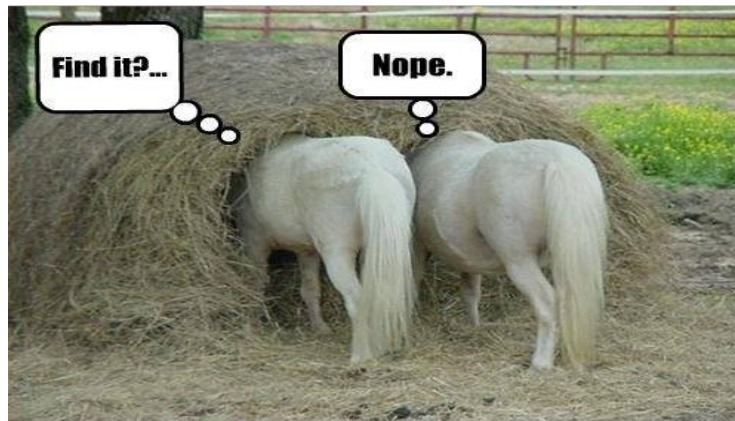
Tryptic Digest of Thyroglobulin

- Tryptic digest of Tg produces more than 400 peptides
- One or more peptides selected and measured as surrogate for Tg
- Sequence: **VIFDANAPVAVR**

```
1 malvleiftl lasicwvsan ifeyqvdaqp lrpcelqret aflkqadyvp qcaedgsfqt
61 vqcqndgrsc wcvgangsev lgsrqgrpv acslfcqlgk qqillsqyin stdtsylpqc
121 qdsgdyapvq cdvqhvcwc vdaegmevyg trqlgrpkrc prsceirnr llhgvqdksp
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2761 gsktyesk
```

Tryptic Digest of Thyroglobulin

- Tryptic digest of human serum contains over **14,000,000 different peptides**
- Concentration of Tg peptides in tryptic digest of human serum billion fold lower than most abundant peptides

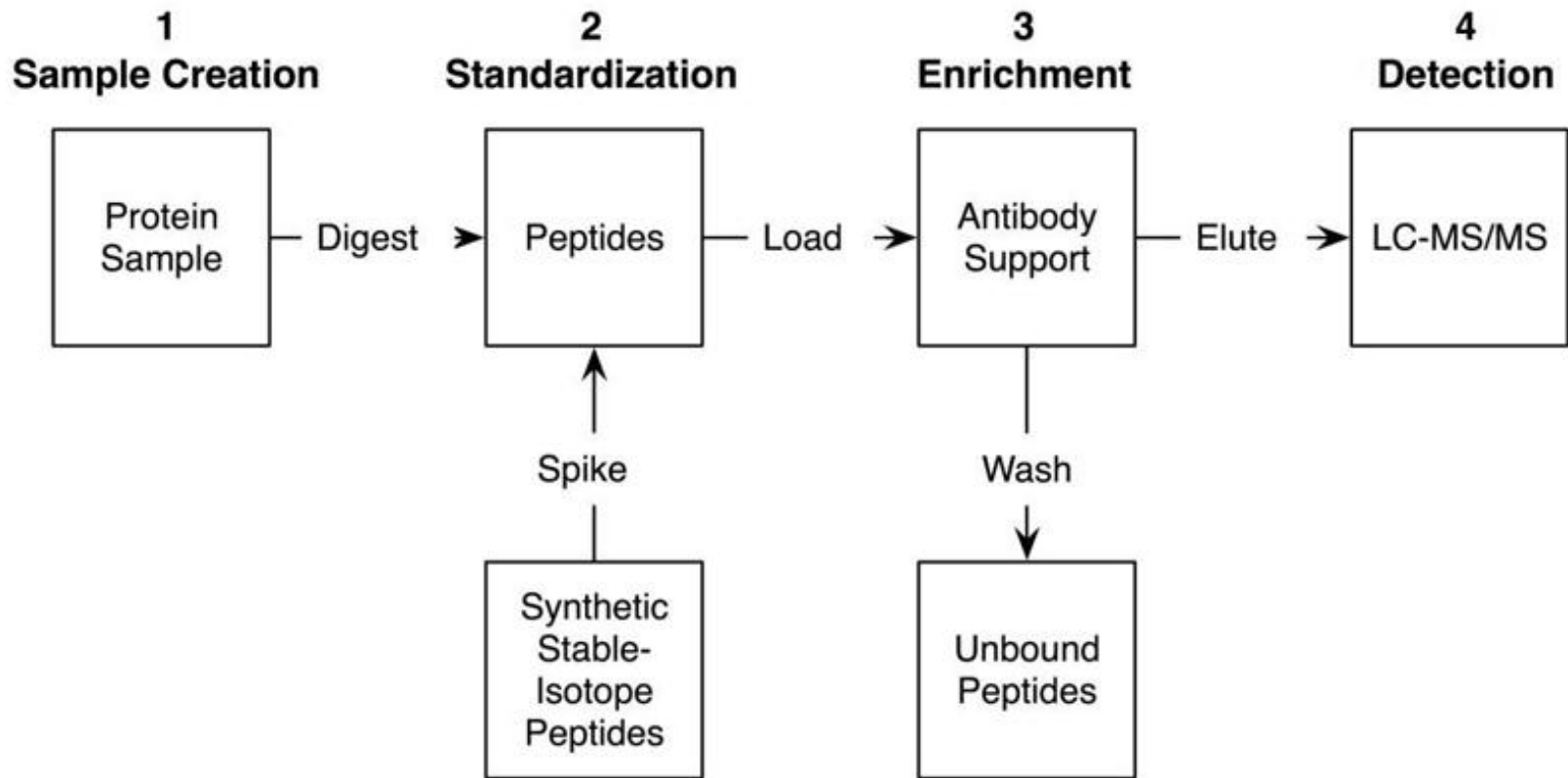


← *Needle in a haystack*

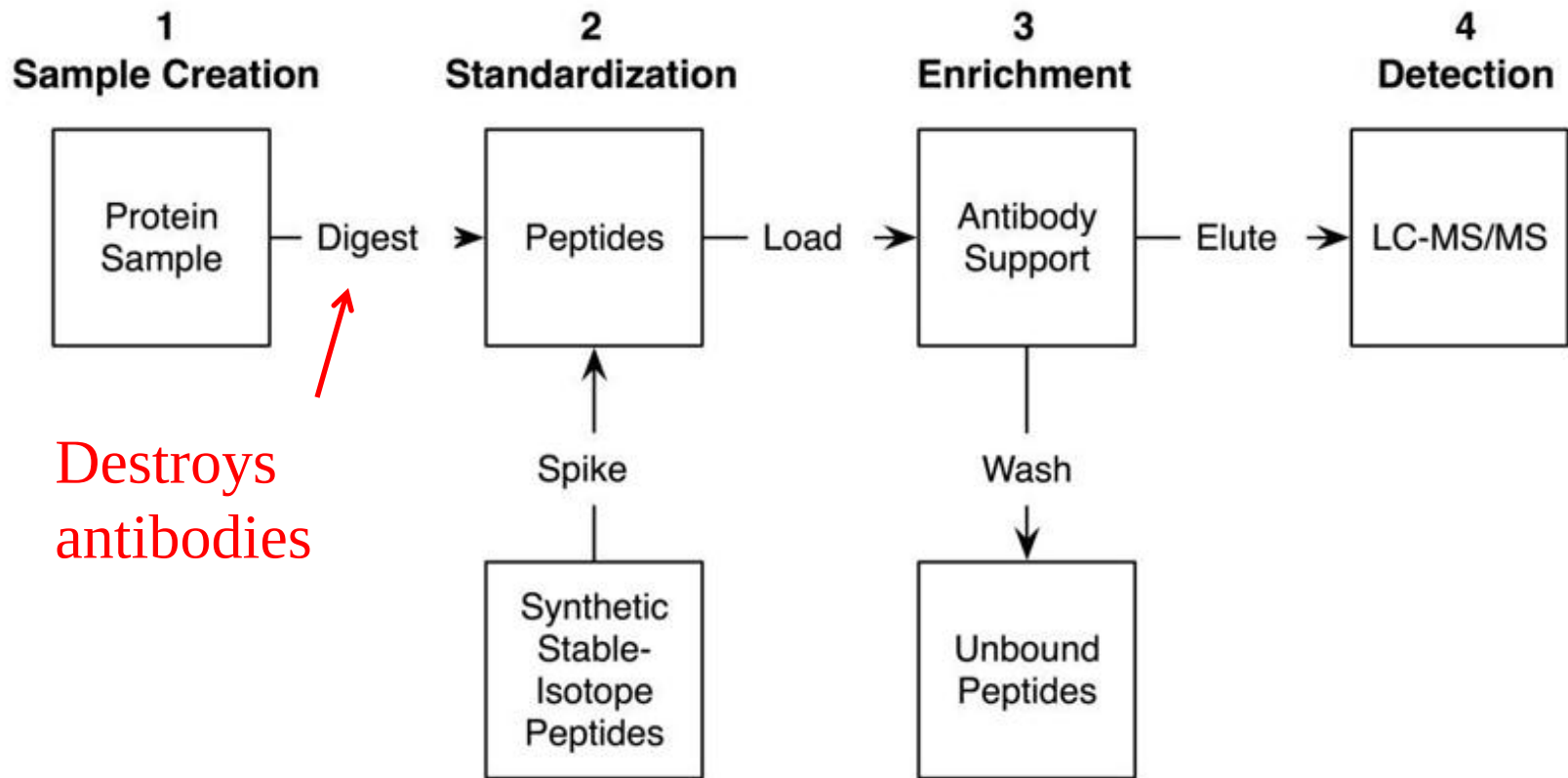
- Affinity purification using anti-peptide antibody
(**SISCAPA**)

