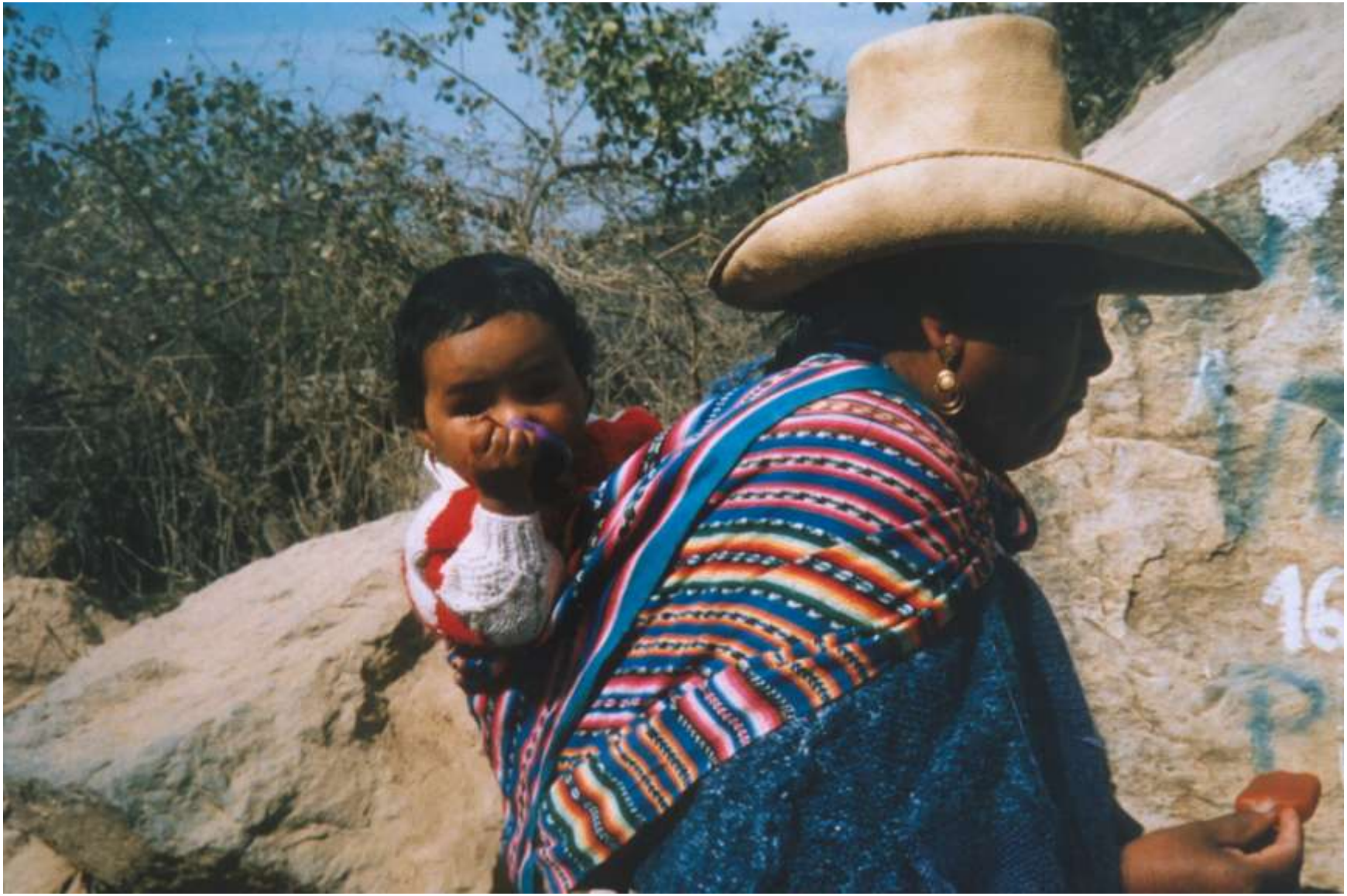


EL PATOLOGO INTERVENCIONISTA EN EL DIAGNOSTICO DEL NODULO TIROIDEO

XX CONGRESO DE LA SOCIEDAD ESPANOLA DE CITOLOGIA
Zaragoza, Mayo 19, 2011

Ricardo H. Bardales, MD
Sacramento, CA
RHBardales@aol.com

Journey

- **Historical perspective**
- Palpation-guided FNA (PG-FNA)
- Ultrasound-guided FNA (USG-FNA)
  - Non-palpable lesions: thyroid, thyroid bed, LNs
- Molecular thyroid cytopathology
- Thyroid cancer – therapeutic targets

Greig EDW & Gray ACH. Lancet 1904;1:1570.

Clinical Notes:

MEDICAL, SURGICAL, OBSTETRICAL, AND THERAPEUTICAL.

NOTE ON THE LYMPHATIC GLANDS IN SLEEPING SICKNESS.[1]

By E. D. W. Greig, M.B., C.M. Edin.,
Captain, I.M.S.,
and
A. C. H. Gray, M.B. Lond., M.R.C.S. Eng., L.R.C.P. Lond.,
Lieutenant, R.A.M.C.

Following a suggestion of Dr. F. W. Mott we have examined the contents of lymphatic glands during life from 15 sleeping sickness patients. In all of them actively motile trypanosomes were very readily found in cover-glass preparations taken from the cervical glands. They were also present in other glands, such as the femoral, but were not nearly so numerous. We found the trypanosomes to be far more numerous in the glands than in the blood or cerebro-spinal fluid and we believe that the examination of fluid removed from lymphatic glands will prove to be a much more rapid and satisfactory method of diagnosing early cases of sleeping sickness than the examination of the blood.

At first the glands were excised but this was soon found to be unnecessary, as it is easy to puncture a superficial gland with a hypodermic syringe and to suck up some of the juice into the needle and to blow this out on a slide.

Martin EH & Ellis EB. Ann Surg 1930;92:169.

BIOPSY BY NEEDLE PUNCTURE AND ASPIRATION

By Hayes E. Martin, M.D.
and
Edward B. Ellis
of New York, N. Y.

From the Pathological Laboratory of the Memorial Hospital of New York

This paper is a presentation of technical procedures employed and results attained by securing tissue from suspected neoplasms for histological examination by needle puncture and aspiration.

The use of some form of trocar or needle to obtain tissue from the living subject is not a new procedure. The Mixter punch, a blunt-tipped trocar sharpened with the bevel on the inside, was devised by S. J. Mixter some twenty-five to thirty years ago and has been quite generally employed to obtain specimens of brain tissue. We have been unable to find any published record of its description. Ward,[1] in 1912, suggested needle puncture and aspiration of lymph nodes in the study of lymphoblastomas, and Guthrie,[2] in 1921, reported his observations on the aspiration of nodes in Hodgkin's disease. Goeller,[3] in 1920, devised a trocar with a spiral cutting tip for securing tissue from the prostate. Forkner,[4] in 1927, presented a method by which he obtains a small amount of tissue by the use of a dental broach inserted through an 18-gauge needle, and summarized the results of study

10.	Carcinoma (?) of thyroid or larynx	Tumor at anterior base of neck	Epidermoid carcinoma, probably from larynx
11.	Carcinoma (?) of thyroid or larynx	Tumor at anterior base of neck	Epidermoid carcinoma
48.	Carcinoma (?) of thyroid	Neck mass	Numerous large spindle tumor cells, some with mitoses. Probably large spindle-cell thyroid carcinoma

Stewart FW. Am J Pathol 1933;9:801.

THE DIAGNOSIS OF TUMORS BY ASPIRATION *

FRED W. STEWART, M.D.

(From the Pathological Laboratory of the Memorial Hospital, New York, N. Y.)

During the past three years the author has studied smears of material from some 2500 tumors obtained by aspiration with an 18 gauge needle. The method of securing the tissue has been described by Martin and Ellis,[1] but since the actual technique is of considerable importance it may be described again in brief. The needle used is an ordinary 18 gauge needle attached to a Record syringe. The skin is infiltrated by a drop of novocaine and a very small puncture wound is made with a bistoury to avoid contamination of the tissue to be aspirated with surface epithelium. The needle is introduced with the piston *closed* until it is felt to enter the neoplasm. Then the piston is partially withdrawn, thus creating a negative pressure within the syringe, and the needle is advanced into the tumor. Portions of tumor are forced by the negative pressure into the needle. The manoeuver is usually repeated once without withdrawing the needle from the mass. When the needle is withdrawn care is taken to release the piston gradually, in order to prevent a sudden spraying of the contents of the needle over the walls of the syringe. Material obtained is placed on a slide and smeared out by pressure with another slide. Gentle heat is applied and the smear is dropped into alcohol for a minute or two, after which it is stained with hematoxylin and eosin, dehydrated, cleared and mounted like any section. If much fluid is obtained small fragments of tissue are selected with a wire loop and smeared on slides, or, if sufficiently large, they are sometimes sectioned. Blood clots may be sectioned in the same manner as tissue.

Historical Perspective II

- Since 1940's Lopes-Cardozo (Holland) & Soderstrom (Sweden) practiced FNA & published books on FNA & hematologic techniques (1954 y 1966)
- 1967-74 Franzen & Zajicek applied FNA to prostate and other superficial organs
  - Franzen: 1960 designed "syringe holder"
- FNA remained almost dormant in USA
- Timid beginning in the early 70's
- Currently PG-FNA or USG-FNA is essential for diagnosing thyroid nodules.

Fig. 2. Joseph Zajicek.

FIGURE 1. Dr. Franzen is survived by his wife, Anne-Lie, 5 children, grandchildren, and the joyful memories of those who knew and worked with him.

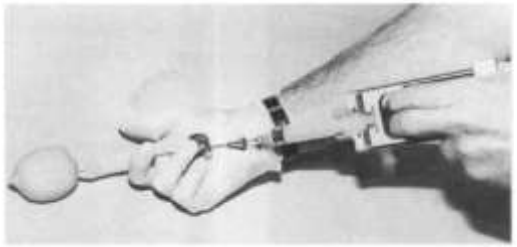
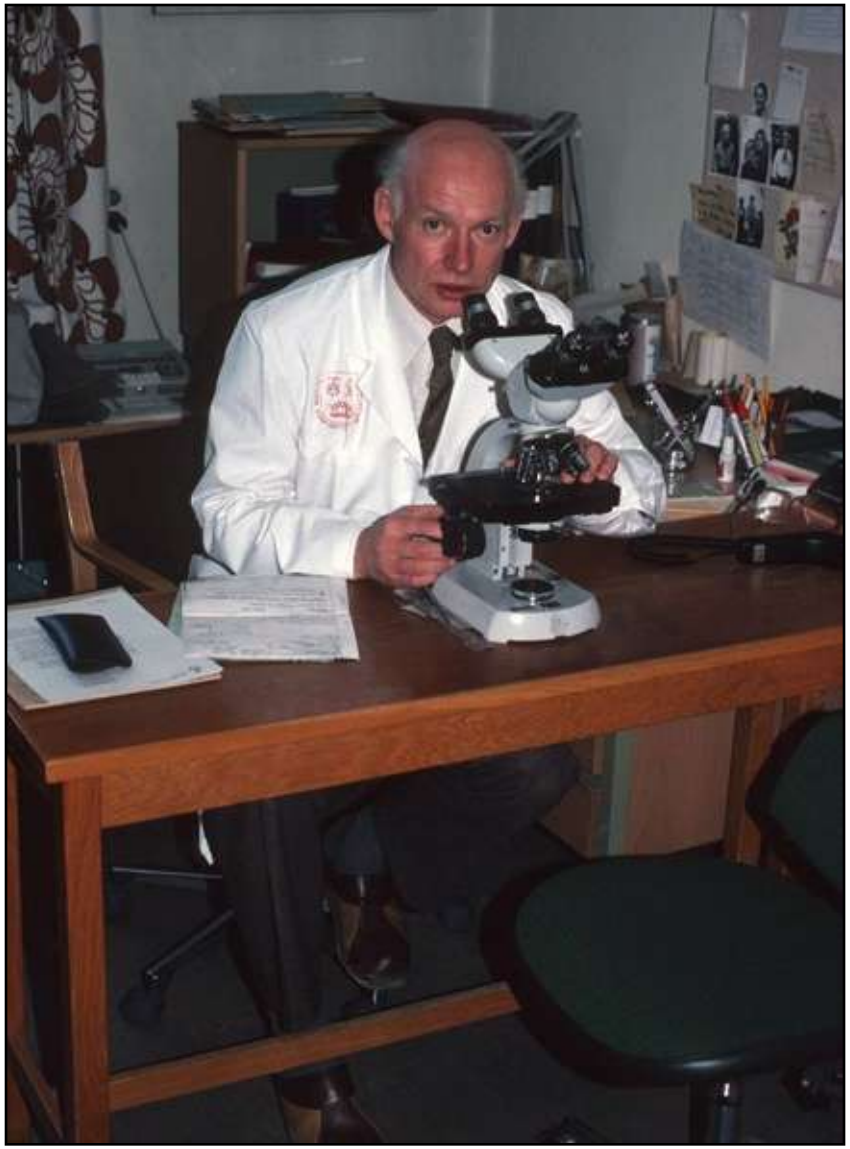


Fig. 5. Torsten Löwhagen. This illustrates aspiration of a portion of liver used as a target organ to obtain cytologic material and demonstrate slide-making.

Diagnostic Cytopathology, Vol 1, No 4, Oct–Dec 1985

CANCER (CANCER CYTOPATHOLOGY) October 25, 2008 / Volume 114 / Number 5

Torsten Löwhagen, M.D., 1929-1999



The Thyroid Nodule

Facts

- Thyroid nodules are common
- Thyroid cancer is uncommon
- Papillary thyroid cancer is a “good” cancer
- Most patients are not biopsied
- Clinical follow-up is unknown by the cytopathologist

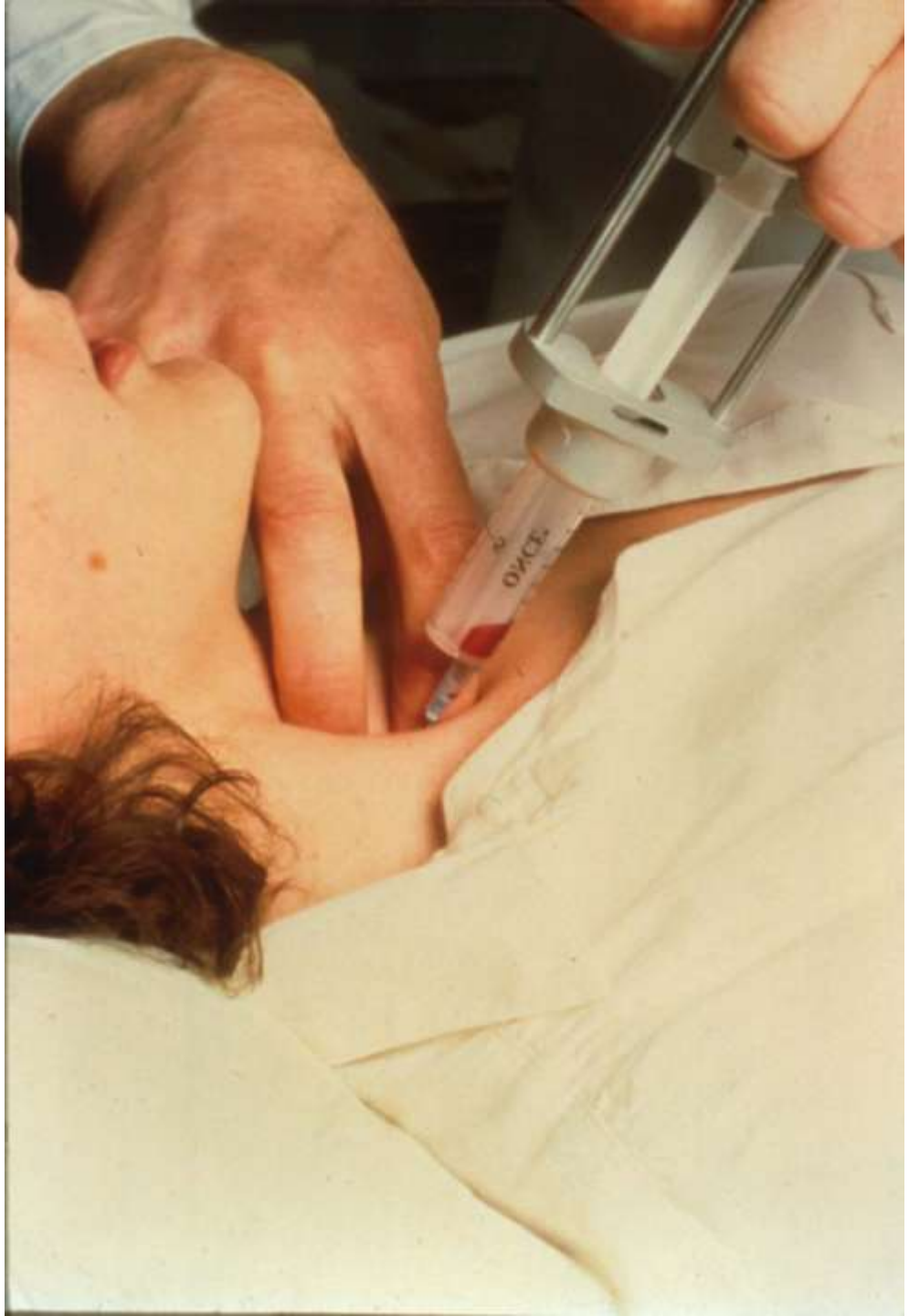
- “Should thyroid nodules be biopsied?”

More Facts

- Follicular carcinoma is very rare
- Most follicular tumors are benign
- Papillary thyroid cancer means thyroidectomy
- Frozen sections, core biopsies, and thin layer techniques are usually helpless
- FNA cytology is highly sensitive and specific except for the “undetermined” diagnosis.
  - 20% - 30% TOO MUCH!!!

Journey

- Historical perspective
- **Palpation-guided FNA (PG-FNA)**
- Ultrasound-guided FNA (USG-FNA)
  - Non-palpable lesions: thyroid, thyroid bed, LNs
- Molecular thyroid cytopathology
- Thyroid cancer – therapeutic targets

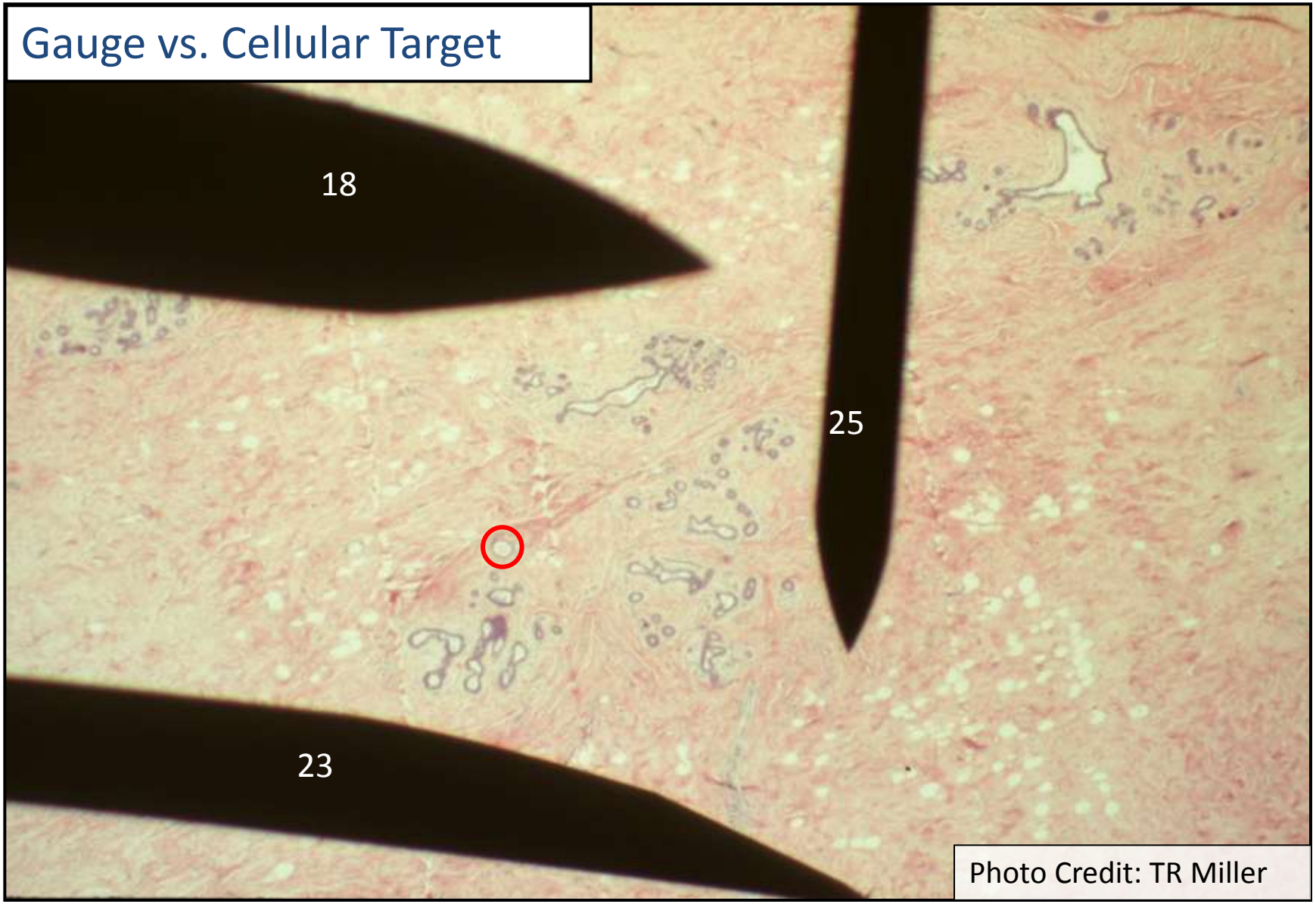


Get Better Before Reaching the Microscope!!!!