SISCAPA

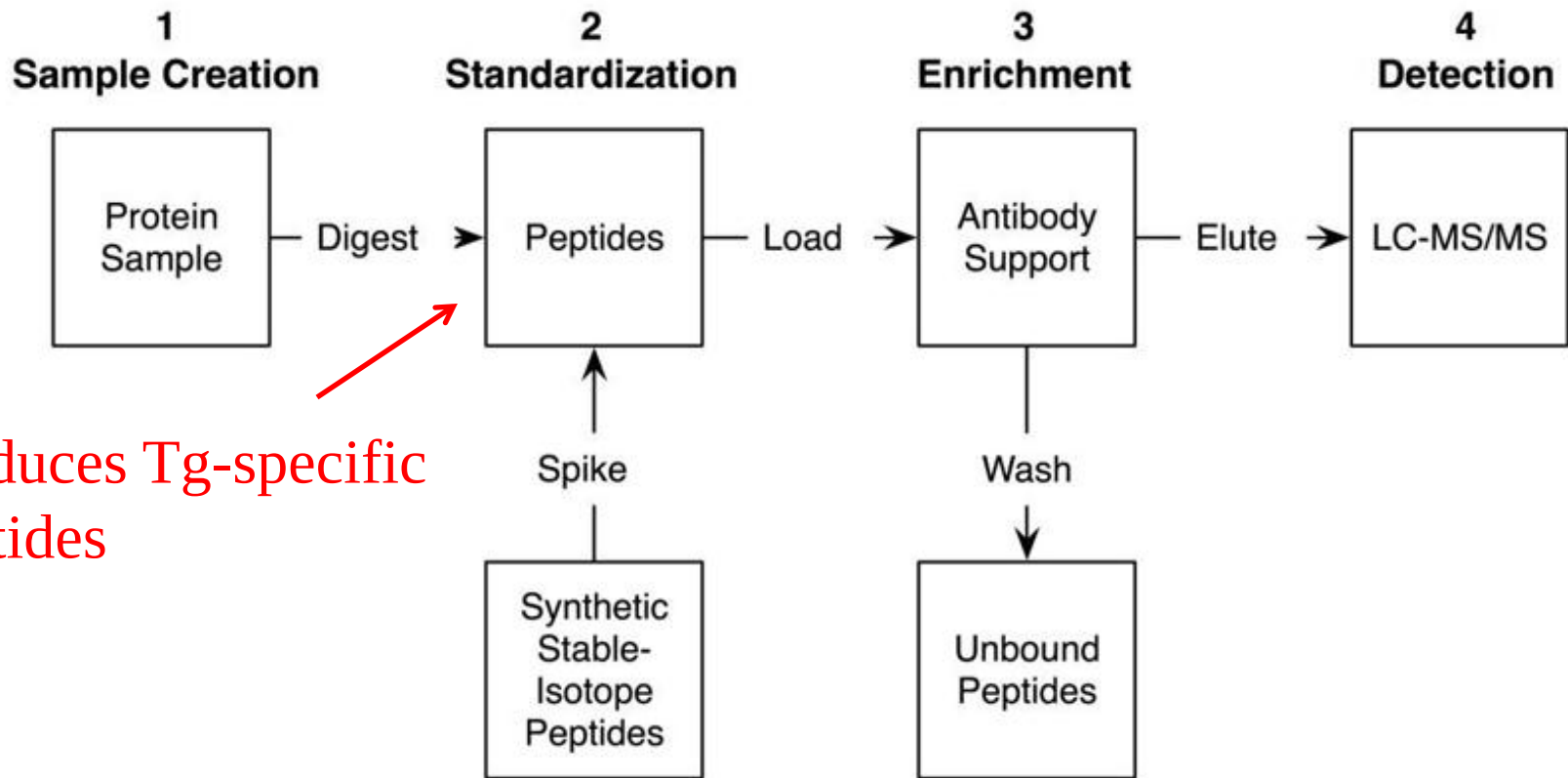
SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration

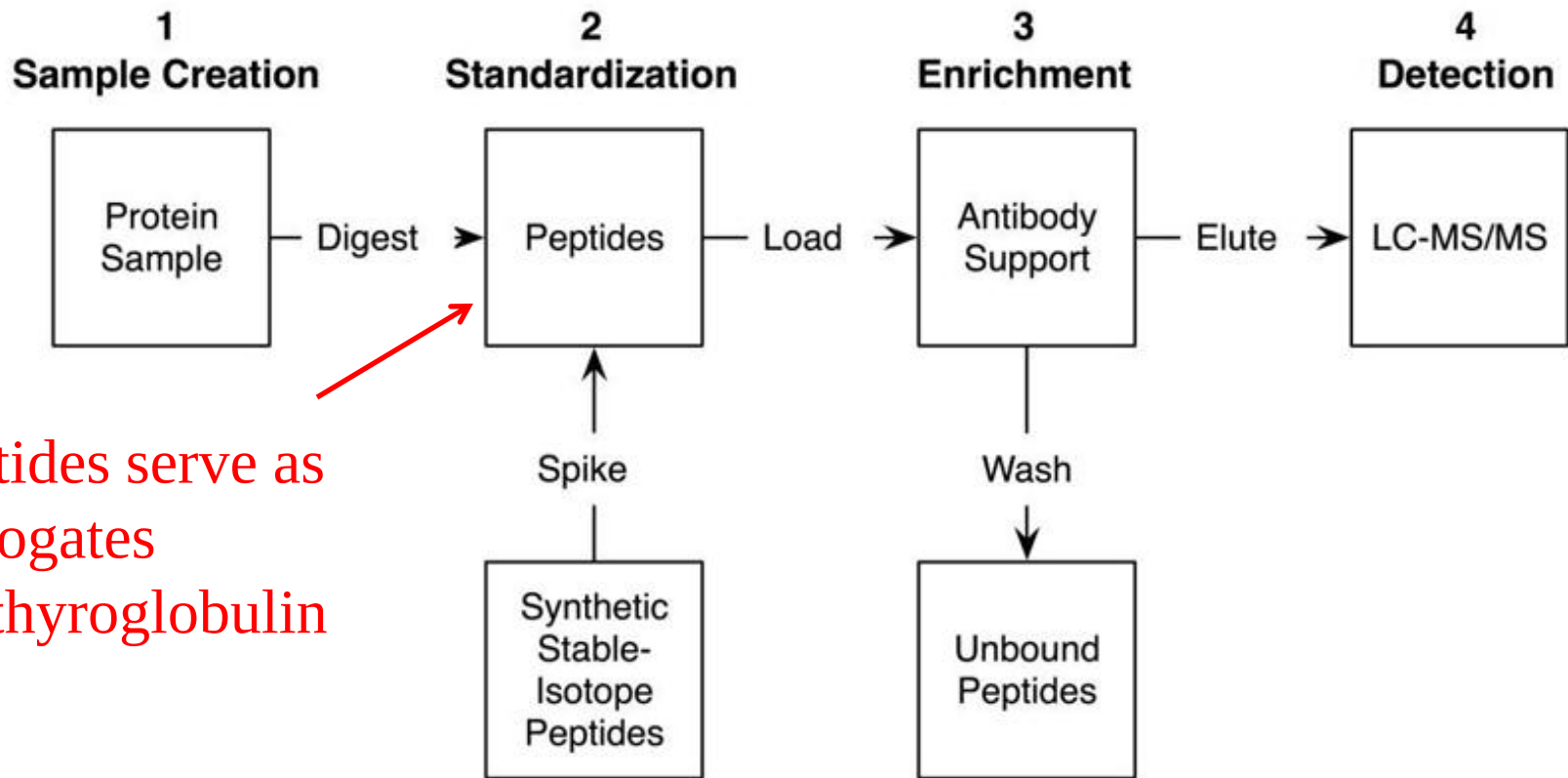


SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



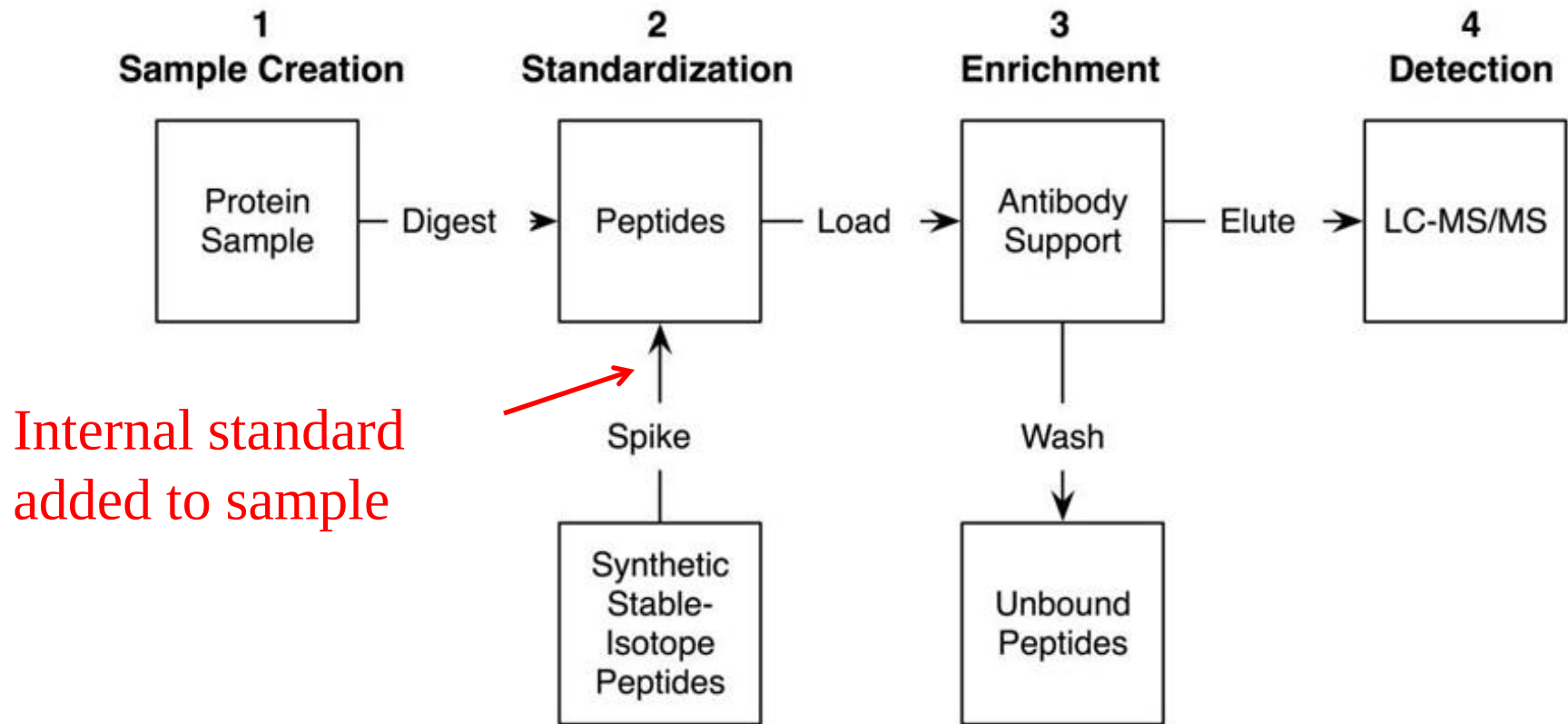
Produces Tg-specific peptides

SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration

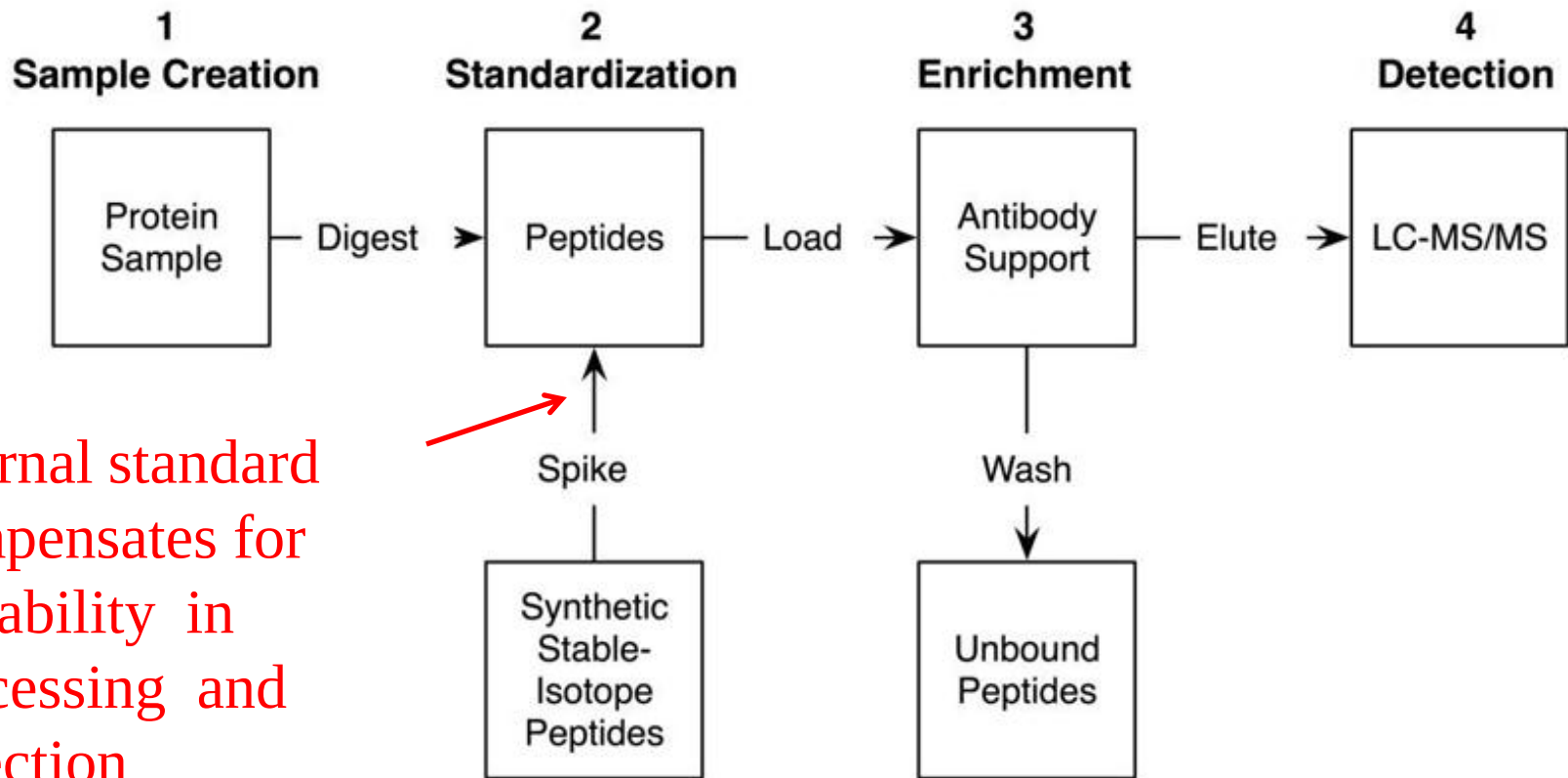


Peptides serve as
surrogates