- Technical Factors
  - PG-FNA and USG-FNA
  - Needle gauge: ideal 27 and 25
  - Number of “passes”
  - Specimen handling
  - Air dried better than OH-fixed slides
  - New processing technologies: monolayer


1
2
3
4
5
6
7
8
9
10
B-D

Gauge vs. Cellular Target



Air-dried Smears: Benefits

- Easy for clinicians, It is hard for them to properly fix a smear
- Colloid is easy to quantify
- Follicular elements are easier to find when the smear is thin, not bloody, and not clotted.

TOO MANY: 36 Slides & No Joy

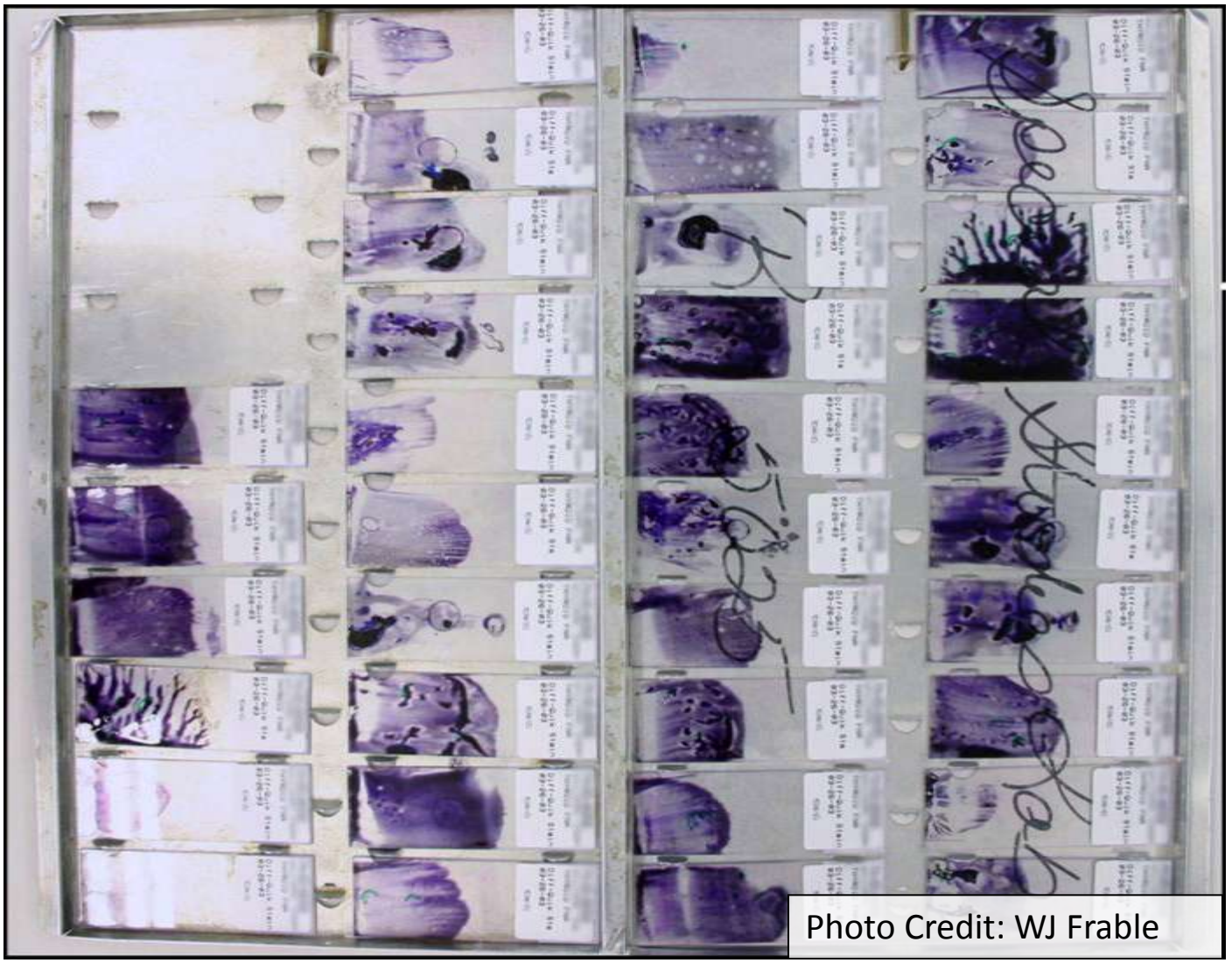
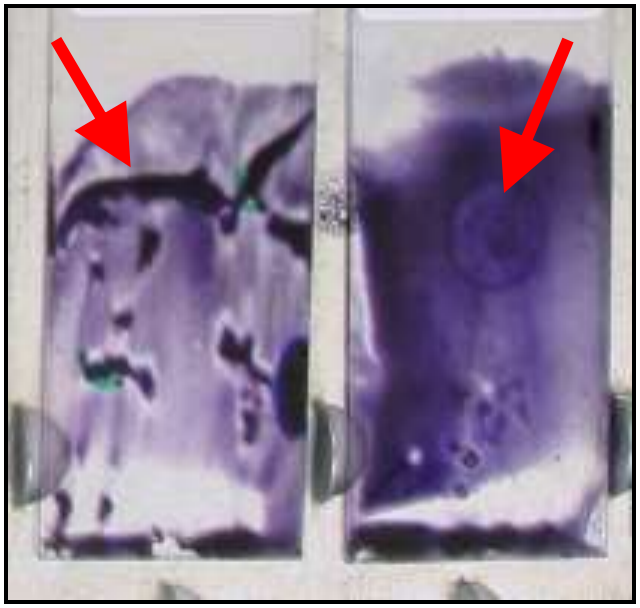
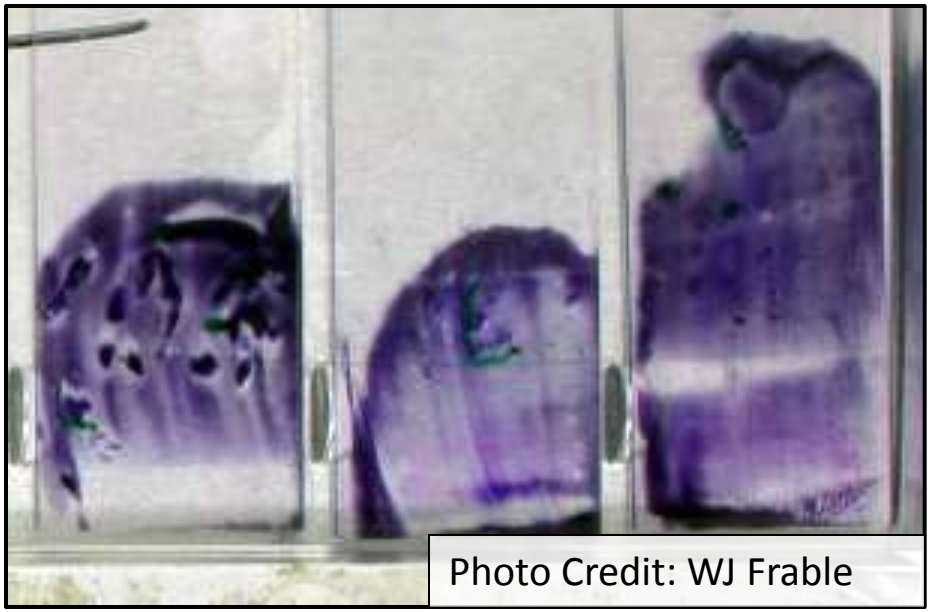


Photo Credit: WJ Frable

36 SLIDES = TOO MUCH BLOOD / CLOT





OPA SLIDE GUIDELINES:

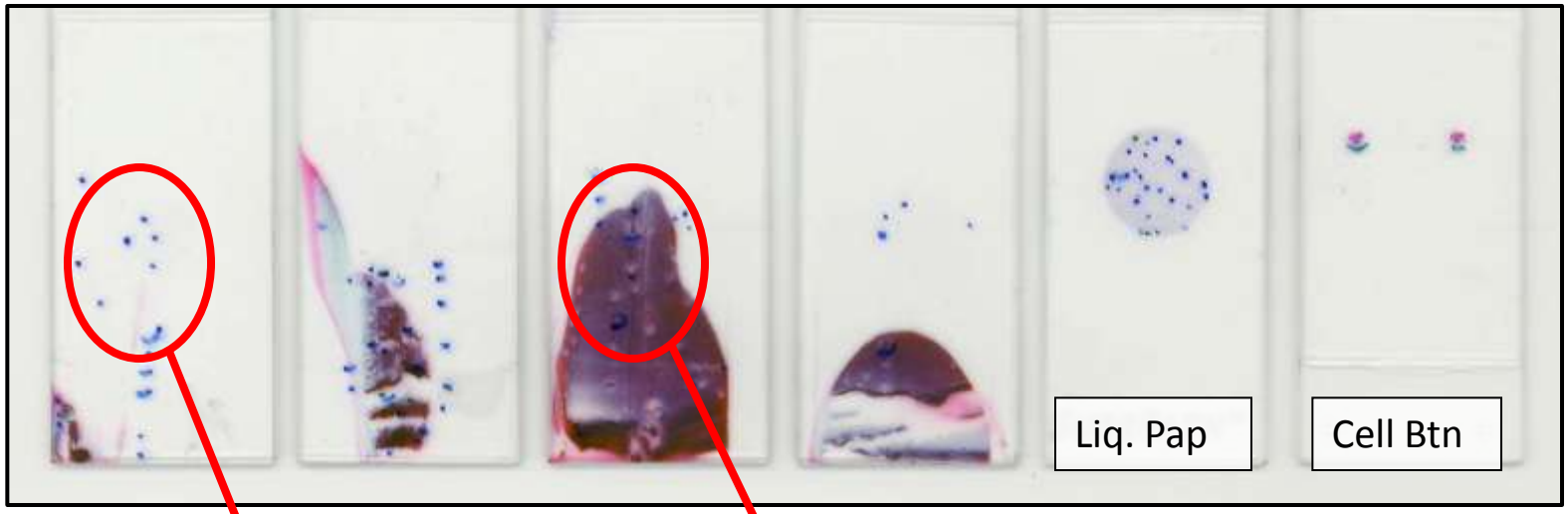
1 BX = 1 SLIDE (2 SLIDES BEGINNING; 1.5 SLIDES)

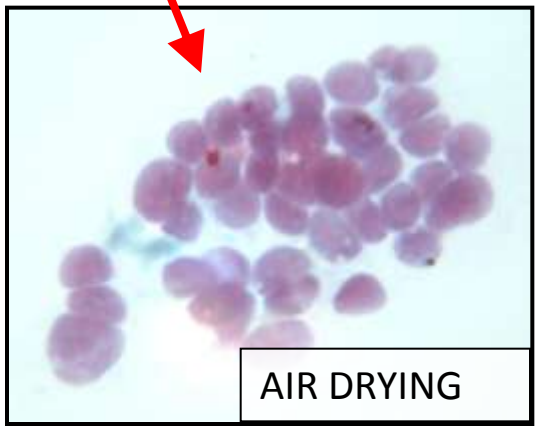
(> 2 = Suboptimal Bx Technique)

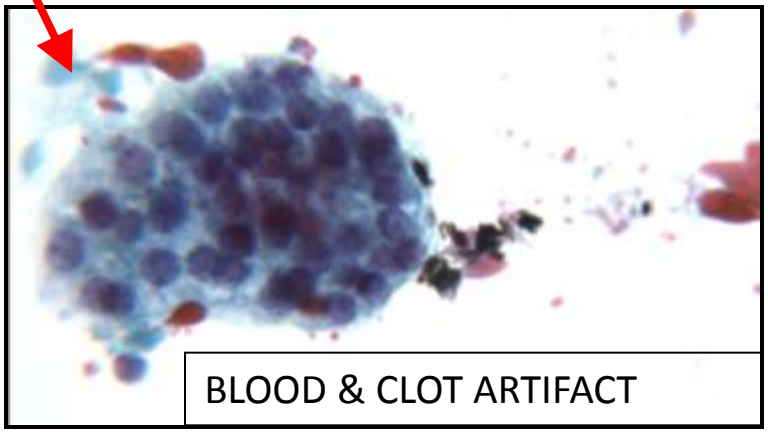
BX Cadence = 3 Seconds @ 3 / Second bml jsa

Technical Smearing Poverty

TOO BLOODY + HORRIBLE SMEARS







Technical Smearing Poverty

LBP & CB DO NOT REPLACE PROPER SMEARS

SMEARING TECHNIQUES

www.papsociety.org

THYROIDOLOGISTS

Experts in the Diagnosis and Treatment of Thyroid Disease

- **>2 cm = Bx**
  - **Exc 'hot' 123-I scan**
- **1.9-1.1 cm usually Bx**
  - **Exc hyperechoic, comet-tail**
- **1.0 & less can be followed exc:**
  - **Radiation neck as child**
  - **FHx papillary, medullary CA**
  - **Previous thyroid CA other lobe**
  - **Worrisome US**

Academy of Clinical Thyroidologist
(www.thyroidologists.com)

Free Thyroid US "Textbook"

Editorials

Radiology

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Orlo H. Clark, MD
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P. Reed Larsen, MD
Susan J. Mandel, MD
William D. Middleton, MD
Carl C. Reading, MD

Management of Thyroid Nodules Detected at US: Society of Radiologists in Ultrasound Consensus Conference Statement[1]

The Society of Radiologists in Ultrasound convened a panel of specialists from a variety of medical disciplines to come to a consensus on the management of thyroid nodules identified with thyroid ultrasonography (US), with particular focus on which nodules should be subjected to US-guided fine needle aspiration and which thyroid nodules need not be subjected to fine-needle aspiration. The panel met in Washington, DC, October 26–27, 2004, and created this consensus statement. The recommendations in this consensus statement, which are based on analysis of the current literature and common practice strategies, are thought to represent a reasonable approach to thyroid nodular disease.

Radiology 2005; 237:794-800

Journey

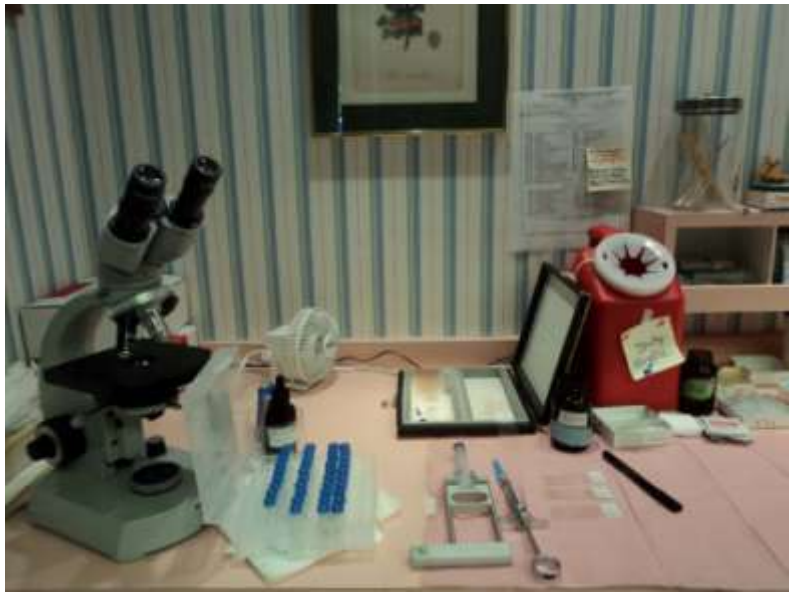
- Historical perspective
- Palpation-guided FNA (PG-FNA)
- **Ultrasound-guided FNA (USG-FNA)**
  - Non-palpable lesions: thyroid, thyroid bed, LNs
- Molecular thyroid cytopathology
- Thyroid cancer – therapeutic targets

Pathologist US Guided Benefits

- **False Physical Image**
  - Posterior Thyroid
  - Lumpy w/o Nodule
- **Edges**
  - Carotid / Larynx
- **Patient Communication**
- **Better Sample Than Radiologist**
- Market Forces – TTHALTS
  - “Pathologists Directed USGFNA”

OPA

OUTPATIENT PATHOLOGY ASSOCIATES

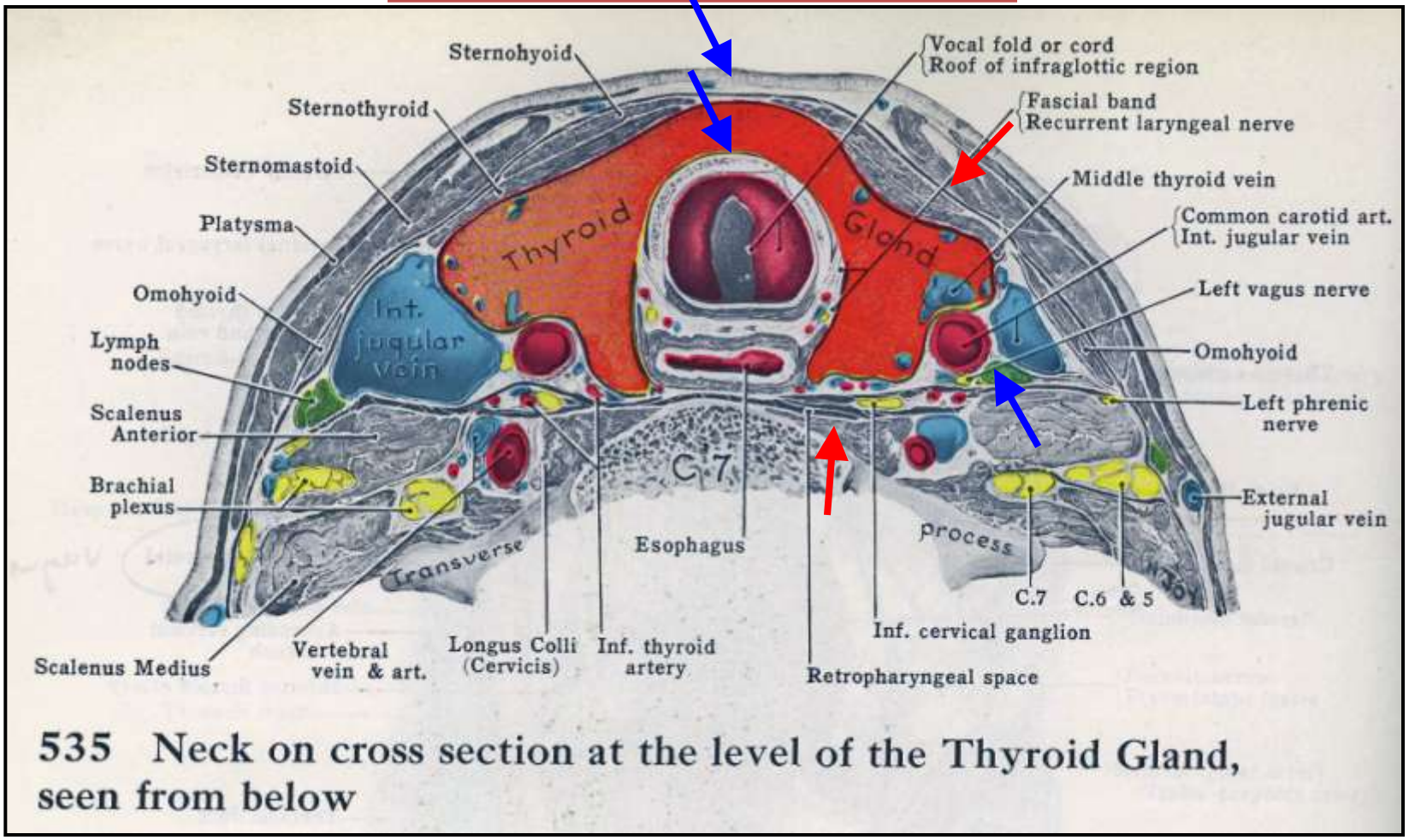


FINE NEEDLE ASPIRATION CASES

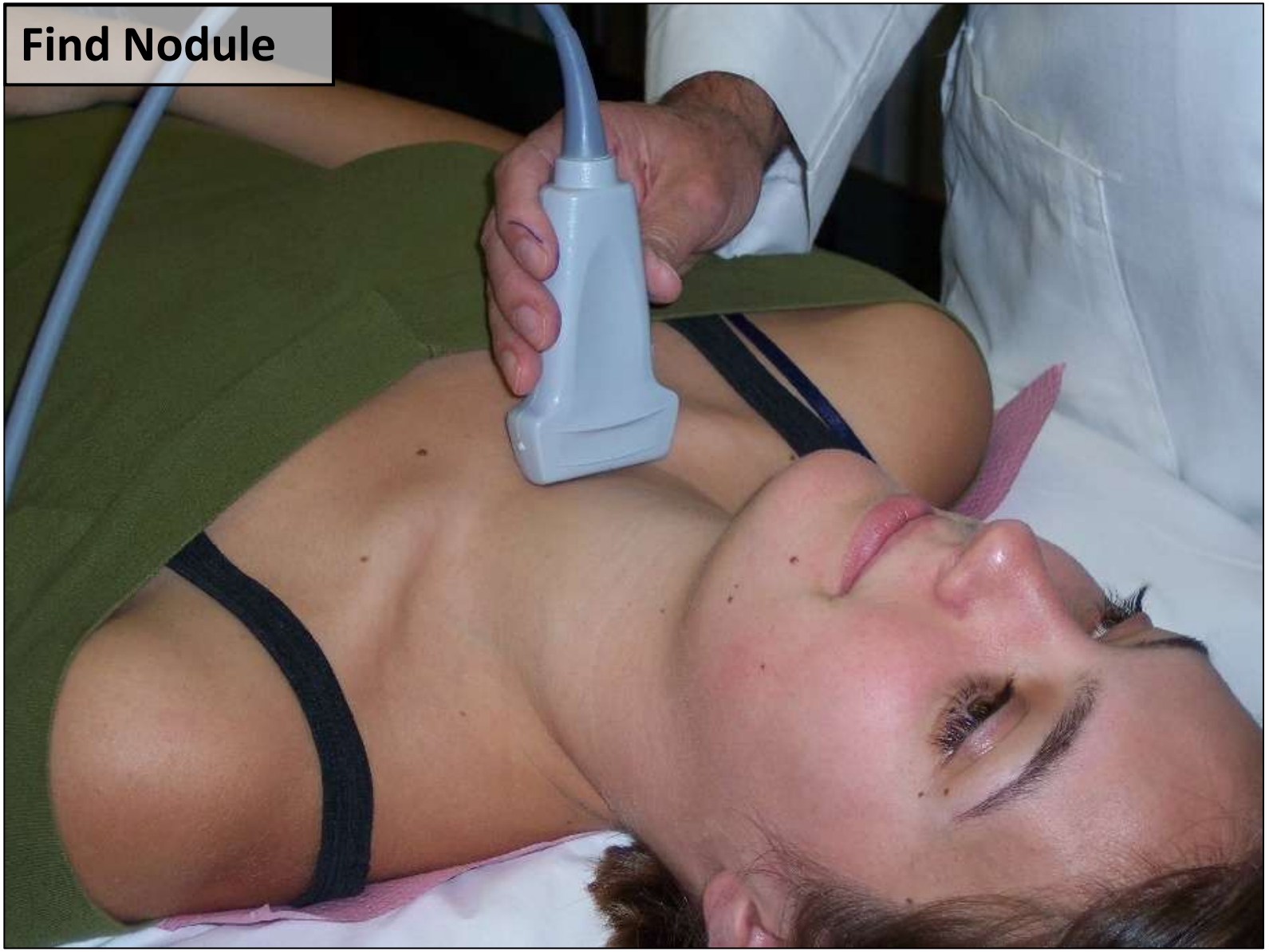
SITE	2009	%	1984-2009	%
BREAST	319	5	31865	25
THYROID	5461	82	59922	47
SOFT/LN	734	11	26089	21
SALIVARY	163	2	4918	4
PROSTATE	0	0	2980	2
MISC	7	0	1140	1
	6,684		126,914	

Outpatient Pathology Associates
Sacramento, CA 95816

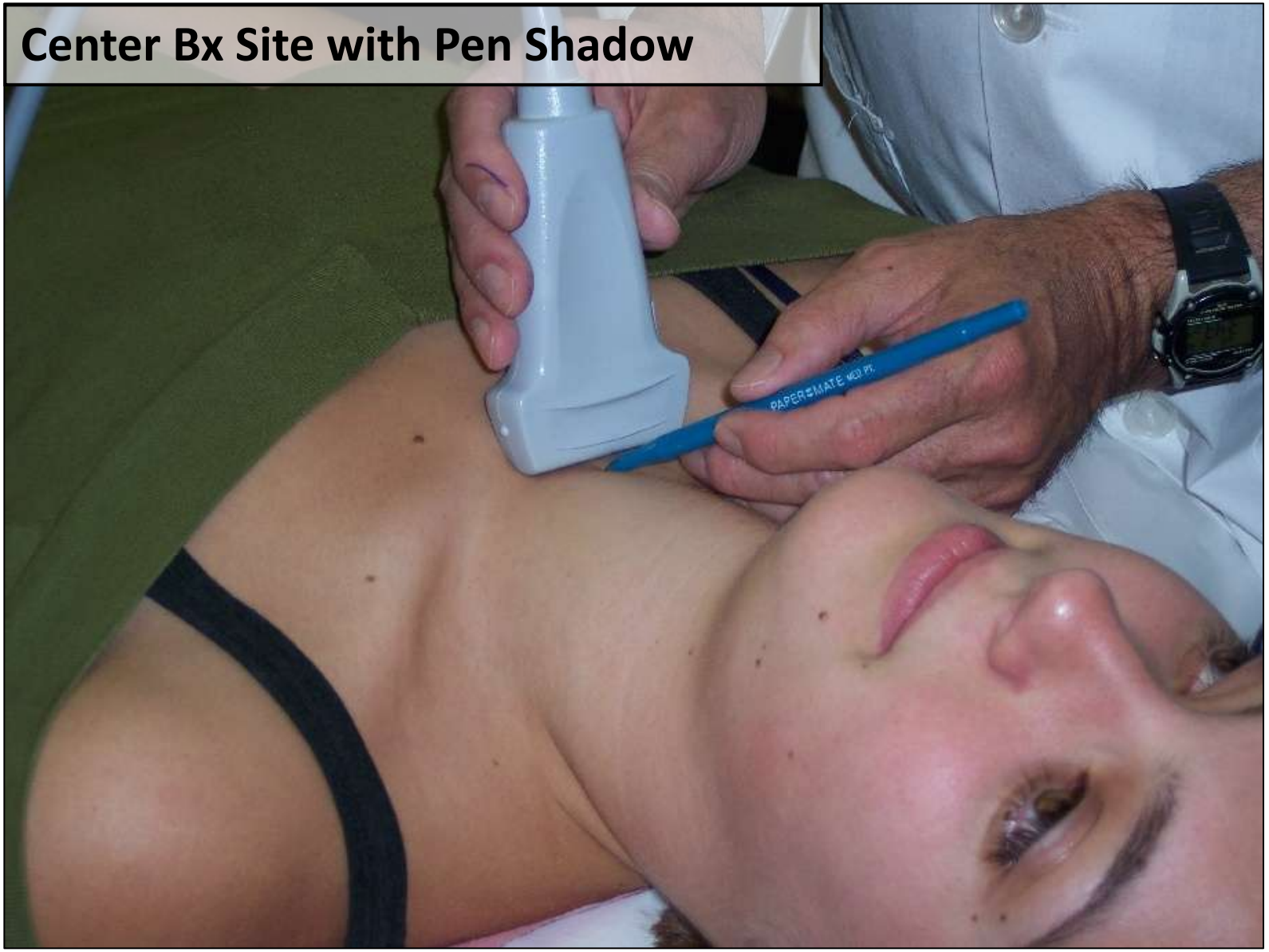
Thyroid Anatomy

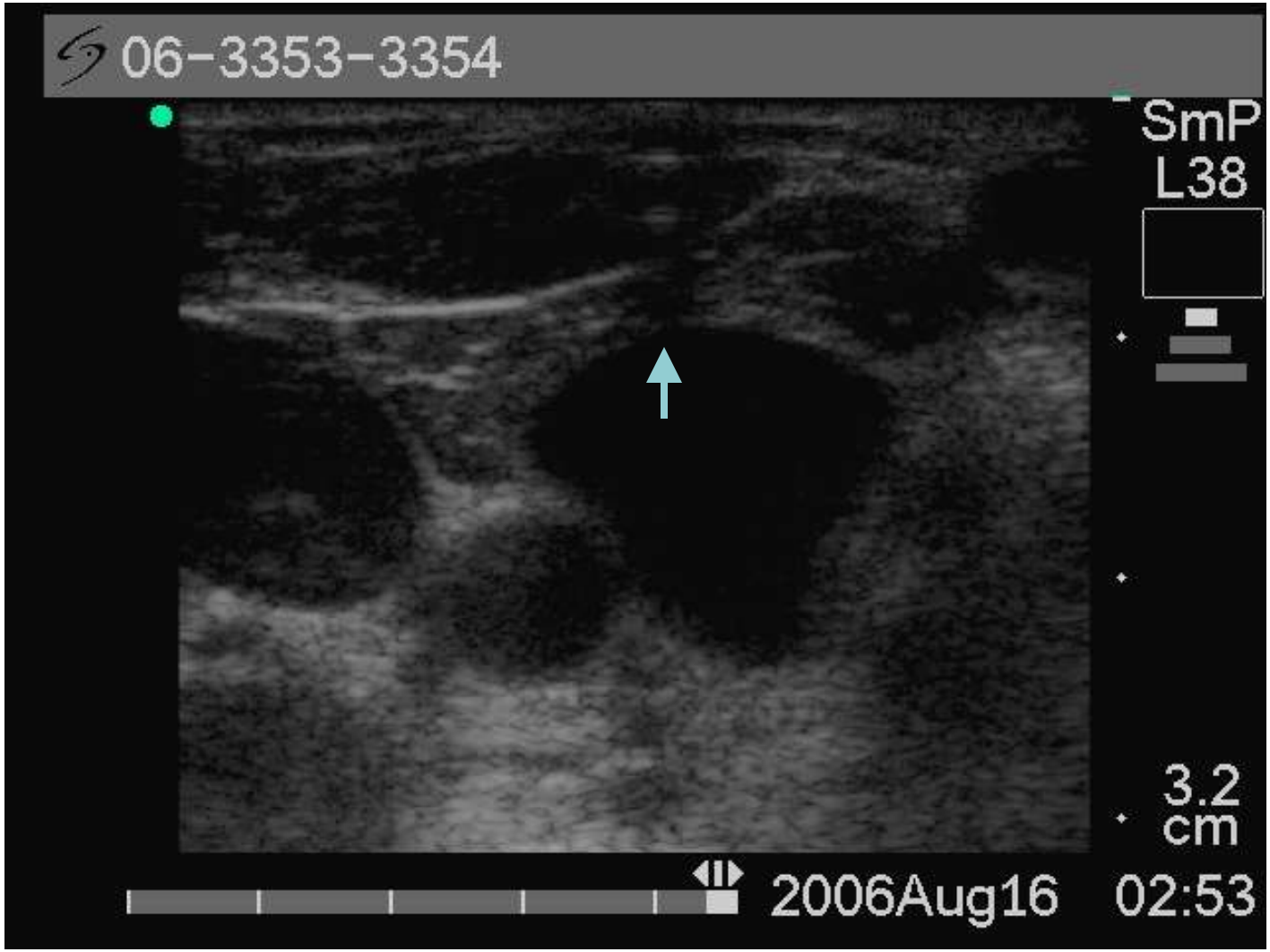


Grant's Atlas of Anatomy 1962


Find Nodule

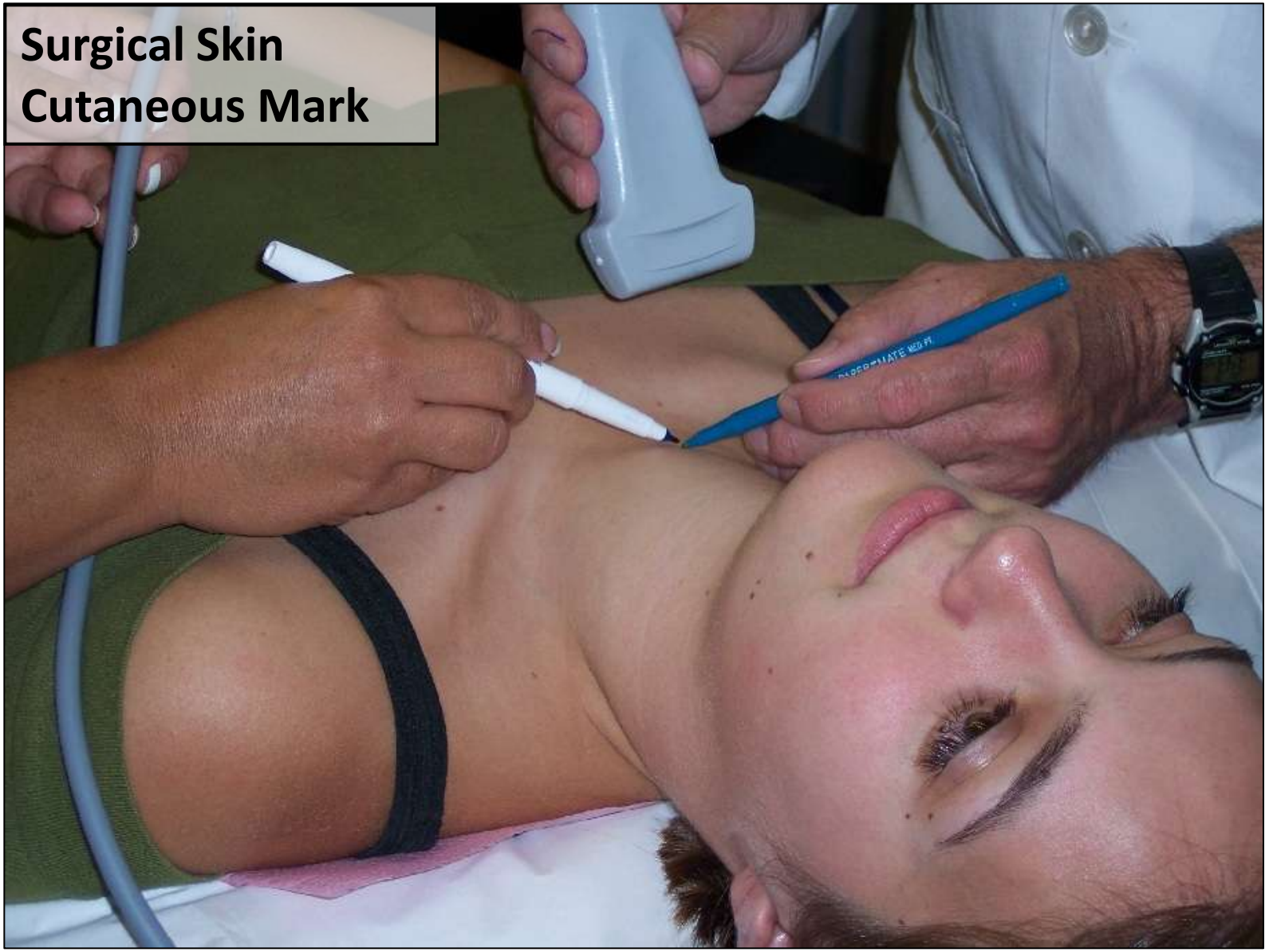
Center Bx Site with Pen Shadow





Pen Shadow to Center Nodule

Surgical Skin Cutaneous Mark



Anesthesia: 30 Gauge

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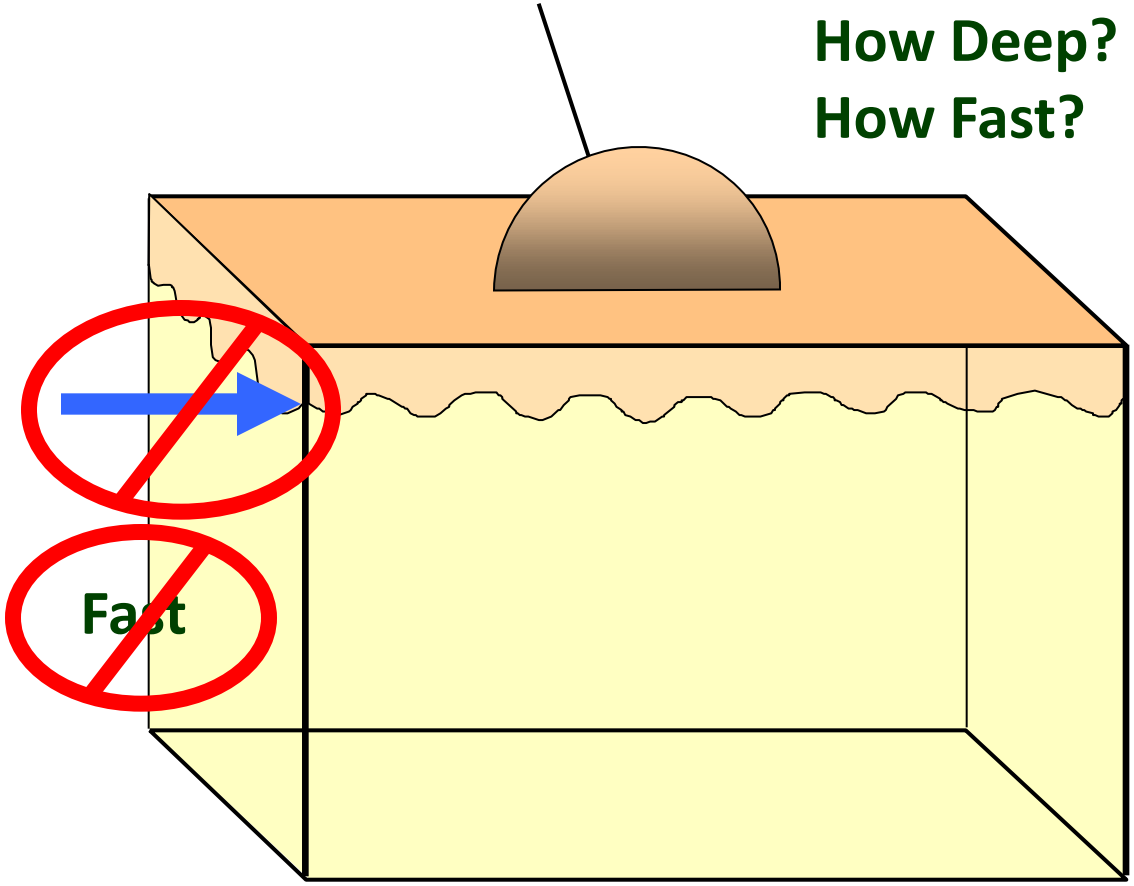
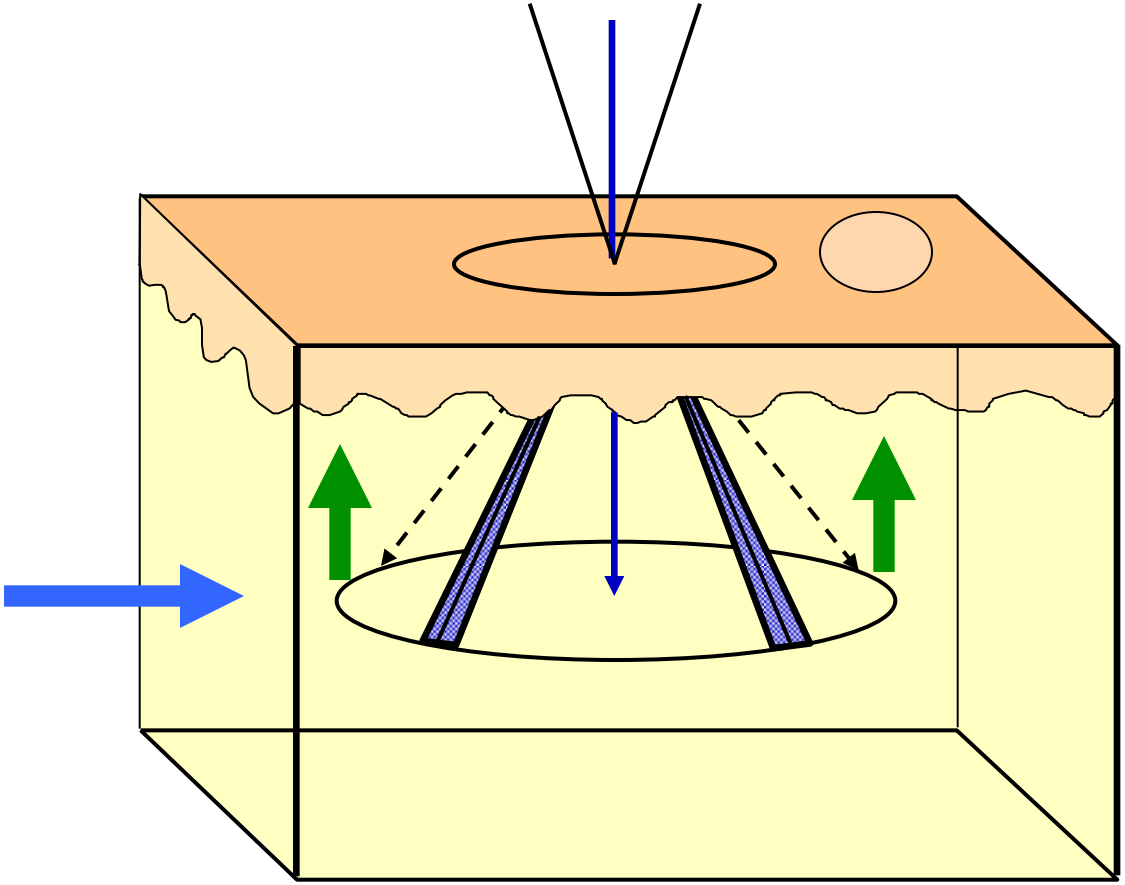
Needles Plastic Hub 30 GA #100 $9 #085-5569
Lidocaine Cart 2% Epi l:100,000 #50 $19 #085-3978
Carbocaine Cart 3% #50 $21 #085-4075
Aspirating Cartridge Syringe Holder $45 #222-4228