for thyroglobulin

SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration

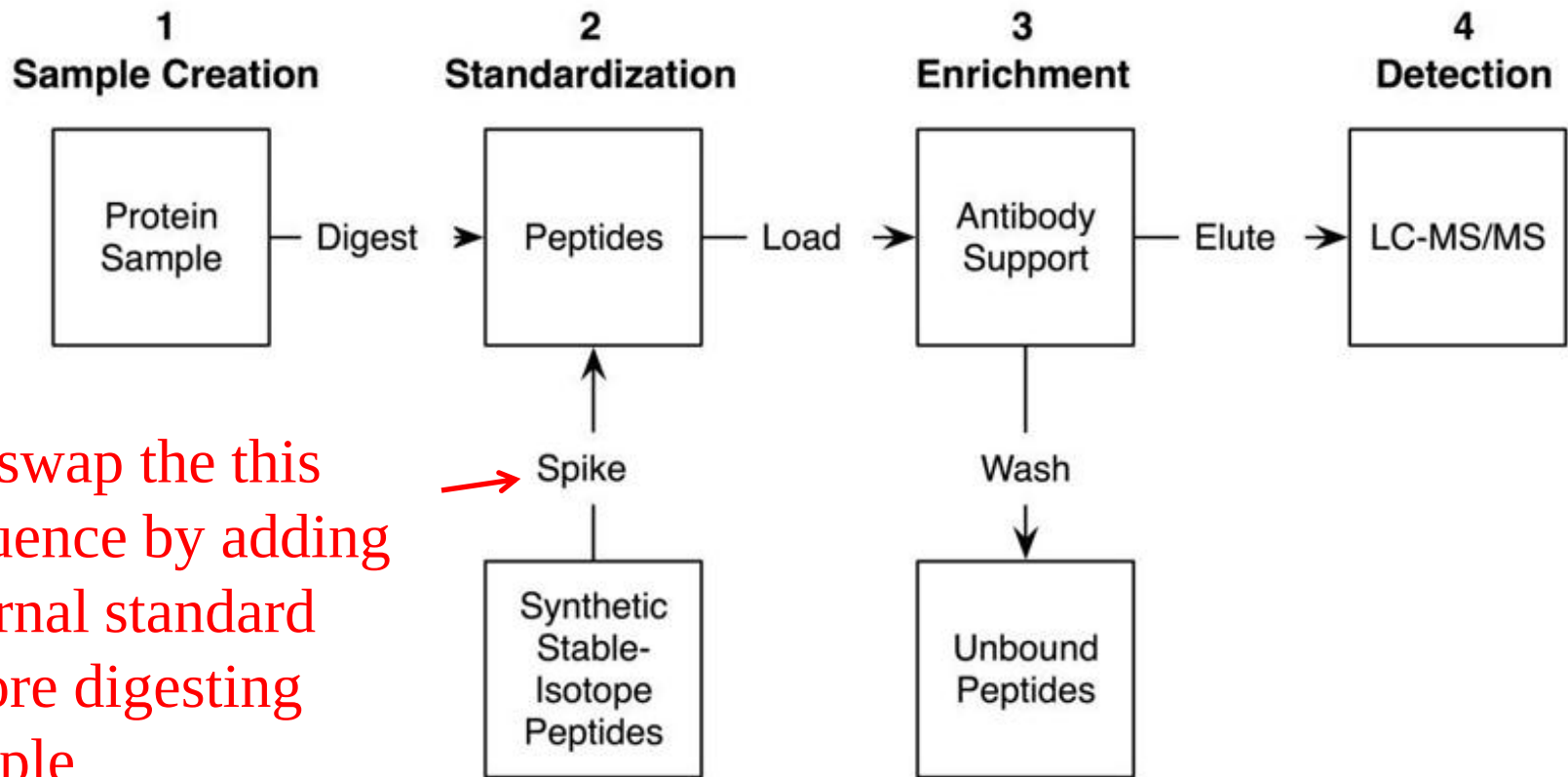


SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



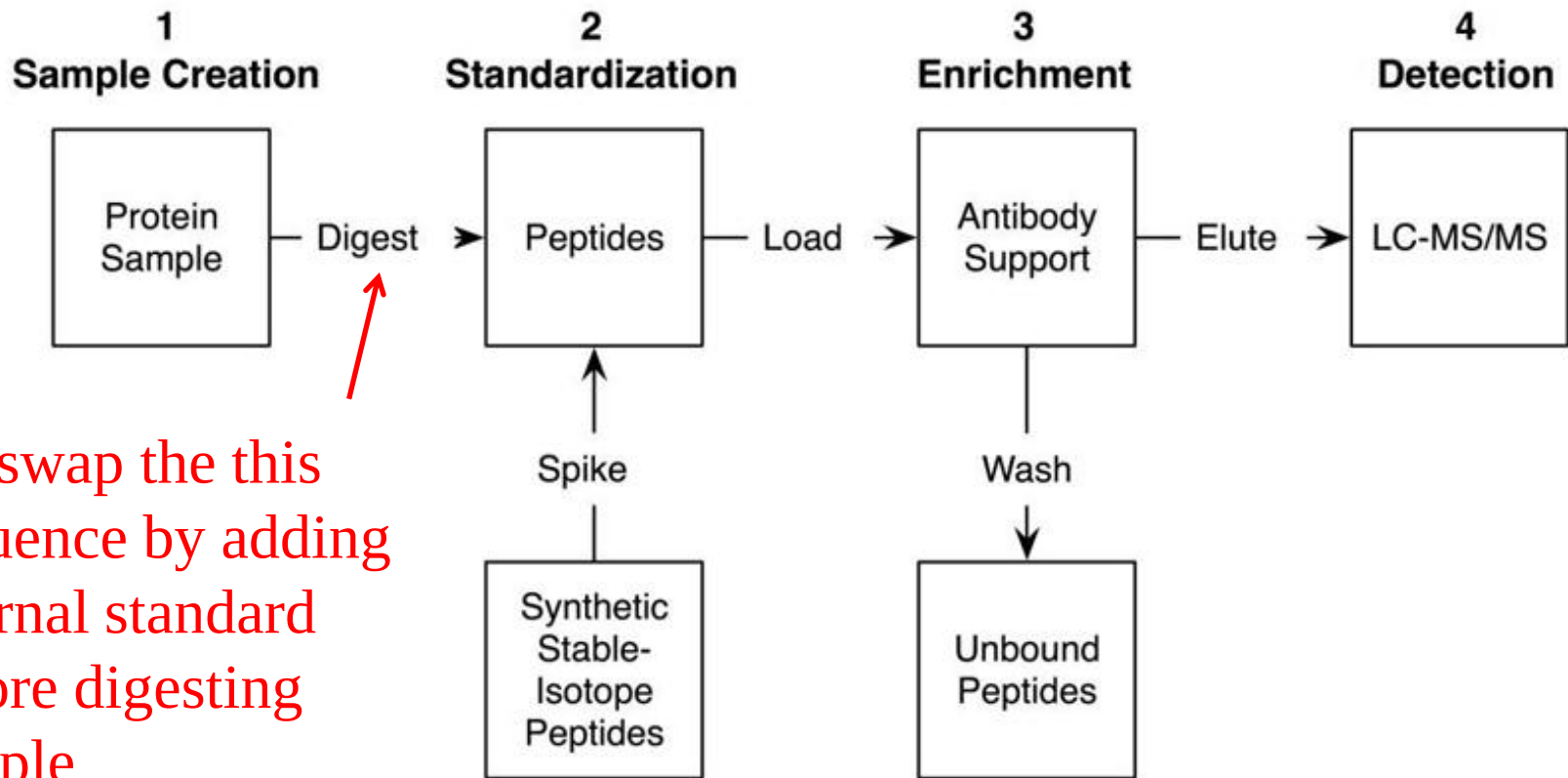
Internal standard
compensates for
variability in
processing and
detection

SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



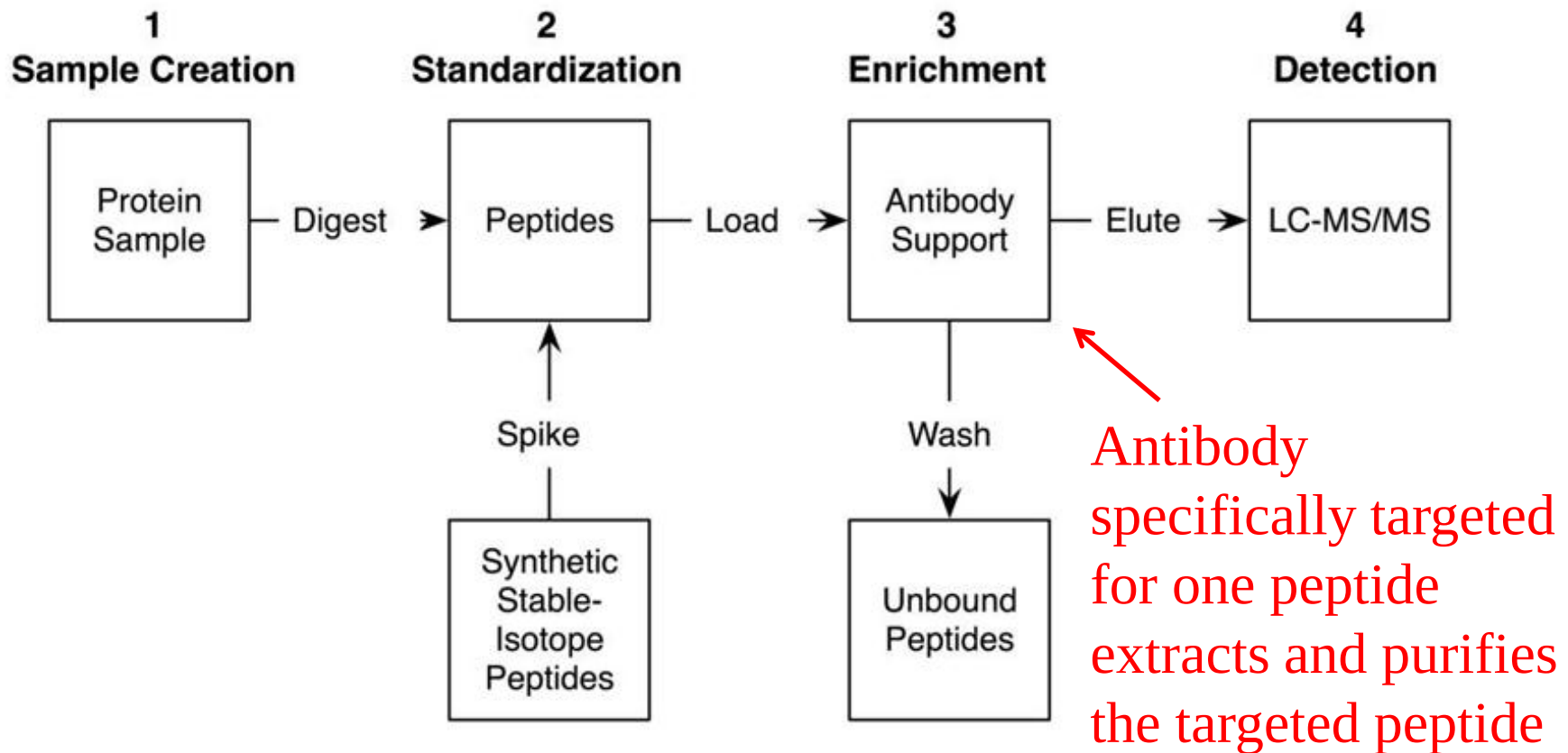
We swap the this sequence by adding internal standard before digesting sample

SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration

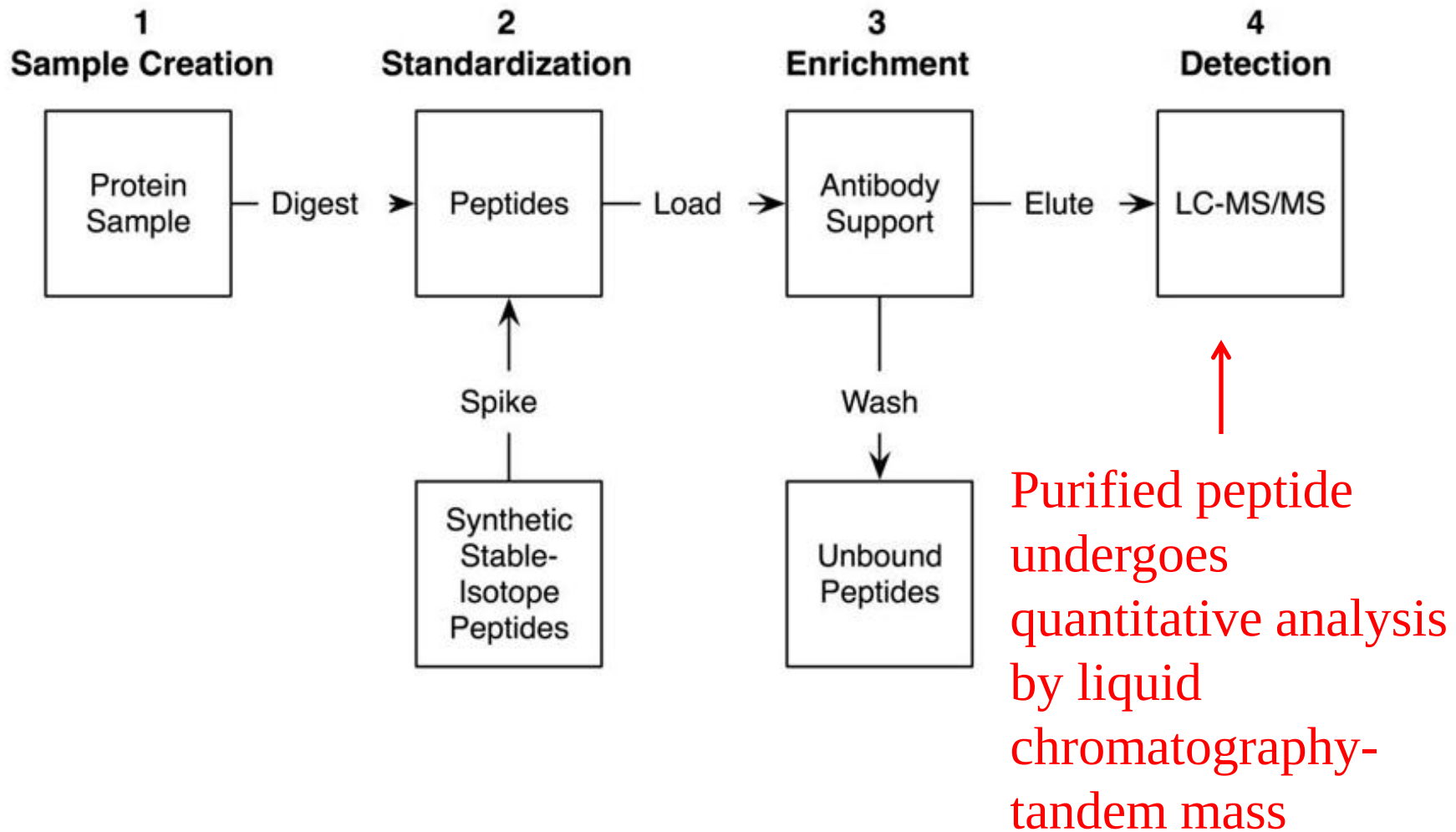


We swap the this sequence by adding internal standard before digesting sample

SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



SISCAPA: using tryptic digests and LC-MS/MS to measure protein concentration



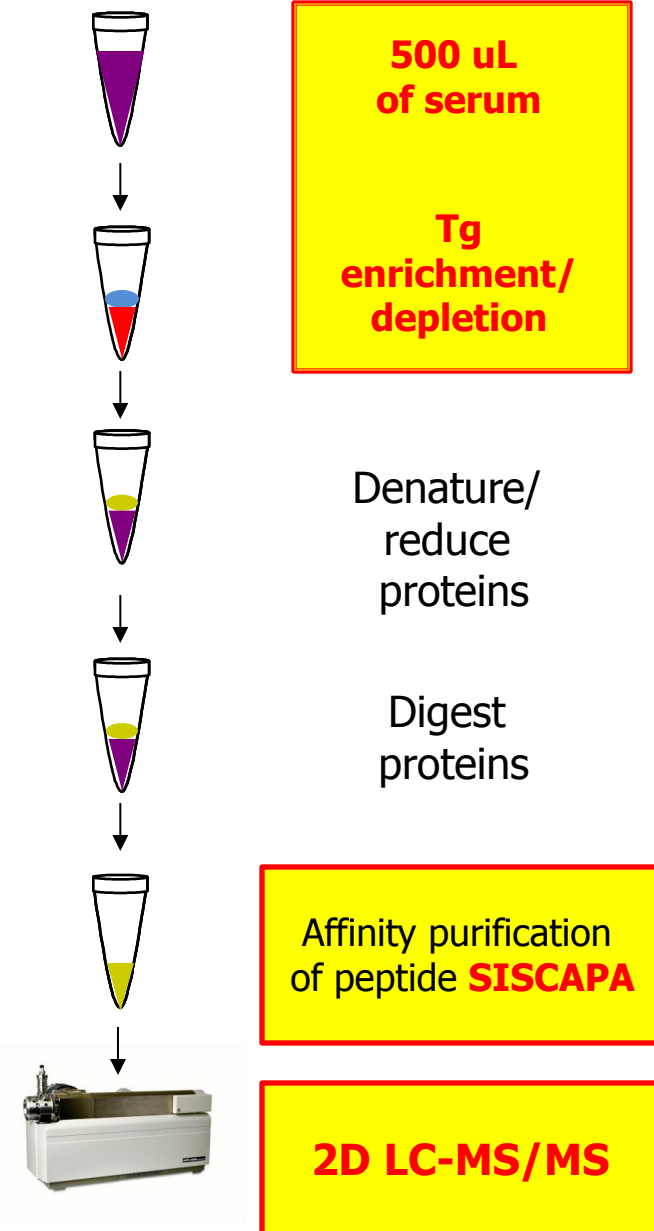
Earlier, Hoofnagle developed MS-based method at U of Washington