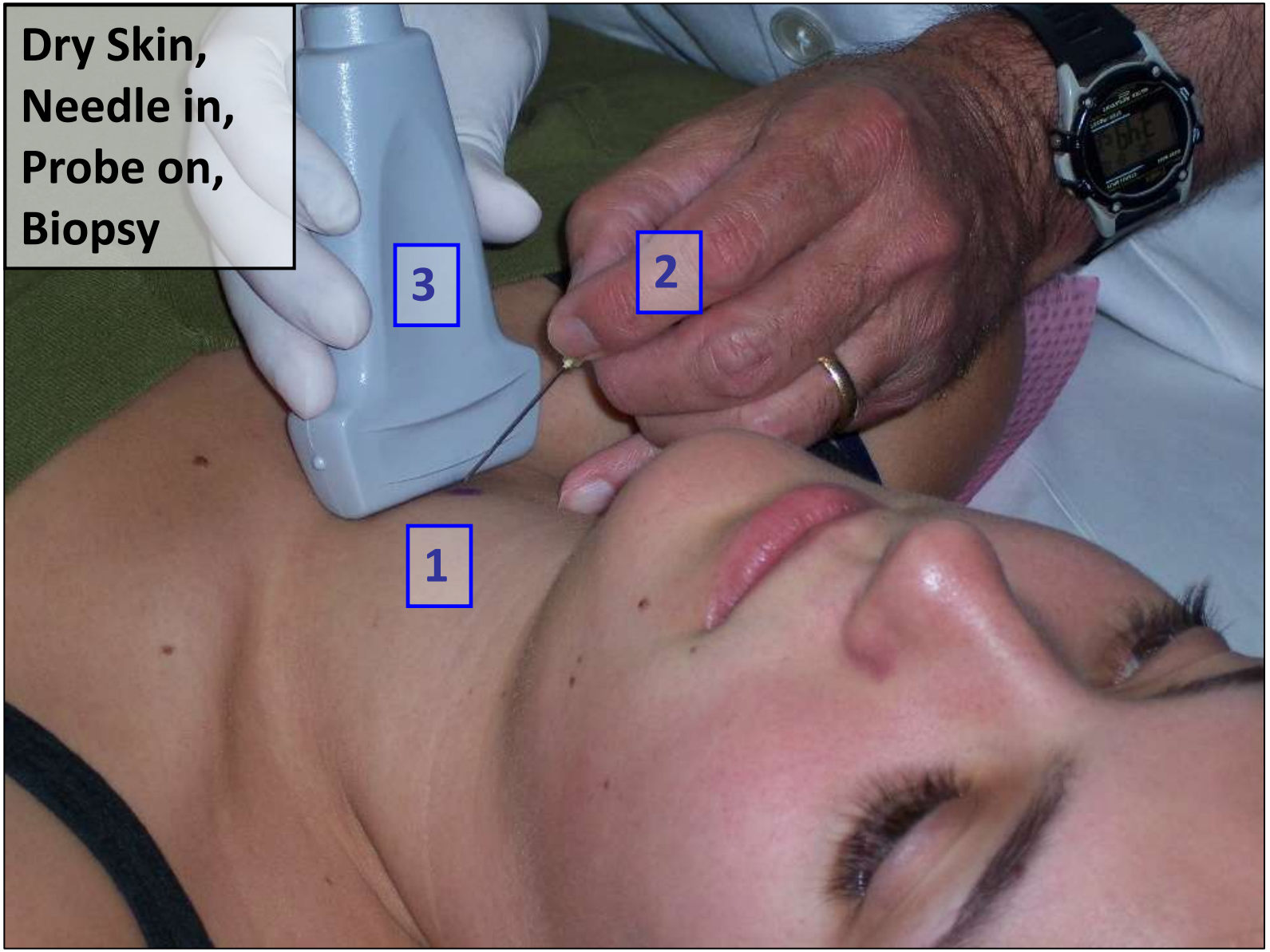
CUTANEOUS ANESTHESIA



CUTANEOUS ANESTHESIA



Diagn Cytopathol. 2008 Jun;36(6):407-24.


Dry Skin,
Needle in,
Probe on,
Biopsy
3
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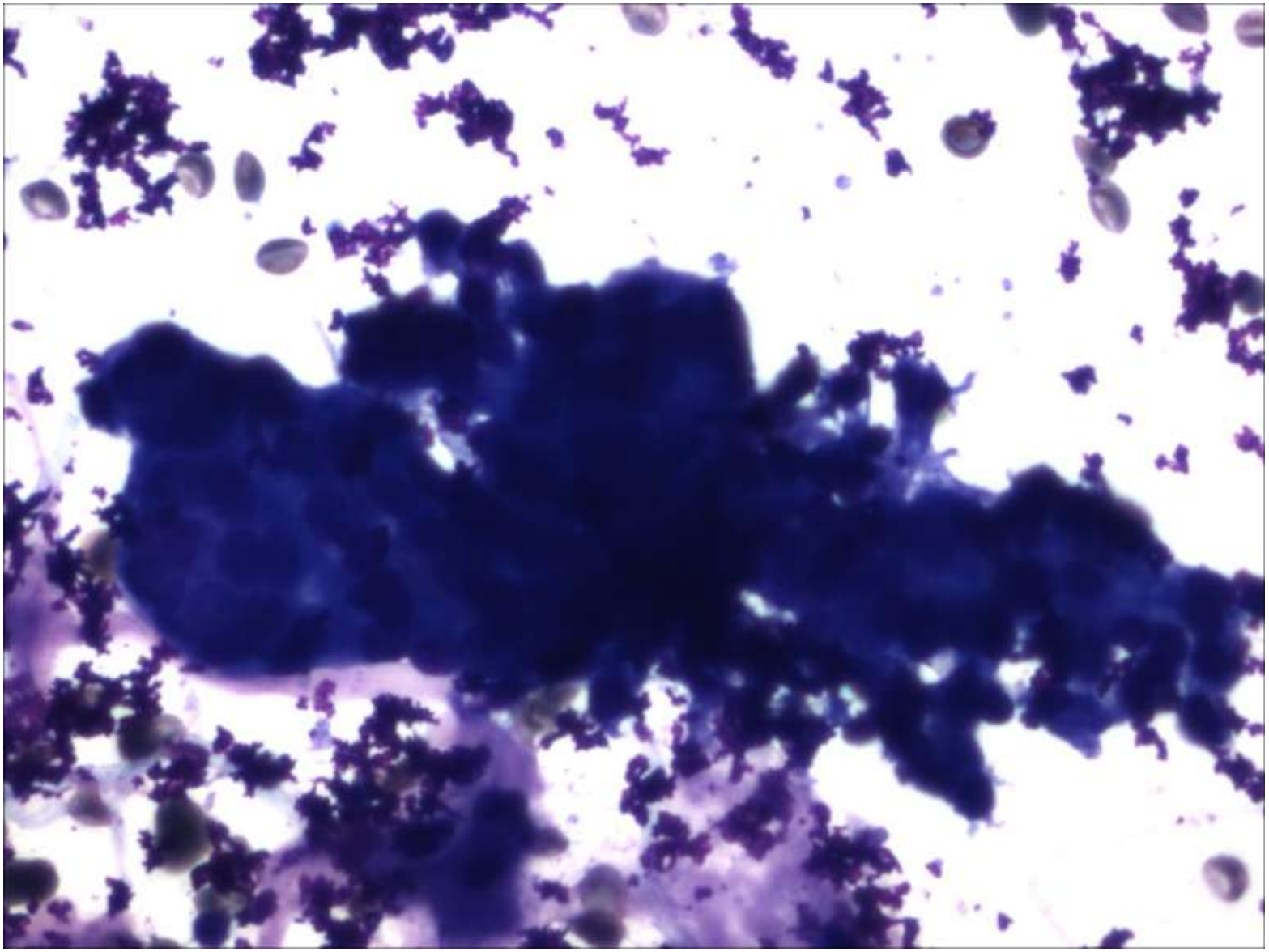
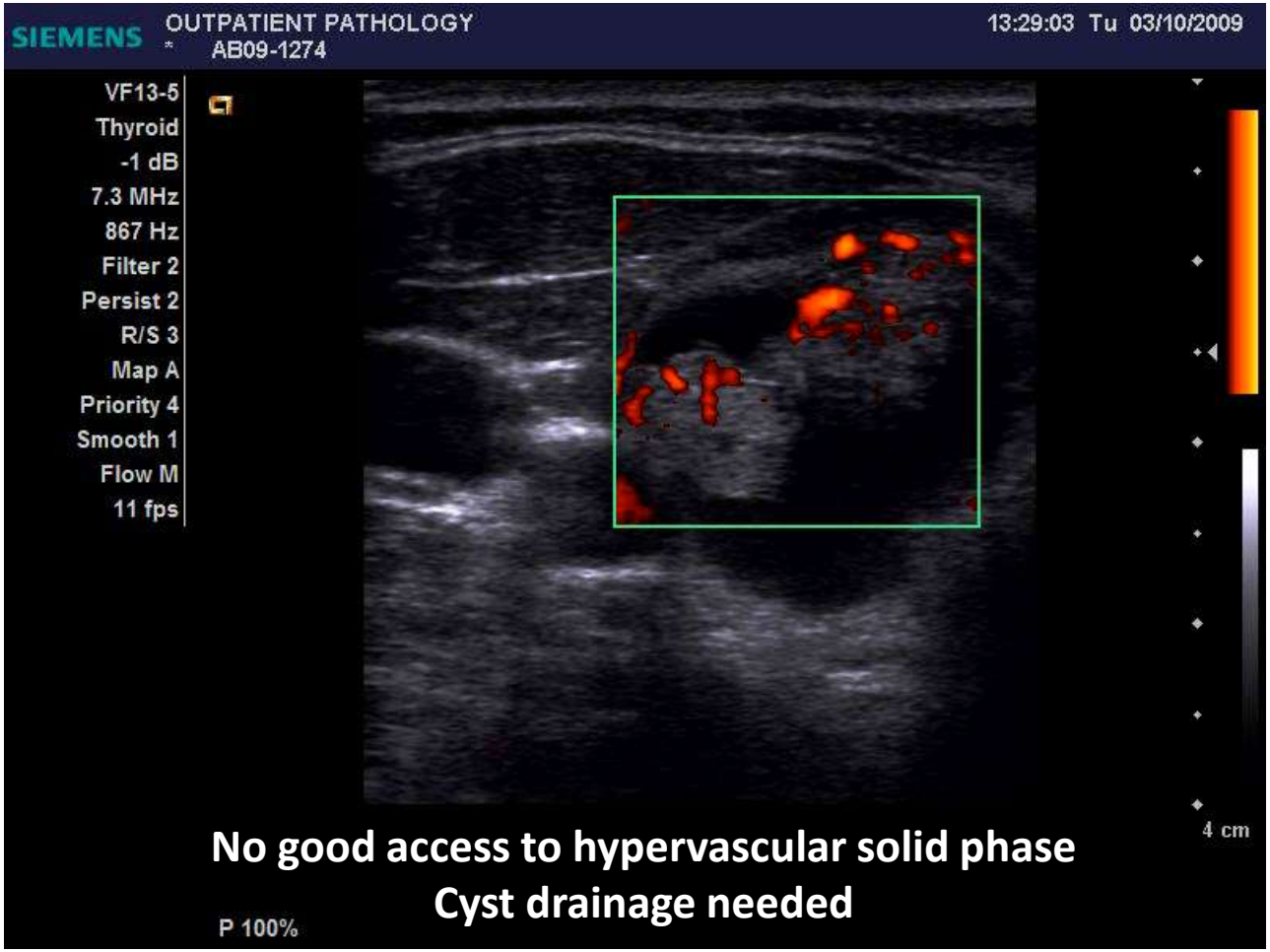
US Gel Obscuration - Heavy

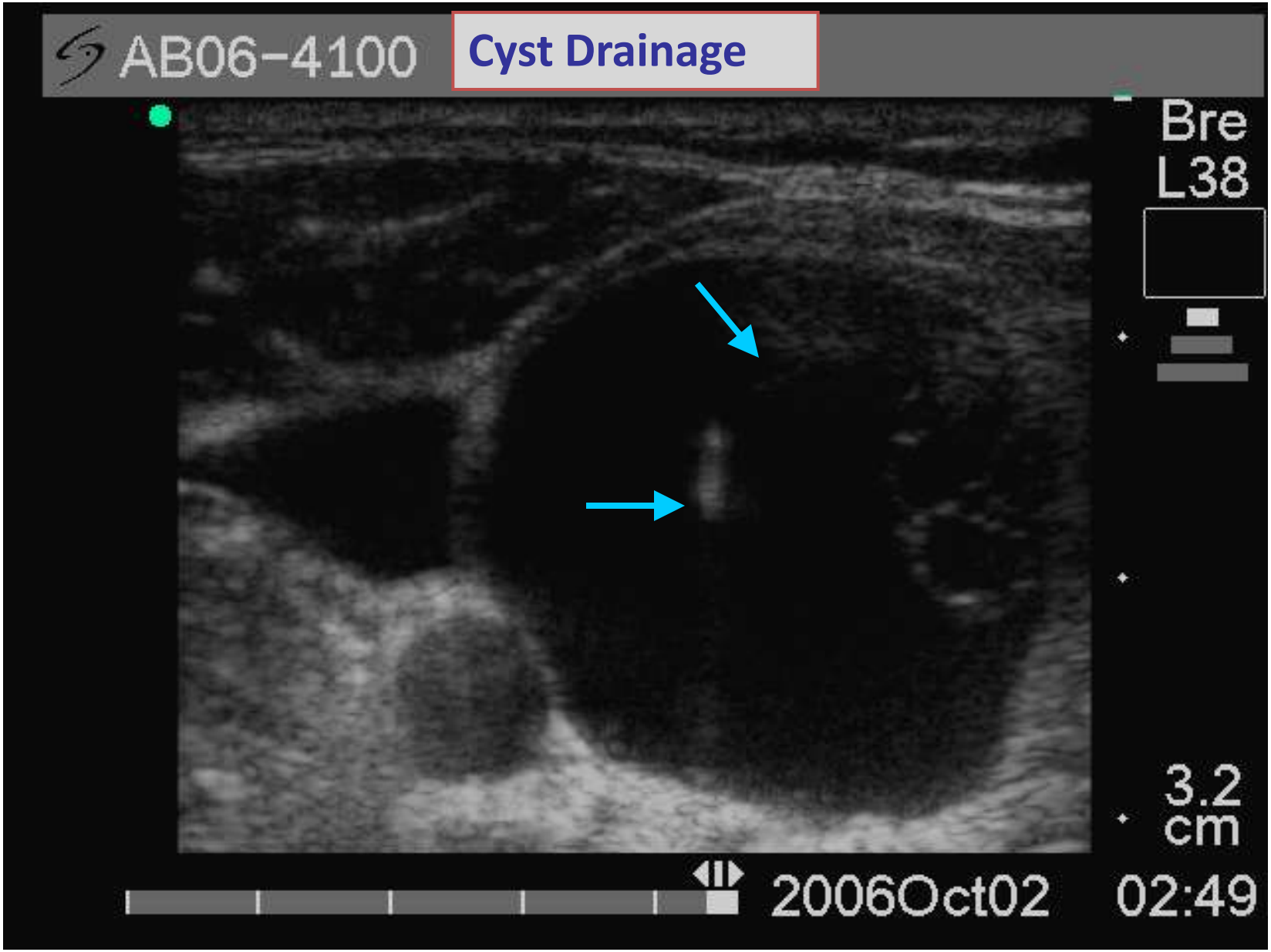


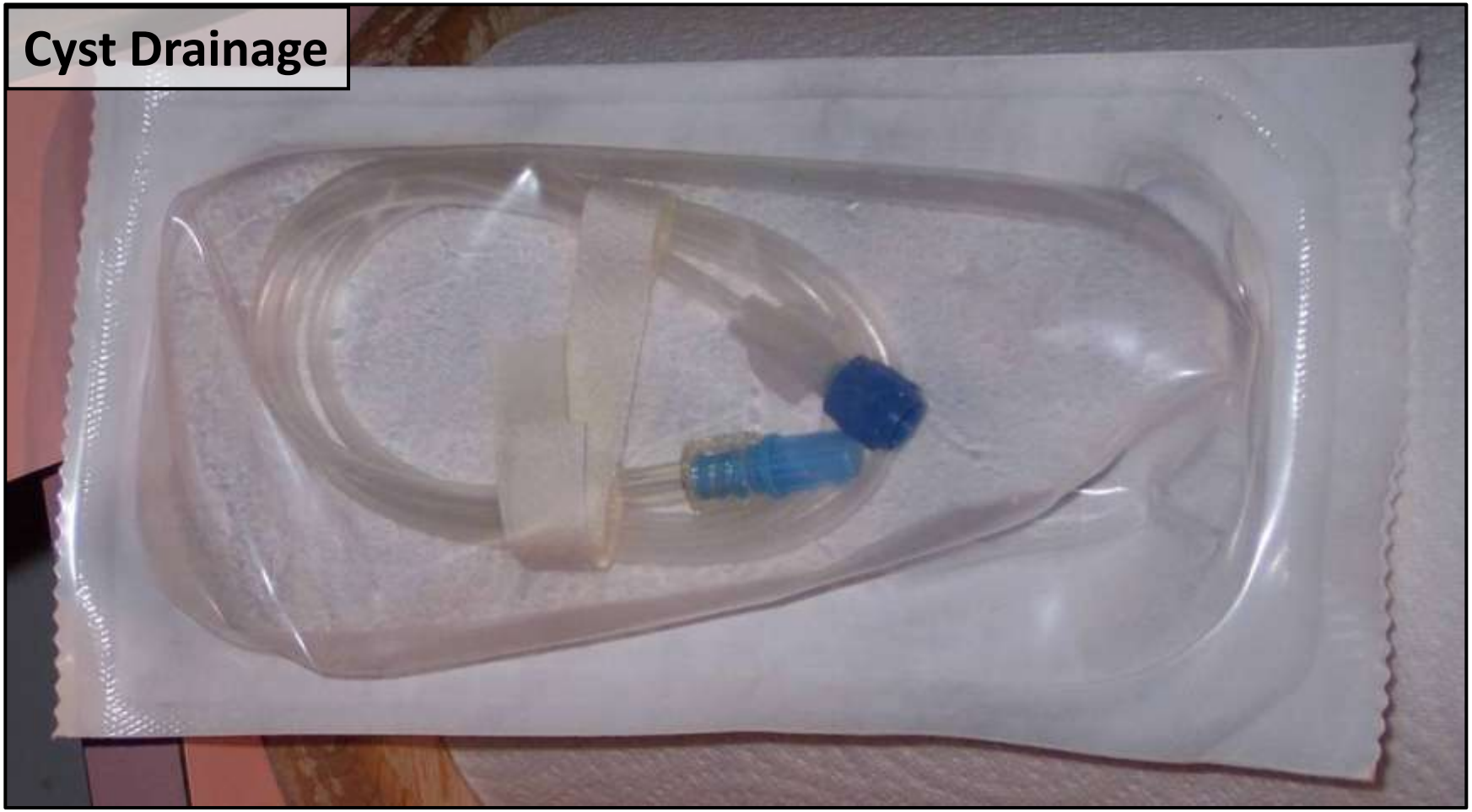
US Evaluation Cystic Nodules - Drainage of Large Cysts



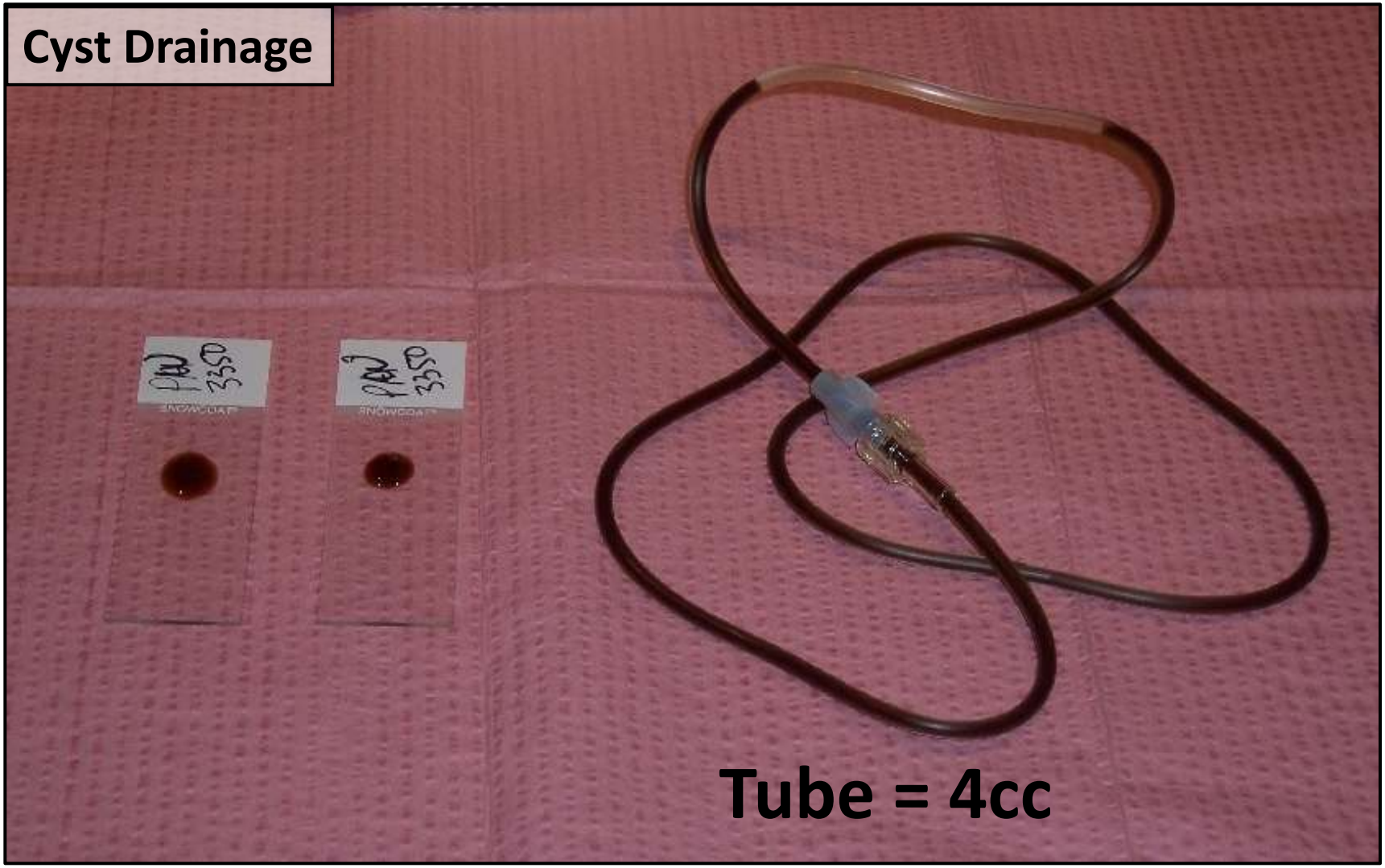
No good access to hypervascular solid phase
Cyst drainage needed

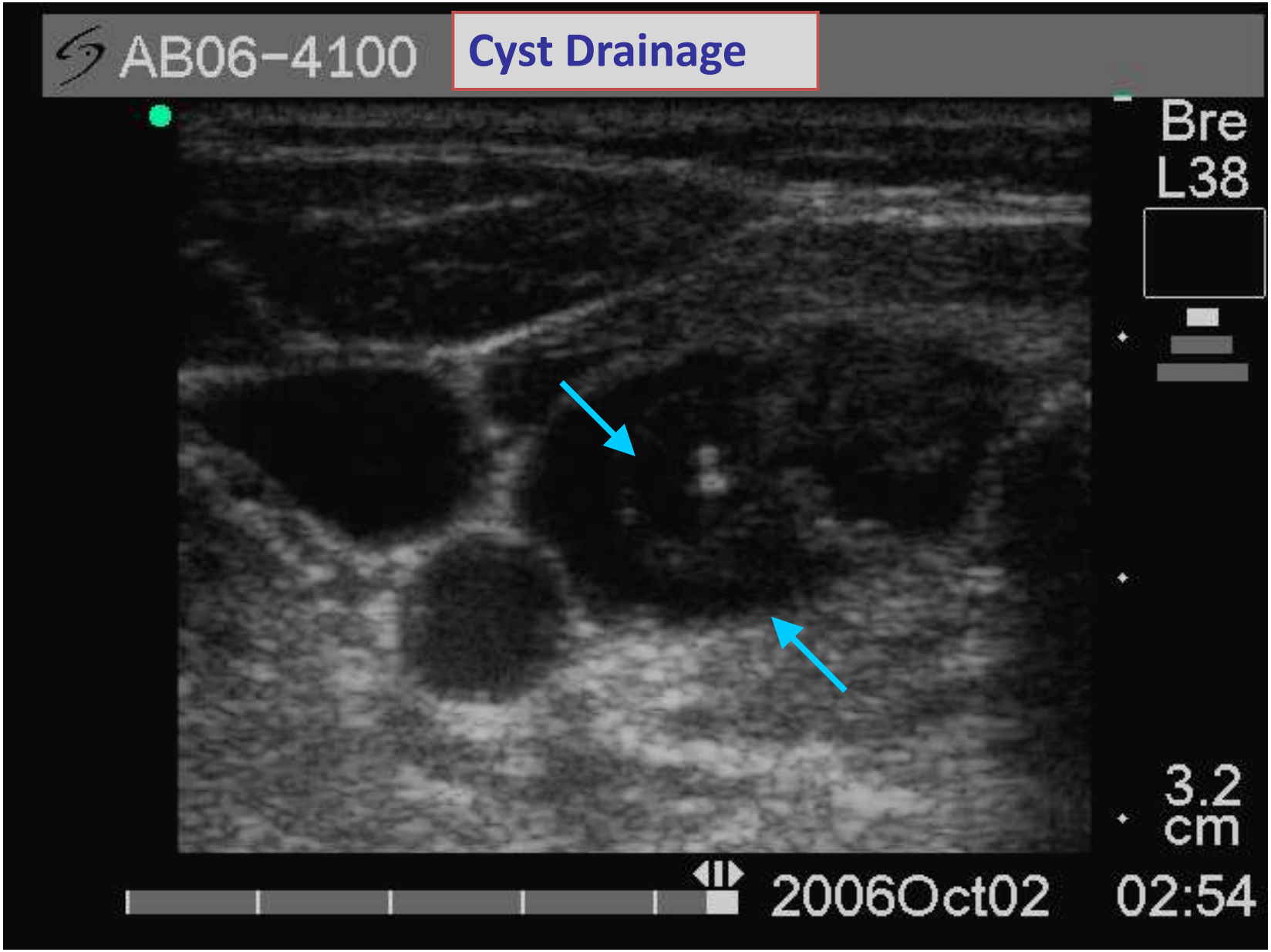
Click for Movie


AB06-4100
Cyst Drainage
Bre
L38
3.2
cm
2006Oct02
02:49

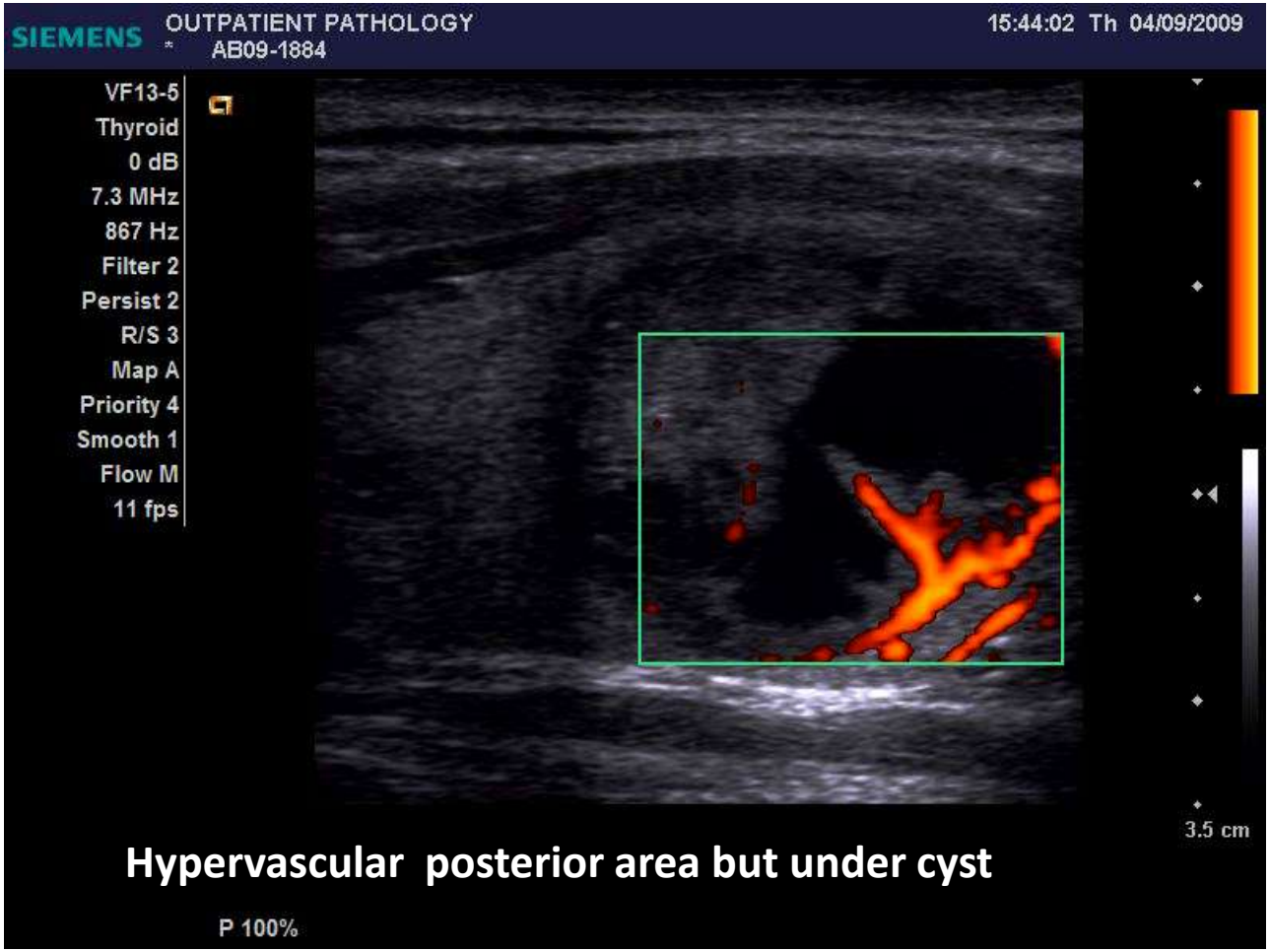


Baxter I.V. Extension 34” Male Luer
4cc Catalogue 2C6227

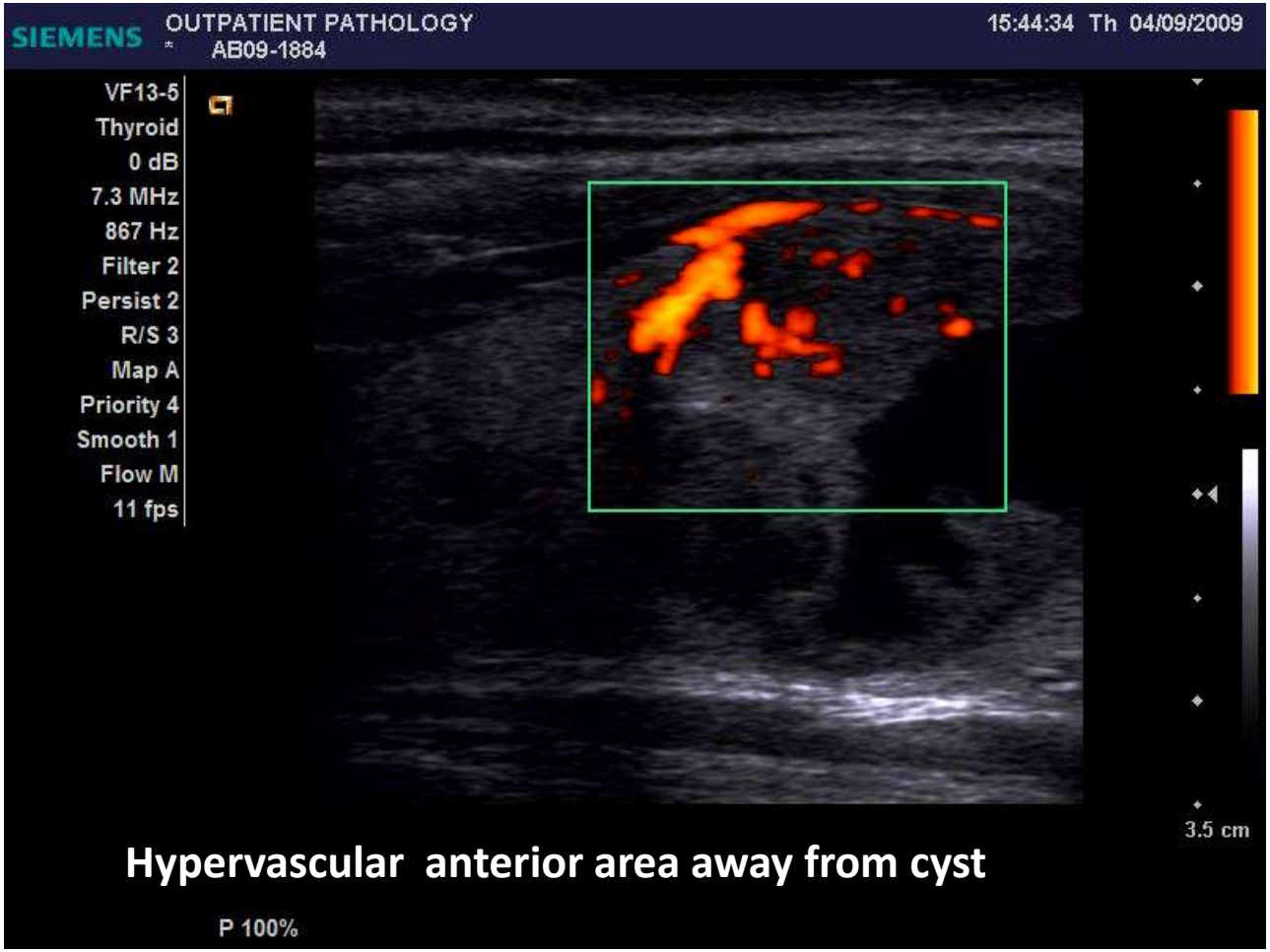

Cyst Drainage
Tube = 4cc


AB06-4100
Cyst Drainage
Bre
L38
3.2
cm
2006Oct02
02:54

US Evaluation
Cystic Nodules -
Avoid Cyst &
Sample Vascular Areas

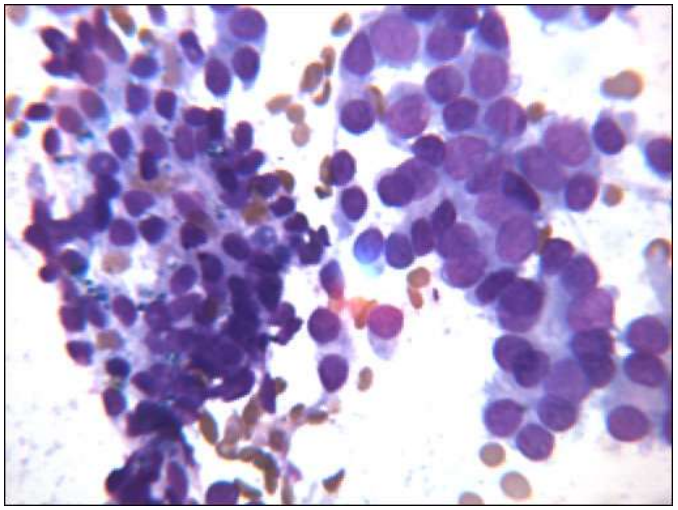
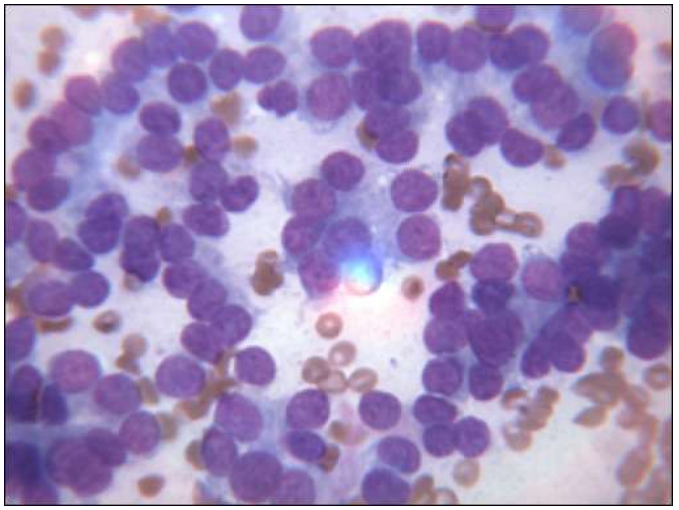
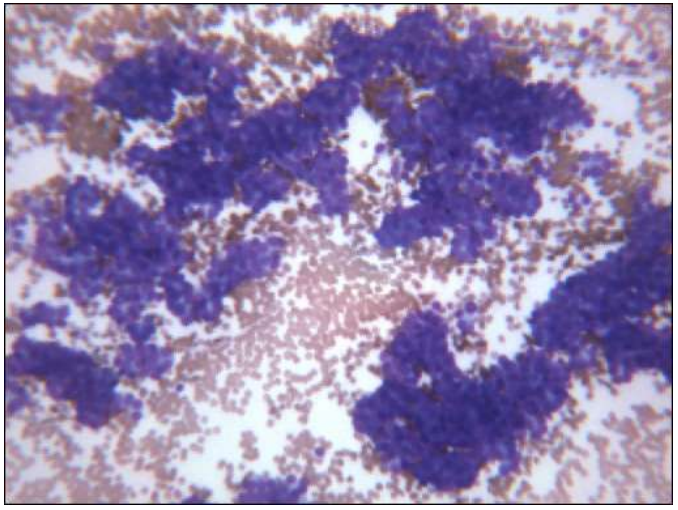
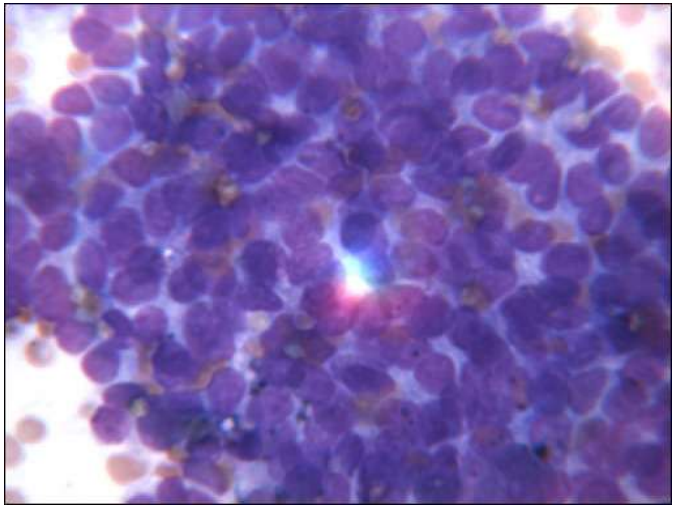