- Insufficiently sensitive for quantitation of Tg at concentrations representative of recurrence of TC
- Low throughput
- Uses nano-HPLC separation

Until recently, MS based methods for Tg were not generally available in clinical laboratories

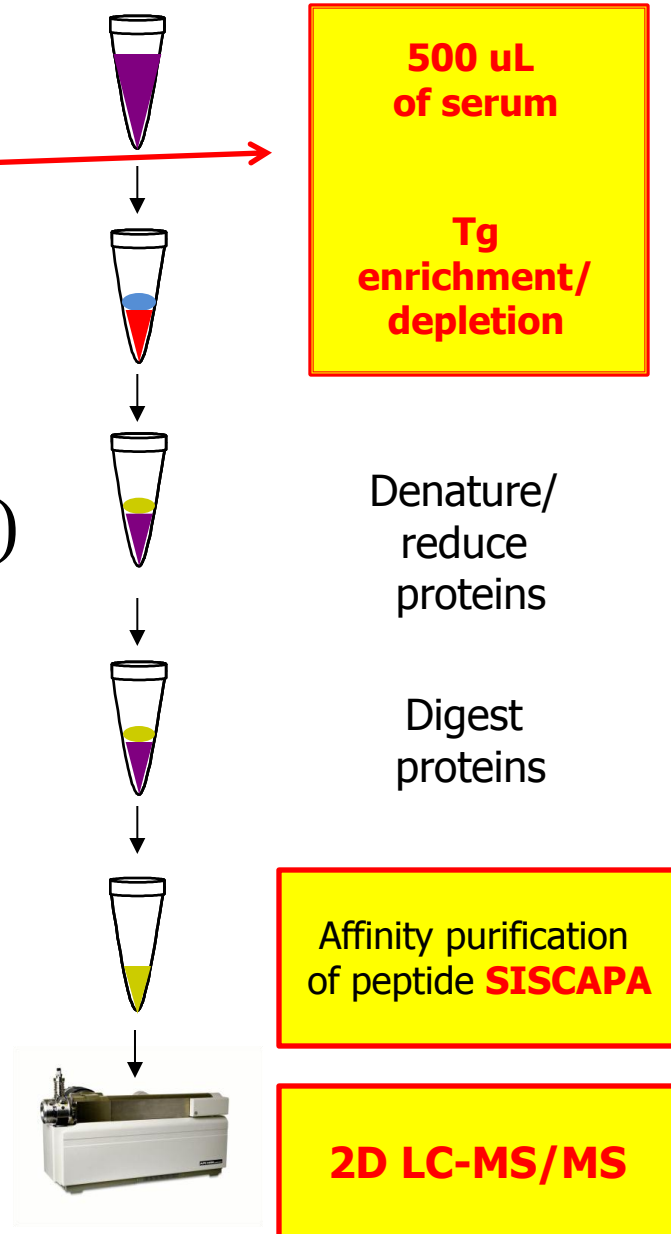
LC-MS/MS, new method overview

- Tg enrichment/ depletion of unrelated proteins
- Every step of sample preparation optimized for enhanced sensitivity/ specificity



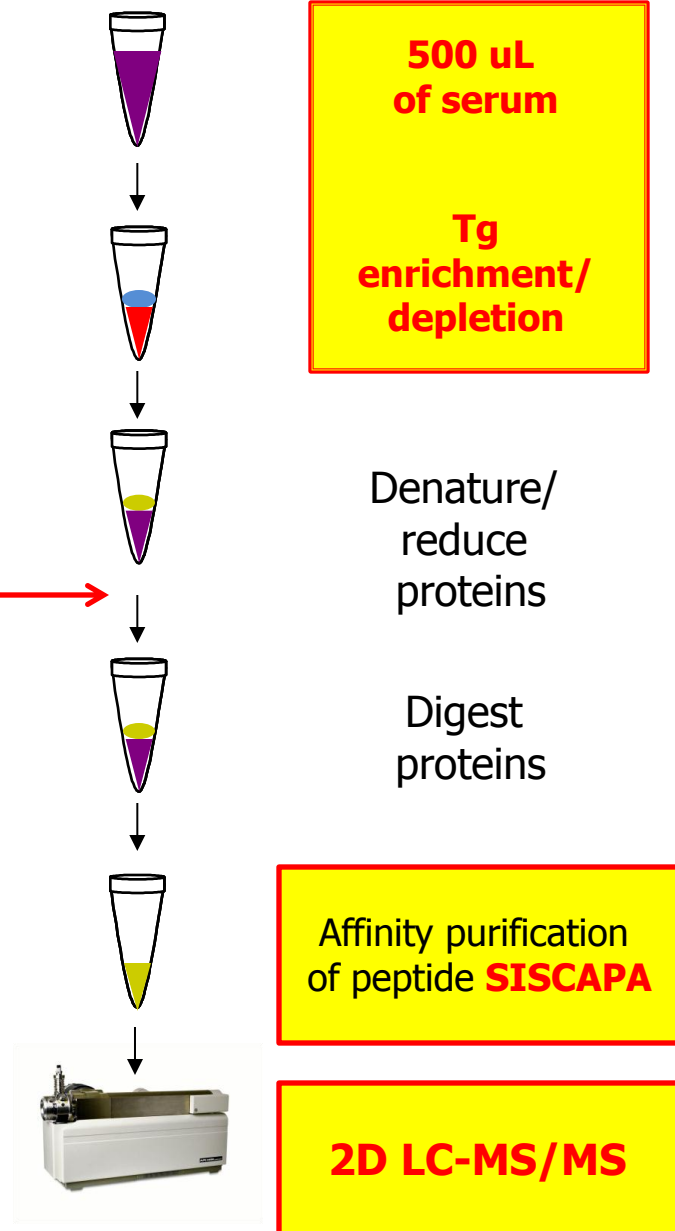
LC-MS/MS, new method – novel aspect

- Tg enrichment/ depletion of unrelated proteins
- Rabbit anti-thyroglobulin antibody added (if you can't lick 'em, join 'em)
- Precipitation of IgG and IgG-bound thyroglobulin using ammonium sulfate
- Rinse precipitate and re-dissolve



LC-MS/MS, new method – novel aspect

- Isotopically labeled internal standard added prior to digestion
 - “Winged” peptide
 - **xxxx-VIFDANAPVAVR-xxxx**
- Winged peptide helps control for recovery during digestion

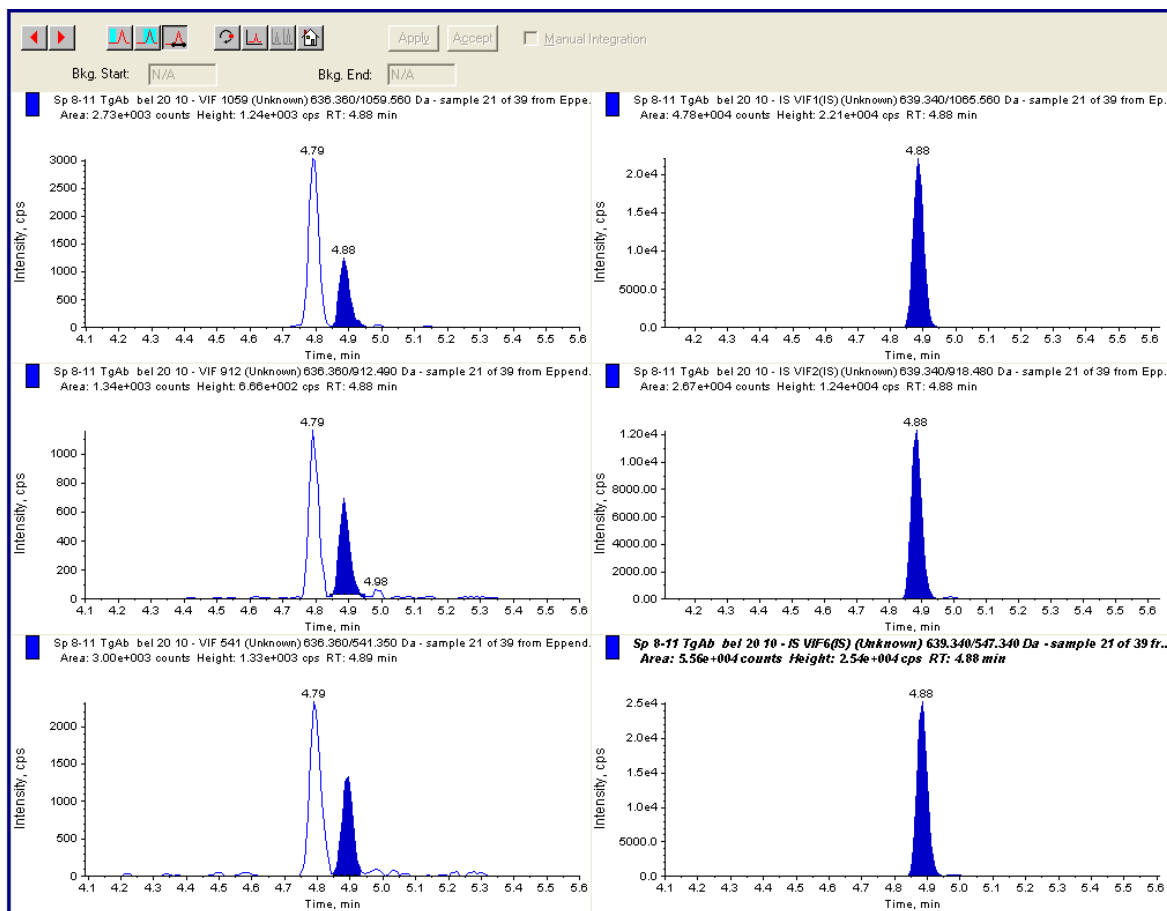


Method performance

Chromatogram of Tg-specific Peptide in Patient Sample Containing 5 ng/mL of Tg

Tg peptide

Internal standard



Clean
chromatograms

Quantitative Calibration

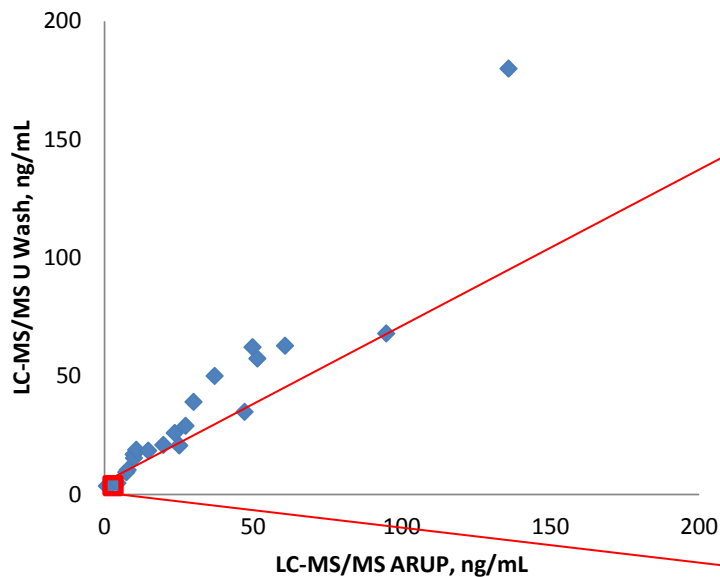


Imprecision

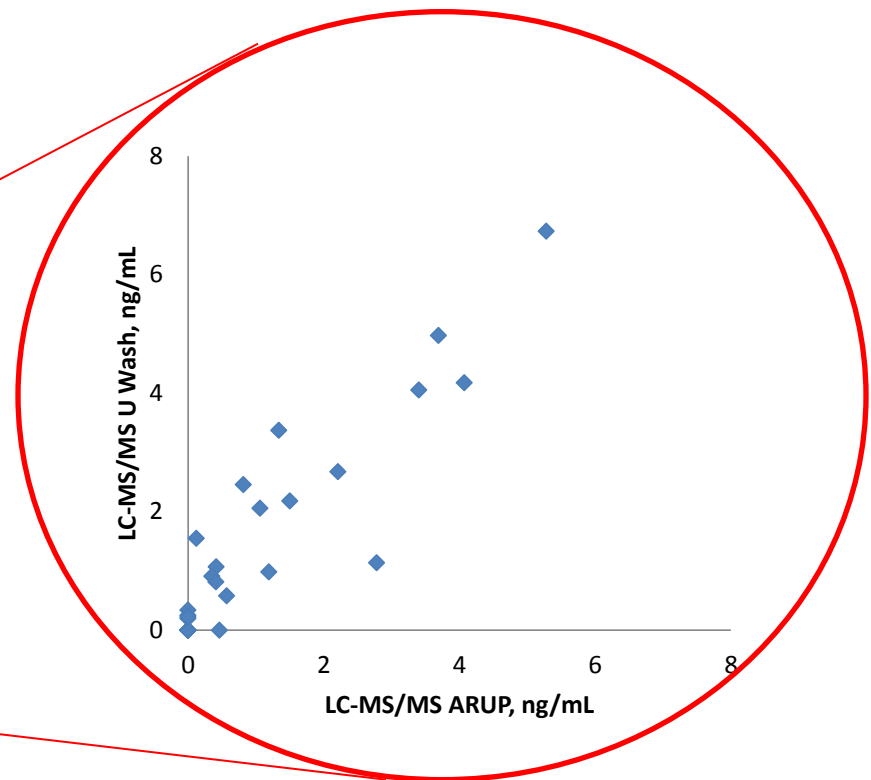
Tg concentration, ng/mL	Within-run CV, %	Between-run/day CV, %	Total CV, %
2.1	6.75	3.67	7.69
5.7	6.87	5.96	9.10
14.8	6.56	5.40	8.50
399	3.56	1.71	3.95

CVs are quite good, especially considering complexity of method.

LC-MS/MS: Our Method vs. University of Washington Method for Tg-AAb Positive and Tg-AAb Negative Samples