Click for Movie

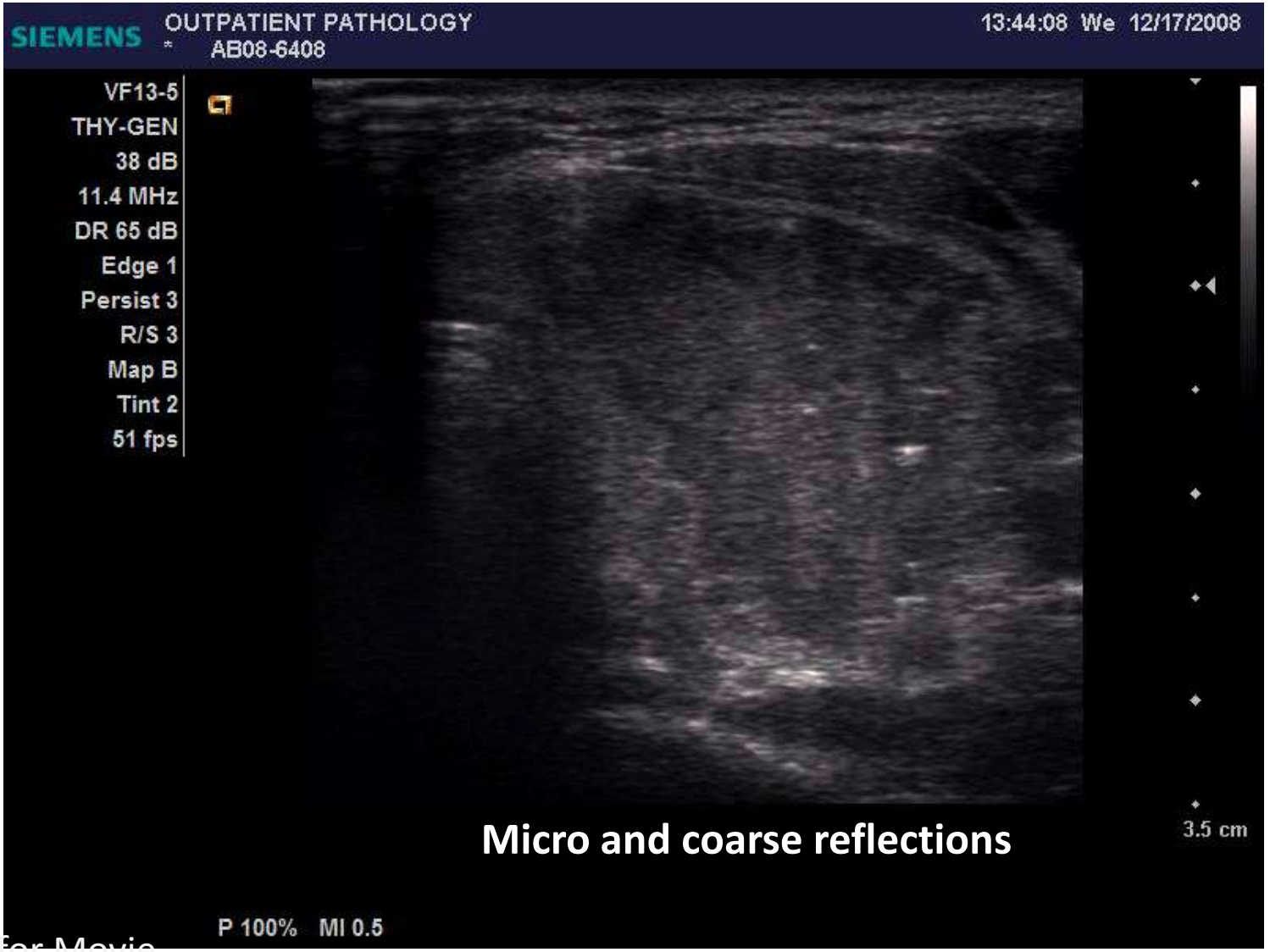


Click for Movie

Follicular Variant PTC AB09-1884

US Evaluation Cystic Nodules - Select Areas of Microcalcifications



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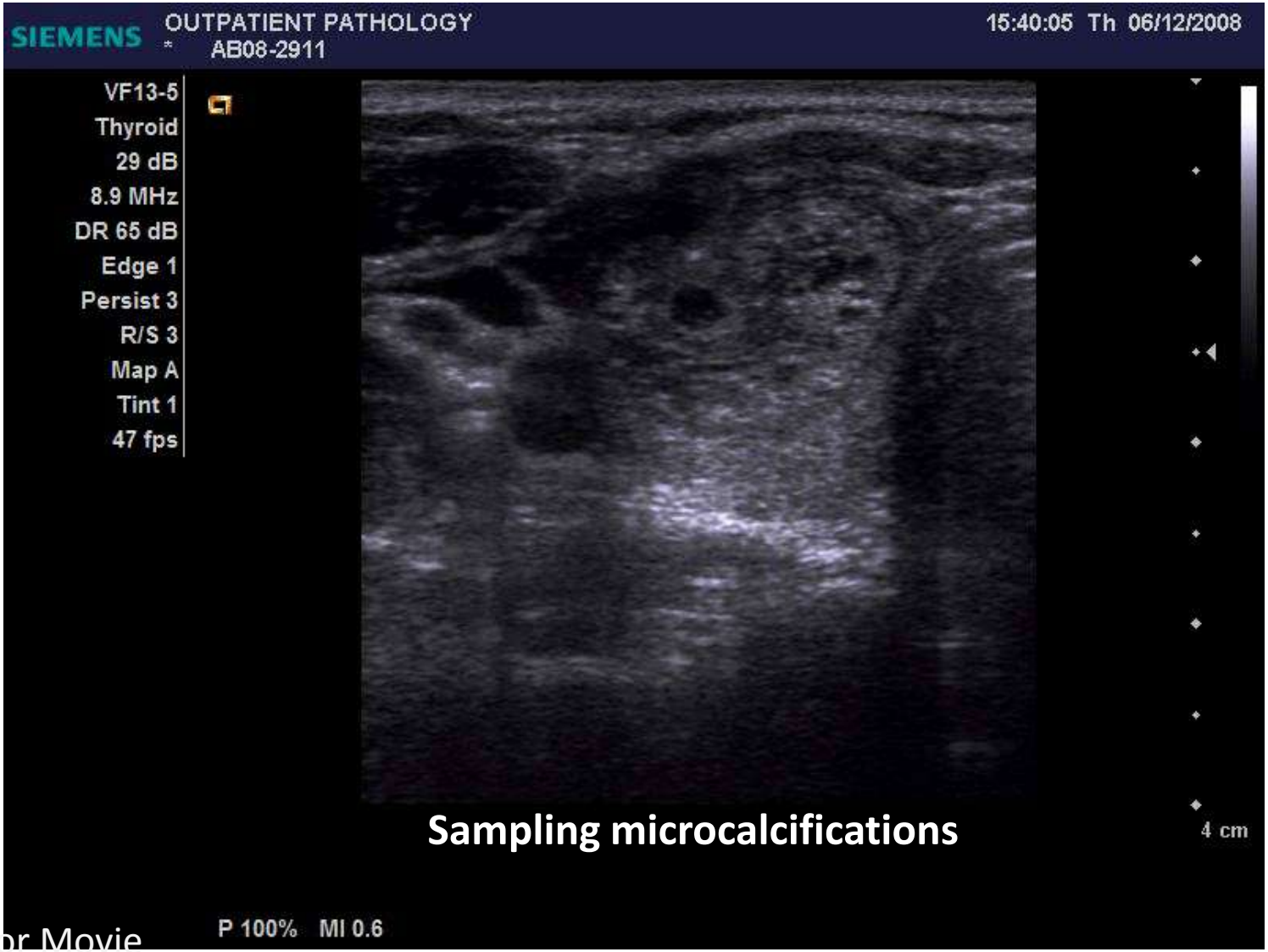
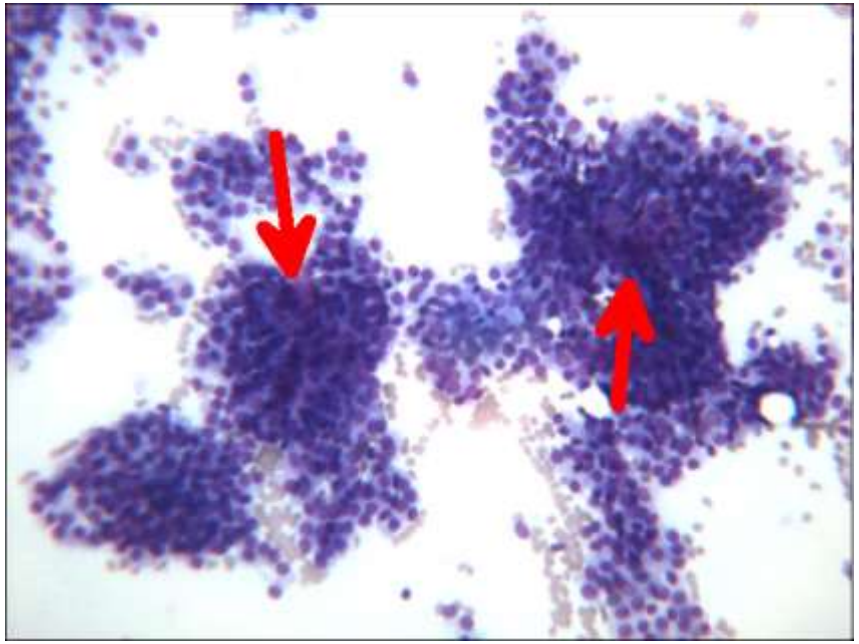
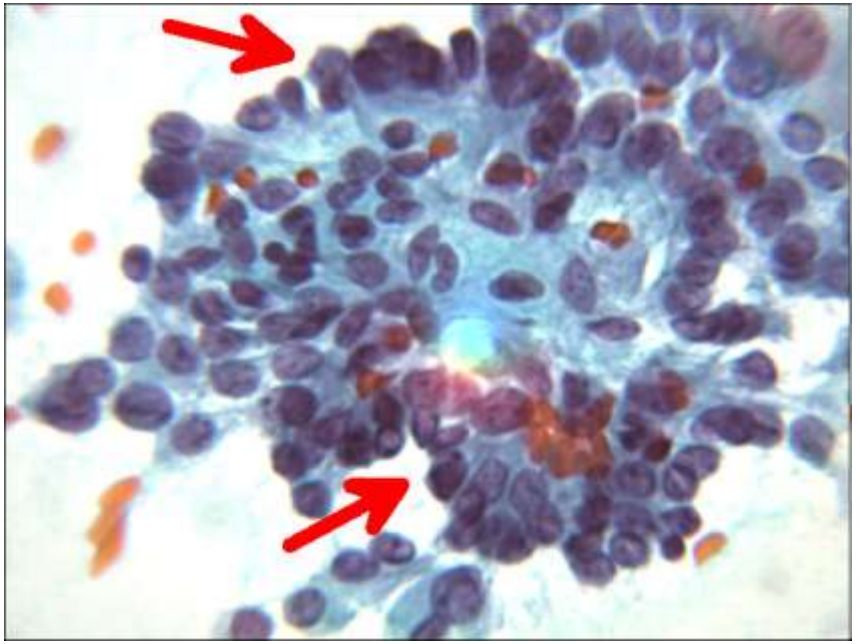
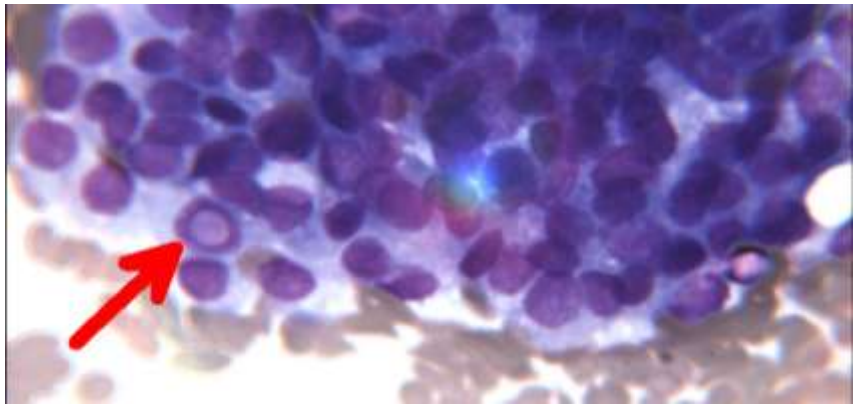


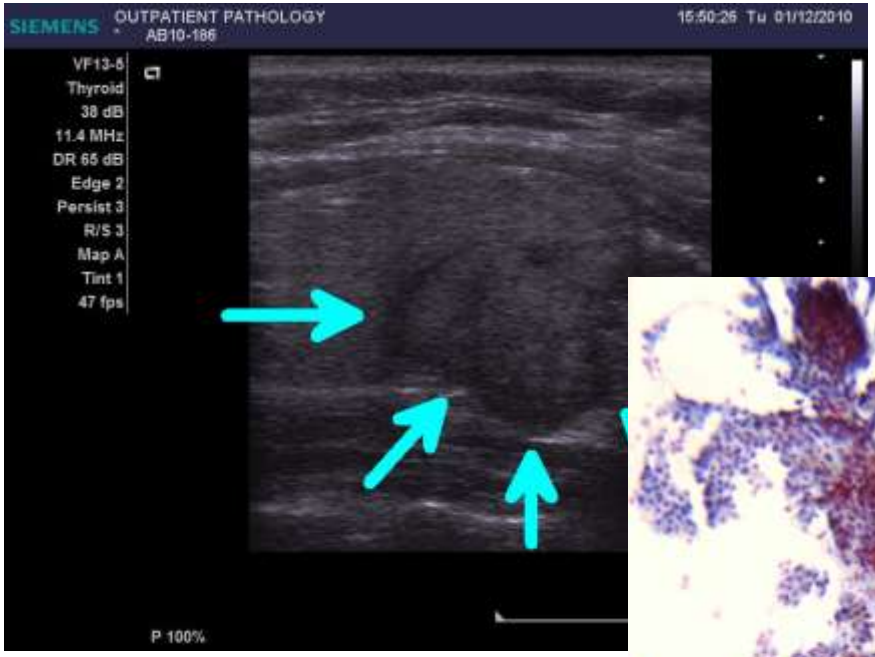
Click for Movie

Papillary Carcinoma AB08-6408





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Thyroid
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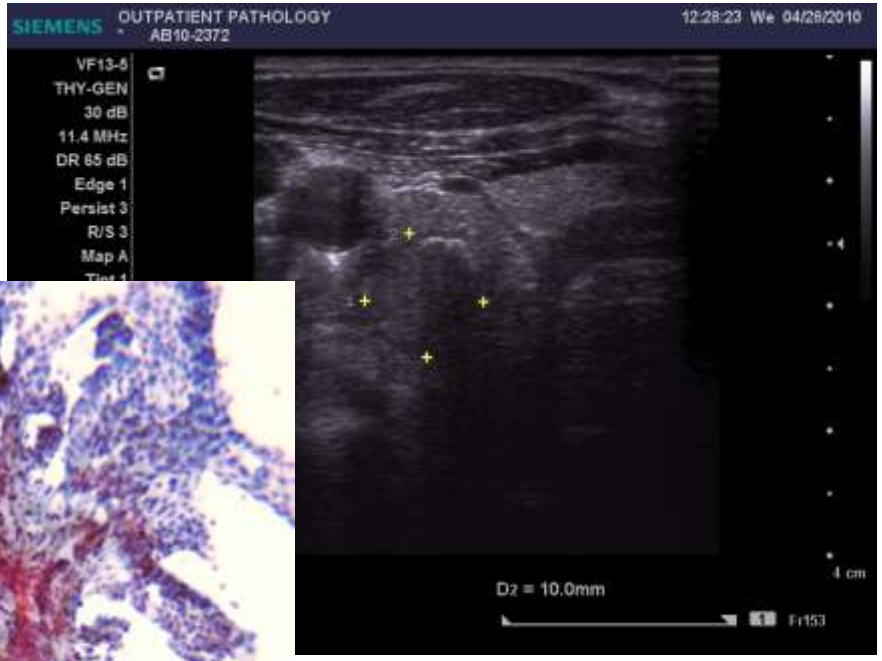
11.4 MHz
DR 65 dB
Edge 2
Persist 3
R/S 3
Map A
Tint 1
47 fps
P 100%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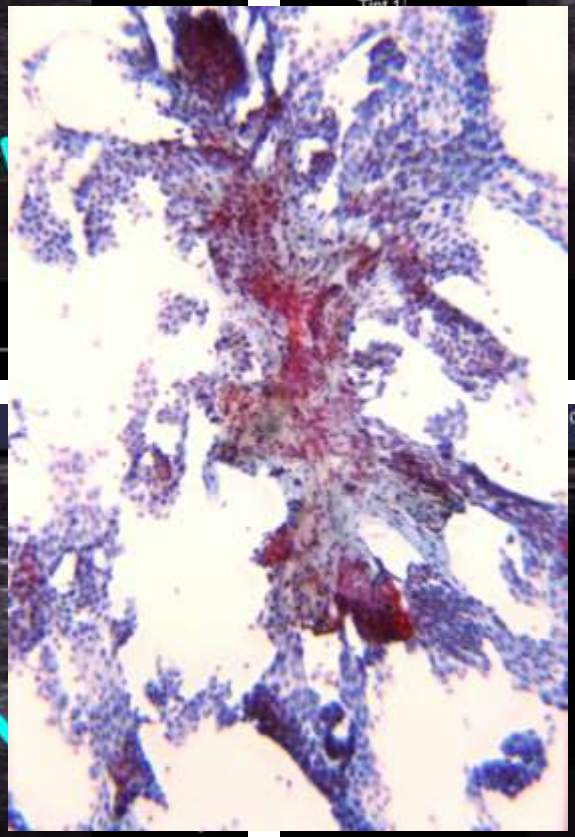
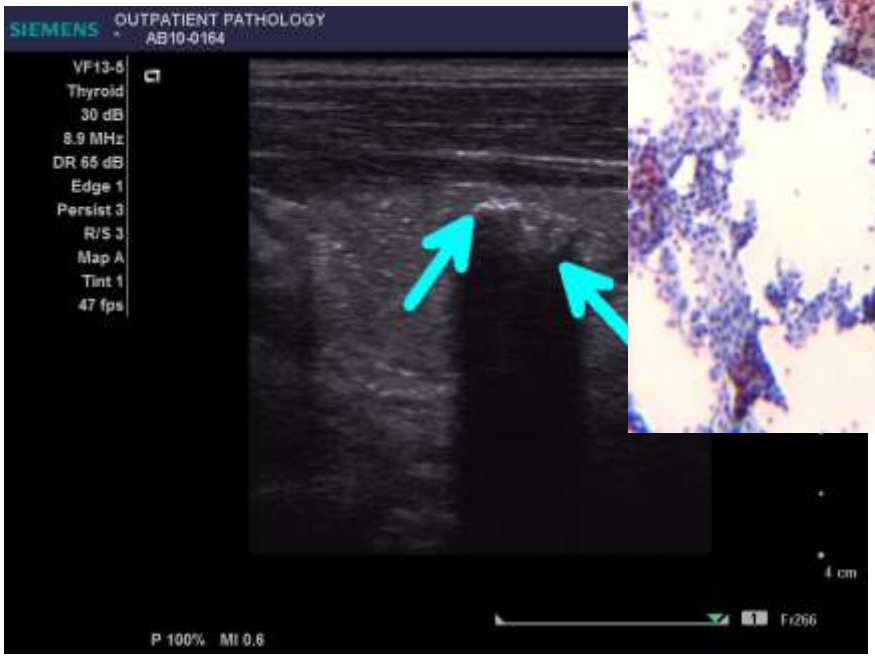
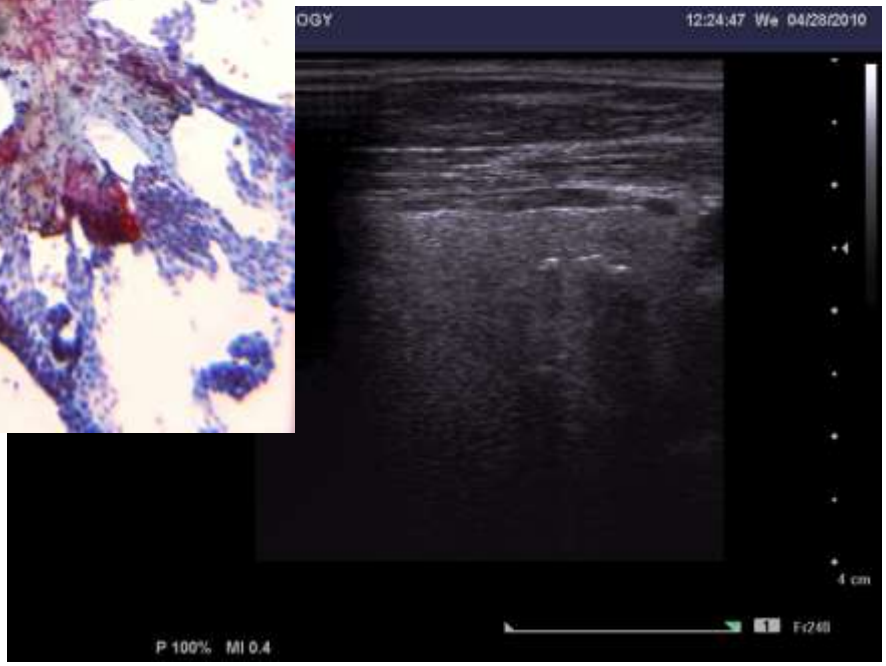
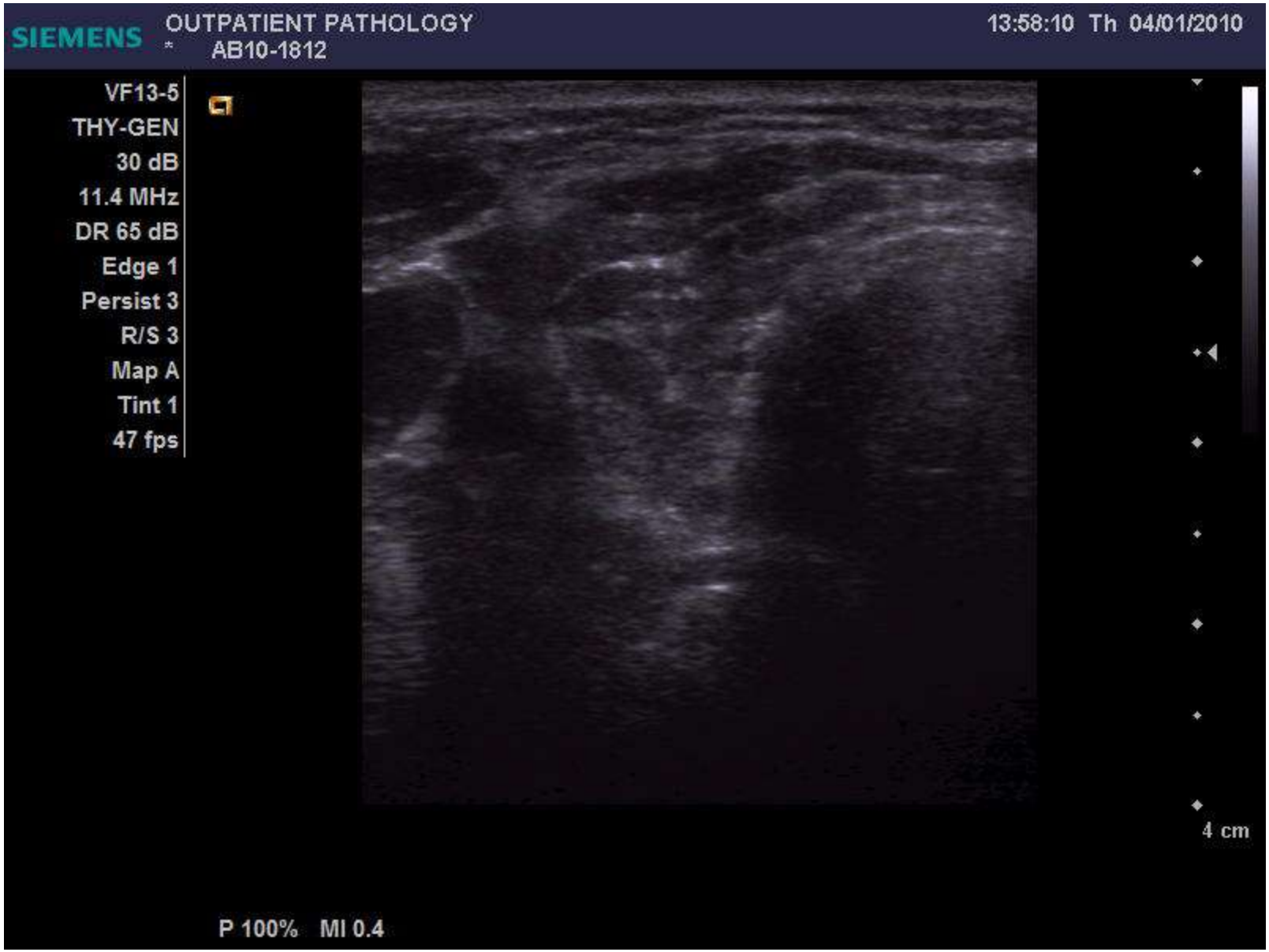
R/S 3
Map A
D2 = 10.0mm
4 cm
Fr153




SIEMENS
OUTPATIENT PATHOLOGY
AB10-0164
VF13-5
Thyroid
30 dB
8.9 MHz
DR 65 dB
Edge 1
Persist 3
R/S 3
Map A
Tint 1
47 fps
4 cm
Fr266
P 100% MI 0.6


OGY
12:24:47 We 04/28/2010
4 cm
Fr248
P 100% MI 0.4

US Evaluation
Solid Nodules - Posterior Dettached


SIEMENS
OUTPATIENT PATHOLOGY
* AB10-1812
13:58:10 Th 04/01/2010
VF13-5
THY-GEN
30 dB
11.4 MHz
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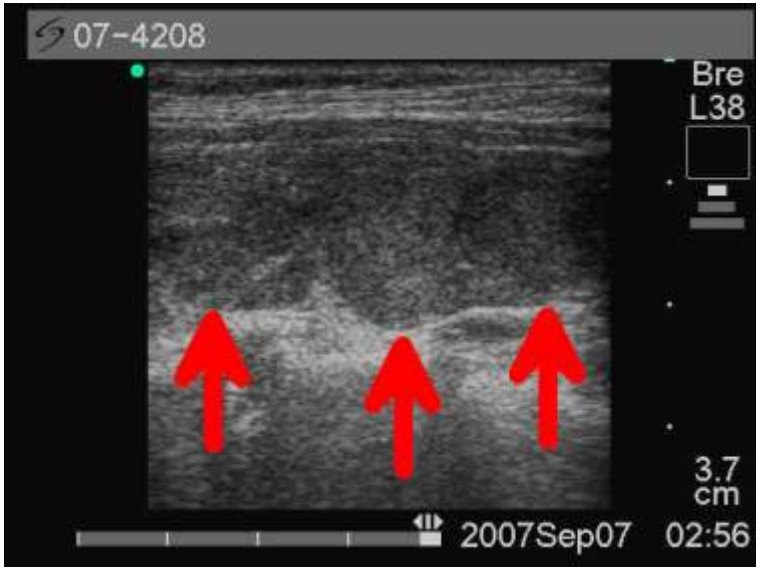
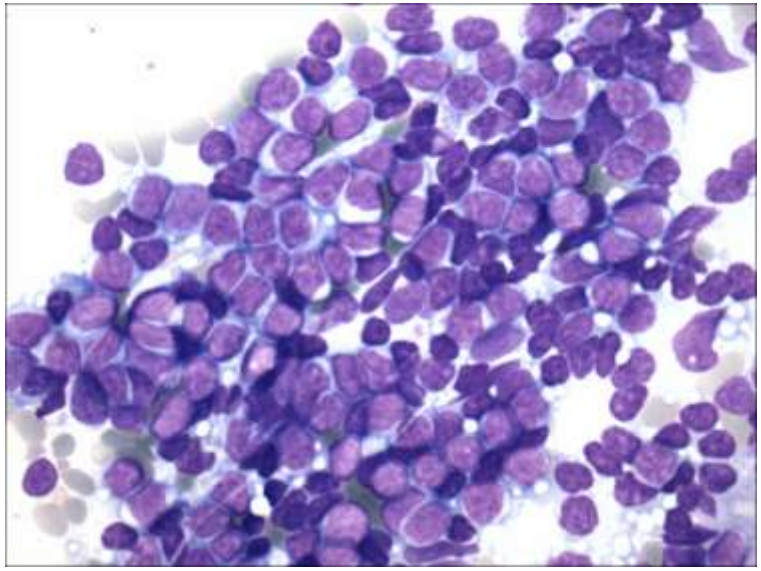
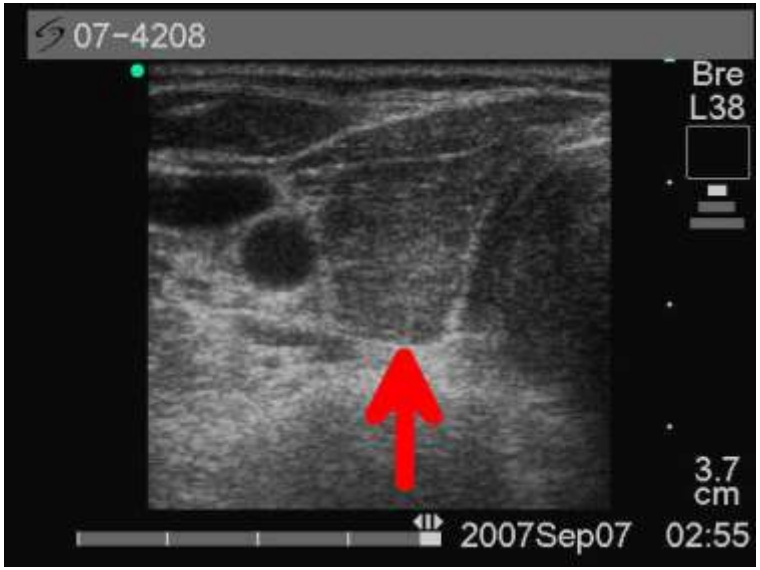
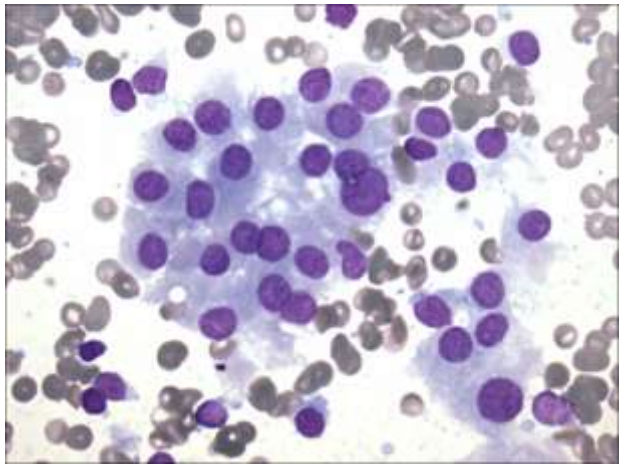
R/S 3
Map A
Tint 1
47 fps
4 cm
P 100% MI 0.4

Lymphocytic Thyroiditis









Cytopathology Interpretation: Can We Do Better?

Adequate Specimens

- Six groups of 10 or more cells, 2 slides?
- Appears to be BTN but with few cells = “Probable Benign Thyroid Nodule”
- “Limited by….”
- Appears to be follicular but has scant cellularity: suggest repeat the aspirate to avoid surgery.

The “Indeterminate Diagnosis” Gost

- Most are bad specimens: bloody, clotted, lubricant, few cells
- “Repeat FNA” is often suggested
- Few colloid and lots of cells is bothersome, but in the absence of microfollicles, “probable BTN.”

REDUCING HIGH MF PATTERN RATES

- **15-30% National Susp Rate**
  - Clot Pseudo Complexity
  - Bloodly Pseudo Complexity
  - Mechanical Distortion Complexity
  - Cellular BTN ≠ MFT
  - Mixed Macro/Micro ≠ MFT
  - Endocrine / Cytopath Discussion
- **3.6% OPA Susp Rate (2006-2009)**

REDUCING HIGH MFT/INDx RATES

- **15-30% National MFT/INDx Rate**

5 Steps to Reduce MFT / INDx Rate

1. **Proper Biopsy Technique**
2. **Proper Smearing Technique**
3. **Restrictive MFT Criteria**
4. **Recognize Artifact ≠ MFT**
5. **MF in Chronic Thyroiditis ≠ MFT**

- **3.6% OPA MFT/INDx Rate**

THYROID FNA
DXs & Cancer Risk Rate

CHRONIC THYROIDITIS	1%
BENIGN THYROID NODULE (BTN)	3%
BTN w/ Complexity (FLUS/AUS)	5-10%
MICROFOLLICULAR TUMOR	20-30%
PAPILLARY CA, POSSIBLE[1]	50%
PAPILLARY CA, PROBABLE[2]	90%
PAPILLARY CA	99+%

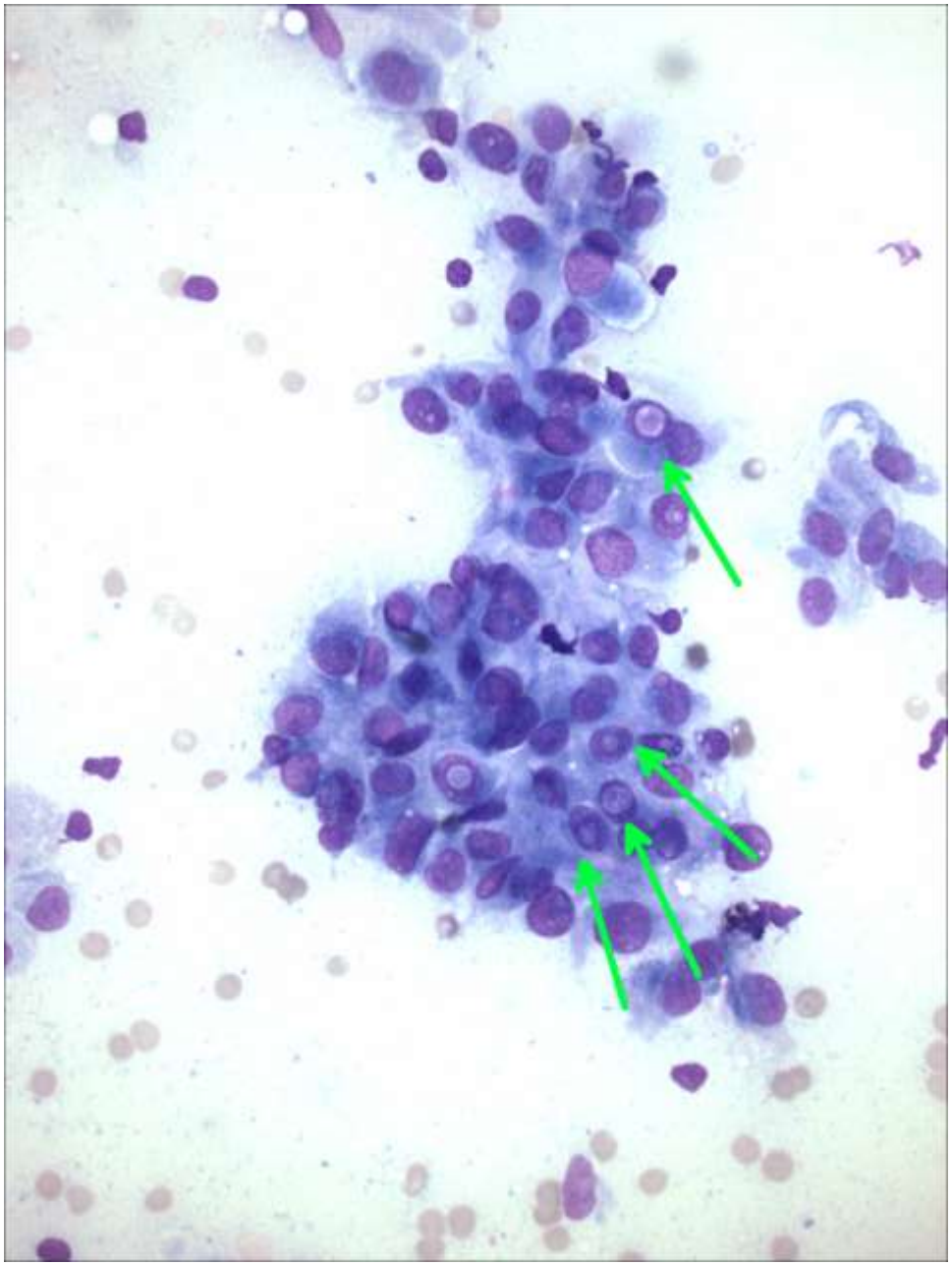
1 = Few 2° features; 2 = 2° features with limited 1°

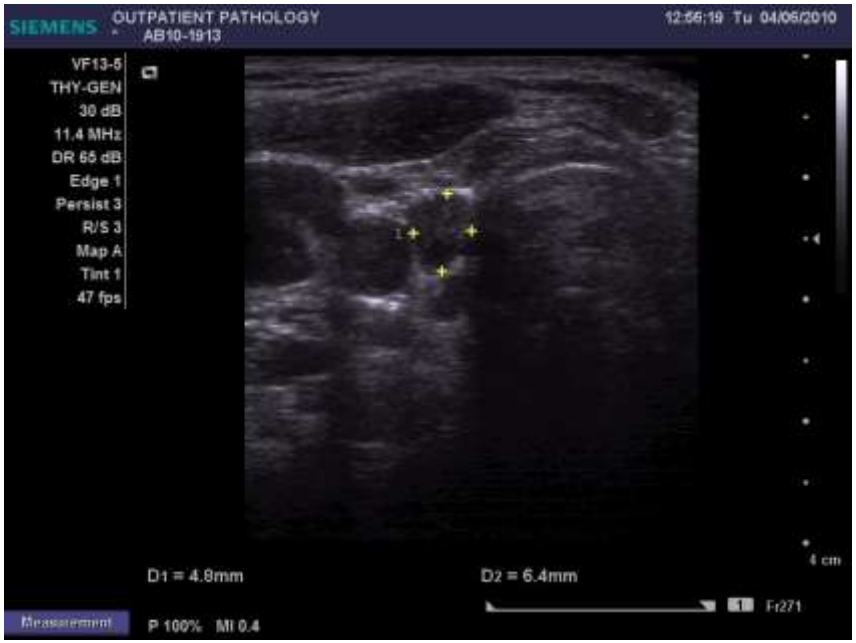
Miller TR, Bottles K, Holly EA, Friend NF, Abele JS.
Acta Cytol. 1986 May-Jun;30(3):285-93.

Baloch ZW, LiVolsi VA, et al.
Diagn Cytopathol. 2008 Jun;36(6):425-37

US Evaluation Thyroid Bed Nodules

Residual BTN versus Parathyroid Tissue versus Recurrent PTC




SIEMENS
OUTPATIENT PATHOLOGY
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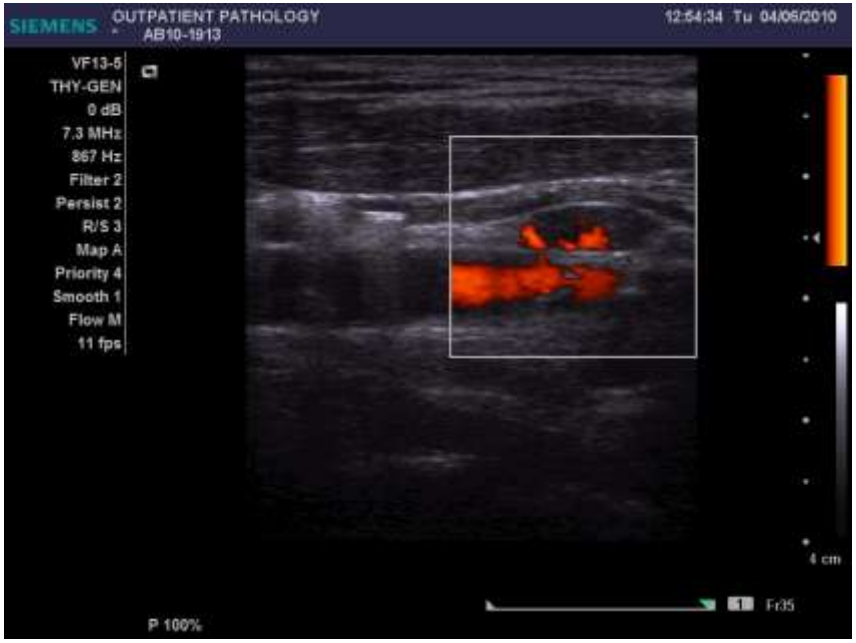
VF13-5
THY-GEN
30 dB
11.4 MHz
DR 65 dB
Edge 1
Persist 3
R/S 3
Map A
Tint 1
47 fps
D1 = 4.8mm
D2 = 6.4mm
4 cm
Measurement
P 100% MI 0.4
Fr271


SIEMENS
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12:54:34 Tu 04/06/2010
VF13-5
THY-GEN
0 dB
7.3 MHz
867 Hz
Filter 2
Persist 2
R/S 3
Map A
Priority 4
Smooth 1
Flow M
11 fps
4 cm
P 100%
Fr35