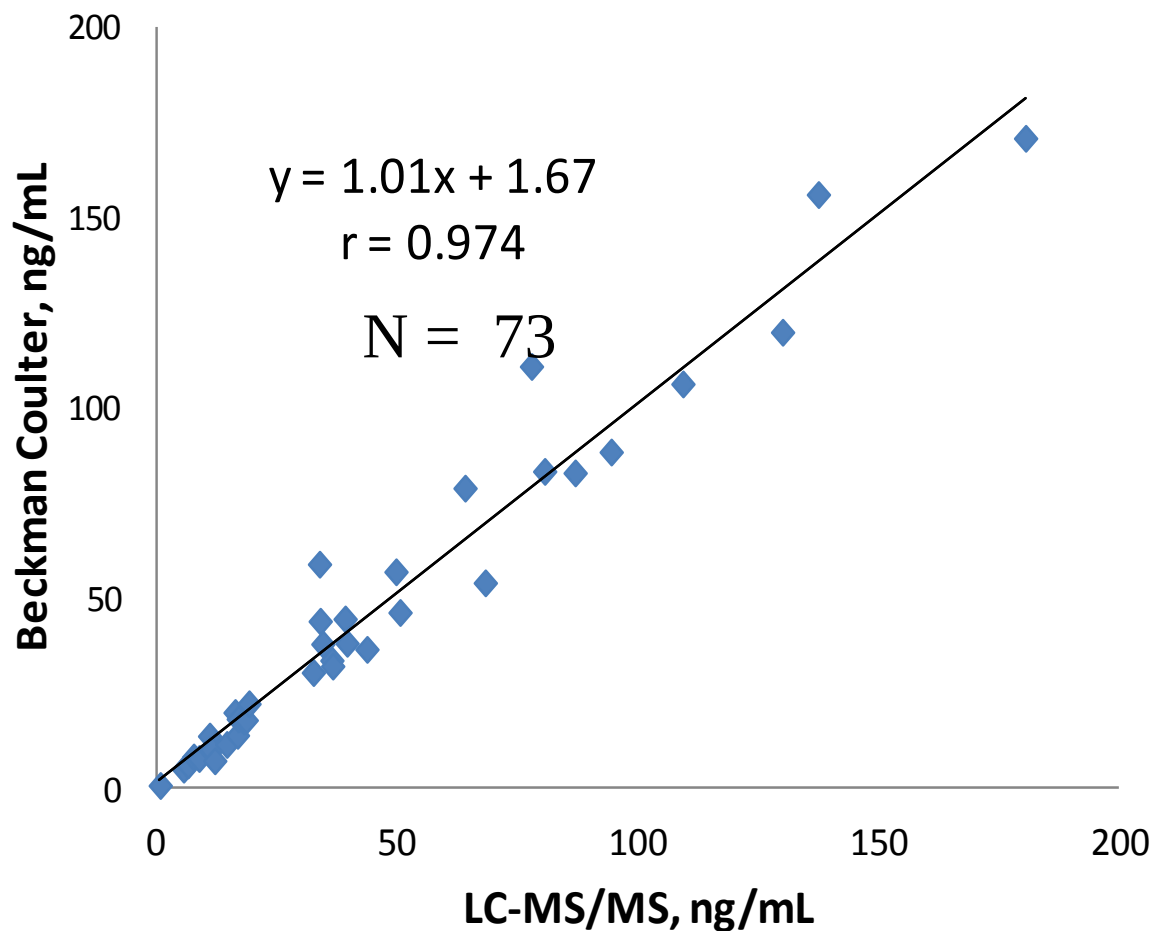


$$y = 1.17 * x - 1.81$$
$$S_{y/x} = 8.14$$
$$r = 0.951$$

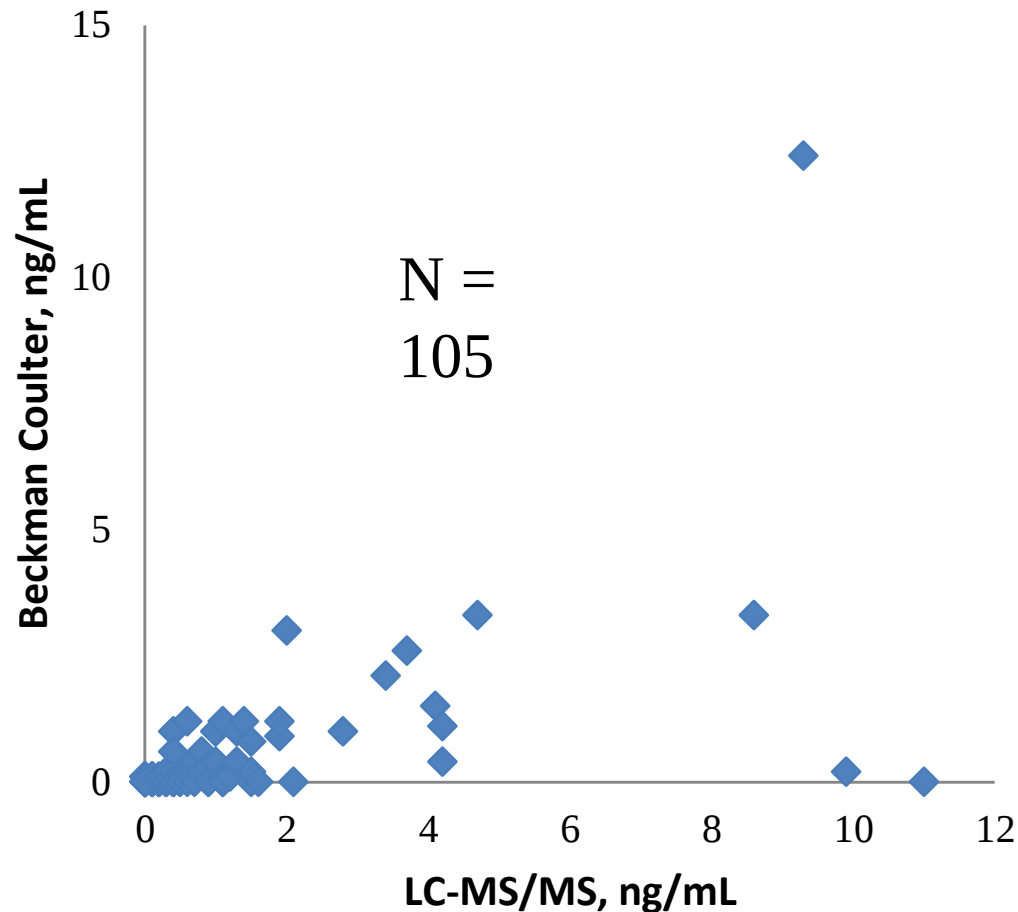


$$y = 1.23 * x + 0.15$$
$$S_{y/x} = 0.475$$
$$r = 0.916$$

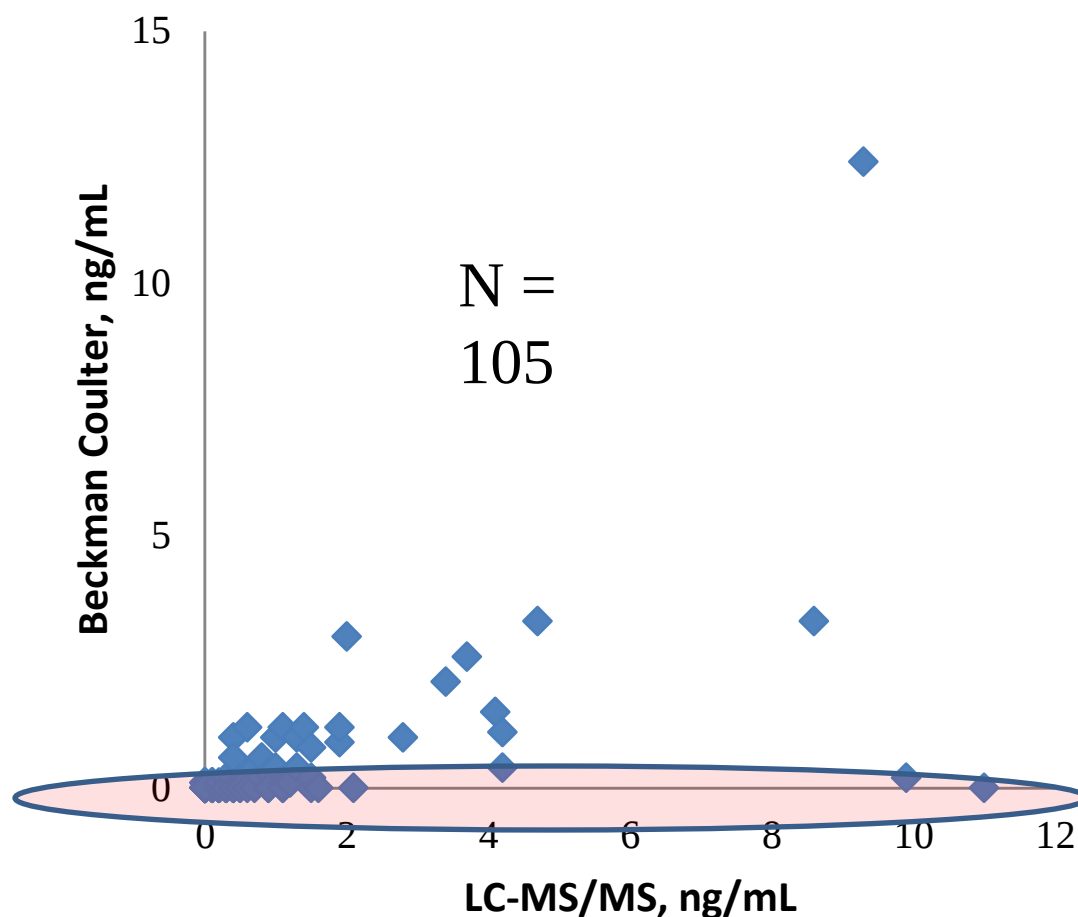
Our LC-MS/MS Method vs. Access[®] Beckman Coulter Method for Tg-AAb Negative Samples



Our LC-MS/MS Method vs. Access[®] Beckman Coulter Method for Tg-AAb Positive Samples



Our LC-MS/MS Method vs. Access[®] Beckman Coulter Method for Tg-AAb Positive Samples



Conclusions

- Serum Tg is the best marker for follow-up of patients with Differentiated Thyroid Carcinoma
- No commercial immunoassays for measuring Tg in samples of patients positive for Tg-AAb
- This LC-MS/MS method allows accurate measurement of Tg in presence of Tg-AAb
- Sensitivity of this method is likely adequate for detection of the recurrence of thyroid cancer
- Method is in routine use for testing autoantibody positive samples (>3000 samples so far)

Acknowledgements

- Andy Hoofnagle: External collaborator on this project and developer of an earlier related method for thyroglobulin
- William Roberts: to whom this presentation is dedicated, for many encouraging and helpful discussions as well as keen insights into method development for clinical pathology