US Evaluation of Lymph Nodes

THYROIDOLOGISTS

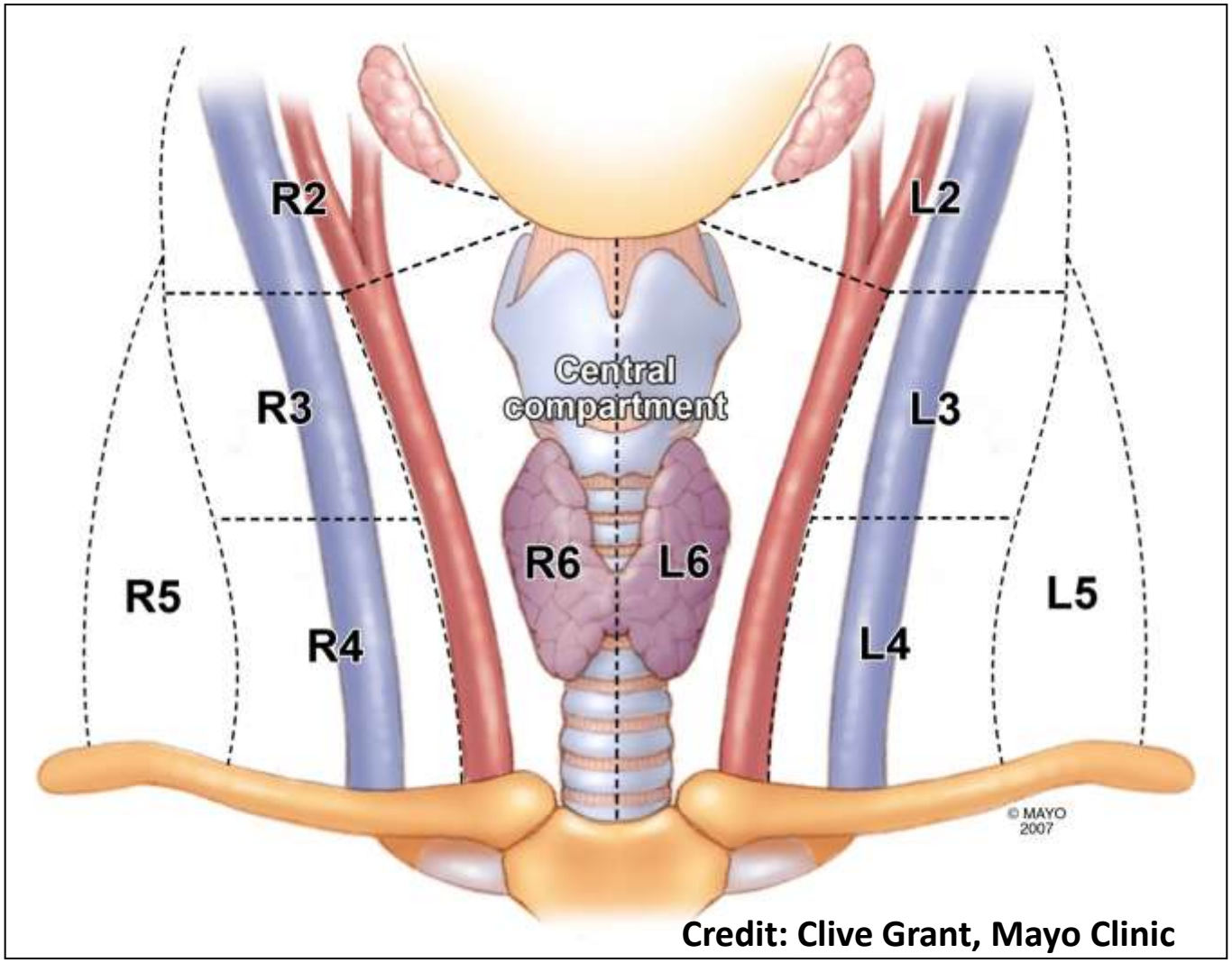
Experts in the Diagnosis and Treatment of Thyroid Disease

INDICATIONS FOR LN FNA IN THYROID CANCER PATIENTS

- **AP/Transverse ratio > 0.5 (transverse view)**
- **Calcifications**
- **Cystic necrosis**
- **Peripheral vascularity**
- **Caused deviation of the IJV**

Academy of Clinical Thyroidologist
(www.thyroidologists.com)

Surgical Neck Compartments

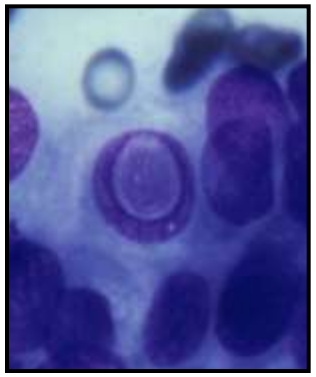


Credit: Clive Grant, Mayo Clinic

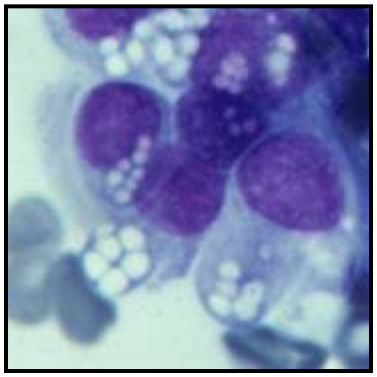
PTC & Lymph Nodes

Basic Issues

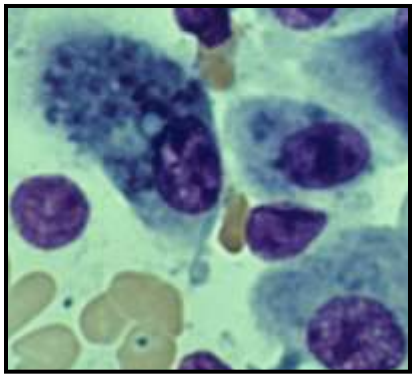
- **Thyroid PTC – Usually Solid**
- **LN Mets – Solid, Cystic, Cystic++**
  - If Solid, Cytology Positive
  - If Partially Cystic – Metaplastic Cells
  - If Purely Cystic – Histiocytes and Blood



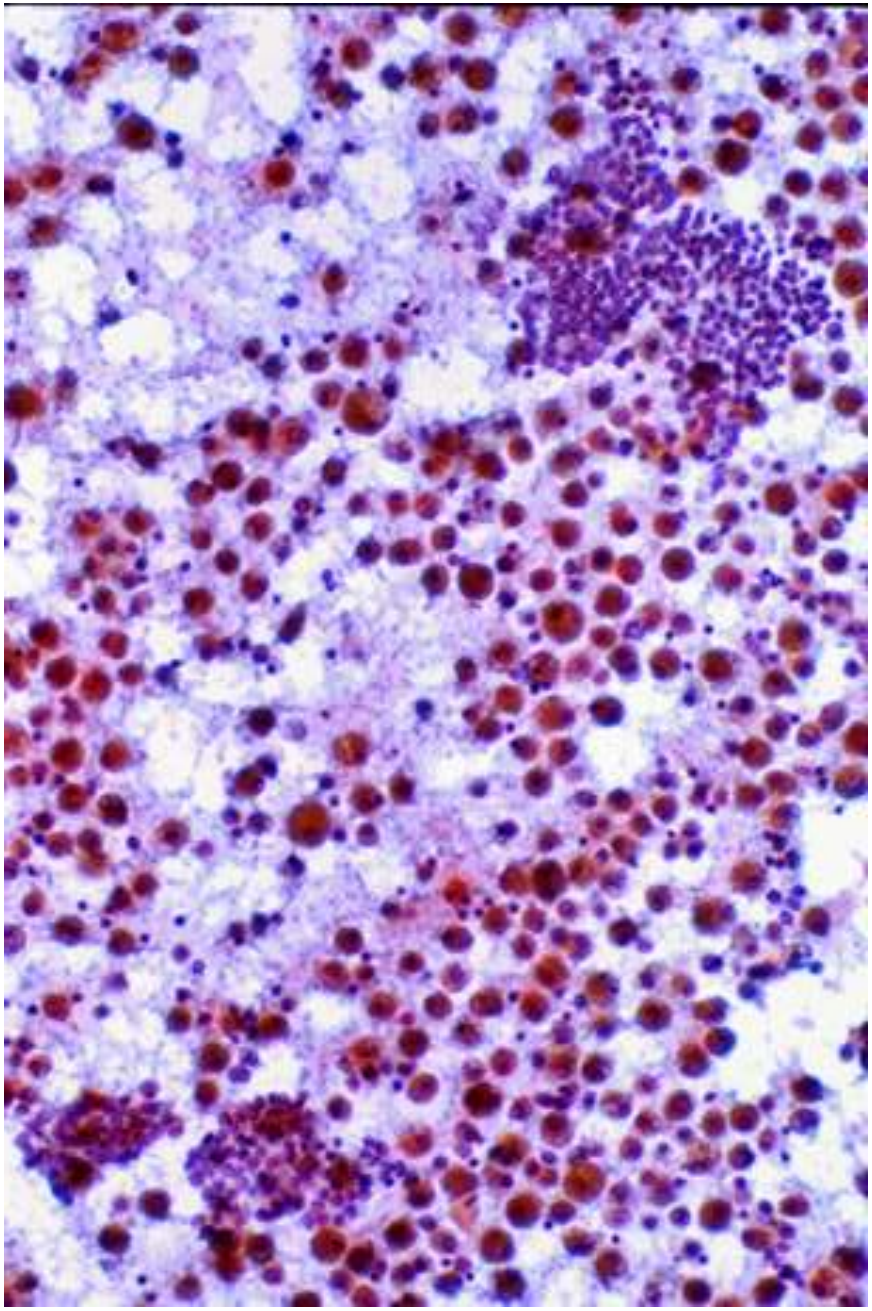
Inclusions

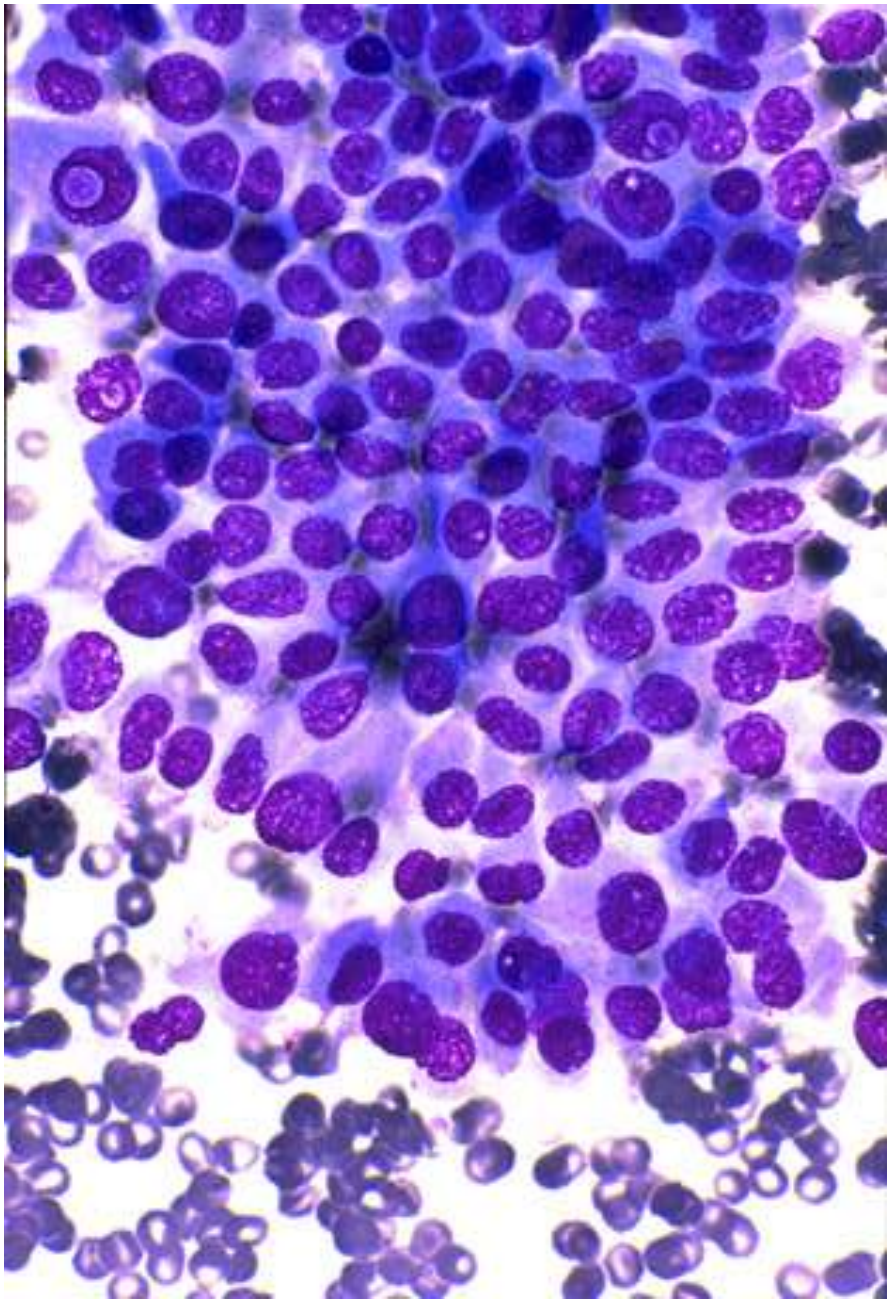


Metaplastic Cells



Histiocytes





TG FNA Needle Washings

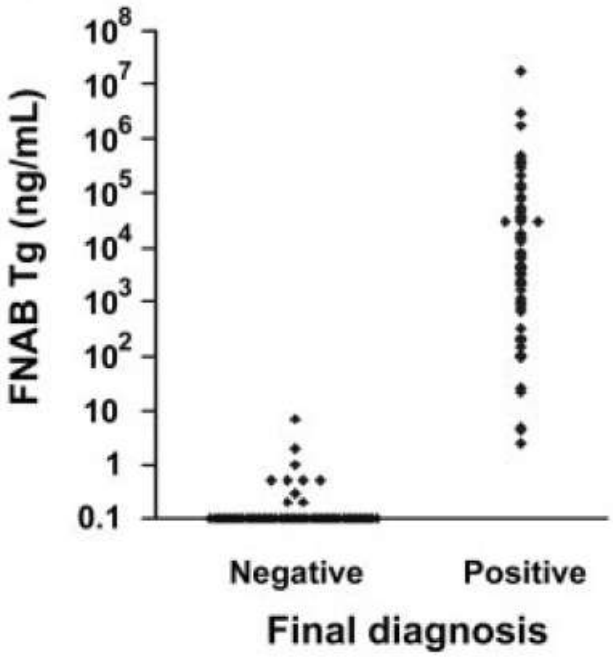


Fig 1A FNA Wash TG vs DX

"The risks associated with false negative results were deemed unacceptable."

"The absence of false negatives ... likely results in part from improved assay performance but mainly from the deliberate choice of a (1ng/ml) cutoff designed to avoid false-negative results."

"A 1 ng/ml cutoff ... provided 100% sens (low = 2.5ng/ml), 96.2% spec (50/52 2.0 and 6.7ng/ml), and 97.2% PPV. This cutoff is well within the range of modern Tg immunoassays"

Snozek CL, Chambers EP, Reading CC, Sebo TJ, Sistrunk JW
J Clin Endocrinol Metab. 2007 Nov;92(11):4278-81.

Journey

- Historical perspective
- Palpation-guided FNA (PG-FNA)
- Ultrasound-guided FNA (USG-FNA)
  - Non-palpable lesions: thyroid, thyroid bed, LNs
- **Molecular thyroid cytopathology**
- Thyroid cancer – therapeutic targets

The “Indeterminate Diagnosis”

- Neoplastic versus non-neoplastic
- Malignant versus benign
- Use of ICC or molecular techniques remains controversial.
- Less “indeterminate diagnoses” less unnecessary surgeries.

Markers

- Galectin-3*
- Cytokeratin-19*
- HBME-1*
- Thyroid peroxidase
- DAP IV

*Not highly specific

- Chromosomal rearrangements
  - *RET/PTC/PAX8/PPARG*
- Genetic mutations
  - BRAF, RAS
  - BRAF mutation: highly specific for PTC (not 100%)
  - Can be done by removing cells from smears.

THYROID
Volume 19, Number 11, 2009

DOI: 10.1089/thy.2009.0110

ORIGINAL STUDIES, REVIEWS,
AND SCHOLARLY DIALOG
THYROID CANCER AND NODULES

Revised American Thyroid Association Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer

The American Thyroid Association (ATA) Guidelines Taskforce
on Thyroid Nodules and Differentiated Thyroid Cancer

David S. Cooper, M.D.[1] (Chair)*, Gerard M. Doherty, M.D.,[2] Bryan R. Haugen, M.D.,[3]
Richard T. Kloos, M.D.,[4] Stephanie L. Lee, M.D., Ph.D.,[5] Susan J. Mandel, M.D., M.P.H.,[6]
Ernest L. Mazzaferri, M.D.,[7] Bryan McIver, M.D., Ph.D.,[8] Furio Pacini, M.D.,[9] Martin Schlumberger, M.D.,[10]
Steven I. Sherman, M.D.,[11] David L. Steward, M.D.,[12] and R. Michael Tuttle, M.D.[13]

Recommendation

The use of molecular markers
(e.g., BRAF, RAS, RET/PTC, Pax8-PPARγ , or galectin-3)
be considered for patients with
indeterminate cytology on FNA

P1-542
A Multi-Gene Test for Accurate Classification of Thyroid Nodules.

JI Wilde Ph.D.1, N Rabbee Ph.D.1, D Chudova Ph.D.1, H Wang Ph.D.1, C Friedlander Ph.D.1, E Wang Ph.D.1, M Pagan Ph.D.1, E Tom1, J Reynolds1, CT Rigl Ph.D.1, CC Wang M.D.1, L Friedman R.N.1, RB Lanman M.D.1, M Zeiger M.D.2, E Kebebew M.D.3, J Rosai M.D.4, VA LiVolsi M.D.5 and GC Kennedy Ph.D.1.

1Veracyte, Inc South San Francisco, CA ; 2Veracyte, Inc South San Francisco, CA ; 3Johns Hopkins Sch of Med Baltimore, MD ; 4Natl Cancer Inst Bethesda, MD ; 5Ctr Consulenze Anatomica Patologica Oncologica Milan, Italy and 6Univ of Pensylvania Sch of Med Philadelphia, PA.

Fine-needle aspiration (**FNA**) of thyroid nodules allows definitive cytopathology diagnoses in 70-80% of cases; however, the remaining **20-30% often lead to ambiguous results**. Since a more definitive diagnosis on FNAs would allow better management of patients with atypical or suspicious thyroid nodules, **we set out to develop a molecular test on thyroid FNAs that provides accurate diagnostic information on nodules with indeterminate cytopathologic features**. Many studies have used molecular analysis to try to determine which indeterminate cytology samples are malignant. We used a different approach; we tried to **identify those indeterminate nodules which are benign**. We used **genome-wide mRNA expression analysis to measure >247,186 transcripts**, including alternatively-spliced genes, in 849 thyroid nodules comprising subtypes which result in indeterminate cytopathology. Thyroid nodules were diagnosed by expert surgical pathology (i.e., gold standard). **With this training set, machine-learning algorithms were used to develop multi-gene molecular classifiers that accurately distinguish benign from malignant thyroid lesions**.

J Clin Endocrin Metab. First published ahead of print September 8, 2010 as doi:10.1210/jc.2010-1087

ORIGINAL ARTICLE

Endocrine Care

Molecular Classification of Thyroid Nodules Using High-Dimensionality Genomic Data

Darya Chudova, Jonathan I. Wilde, Eric T. Wang, Hui Wang, Nusrat Rabbee, Camila M. Egidio, Jessica Reynolds, Ed Tom, Moraima Pagan, C. Ted Rigl, Lyssa Friedman, C. Charles Wang, Richard B. Lanman, Martha Zeiger, Electron Kebebew, Juan Rosai, Giovanni Fellegara, Virginia A. LiVolsi, and Giulia C. Kennedy

Objective: We set out to develop a molecular test that distinguishes benign and malignant thyroid nodules using fine-needle aspirates (FNA).

Design: We used mRNA expression analysis to measure more than 247,186 transcripts in 315 thyroid nodules, comprising multiple subtypes. The data set consisted of 178 retrospective surgical tissues and 137 prospectively collected FNA samples. Two classifiers were trained separately on surgical tissues and FNAs. The performance was evaluated using an independent set of 48 prospective FNA samples, which included 50% with indeterminate cytopathology.

Conclusions: The FNA-trained classifier was able to classify an independent set of FNAs in which substantial RNA degradation had occurred and in the presence of blood. High tolerance to dilution makes the classifier useful in routine clinical settings where sampling error may be a concern. An ongoing multicenter clinical trial will allow us to validate molecular test performance on a larger independent test set of prospectively collected thyroid FNAs. (*J Clin Endocrinol Metab* 95: 0000–0000, 2010)

J Clin Endocrin Metab. First published ahead of print September 8, 2010 as doi:10.1210/jc.2010-1087

algorithm training process. In conclusion, we show cross-validated molecular test performance and validation on a modest-sized independent validation set with high enough specificity to reclassify over half of indeterminate FNAs as benign, making the test clinically useful. In addition, the high NPV we observe indicates that nodules with a benign molecular test result carry a risk of malignancy similar to that of nodules with benign cytopathology (29). Future ongoing studies are aimed at evaluating the performance of this classifier on a larger independent set of FNAs with indeterminate cytopathology to assess its clinical utility in thyroid nodule management.

Molecular Diagnostics and Cytology

- Could it be an adjunct to the “indeterminate” diagnosis?... Not yet
  - There is not an HPV-like test for thyroid to avoid surgery [avoid colposcopy in case of HPV(-)]
  - Standarization of techniques (from FNA to molecular assay) = reliable results
  - Molecular technique: the simpler, the better (able to be done by most labs)
  - Chose the adequate biomarkers (BRAF, RAS, RET/PTC, PAX8-PPARγ) …. Standard report format

Clark DP. Cancer Cytopathol 2010;118:14-.

Journey

- Historical perspective
- Palpation-guided FNA (PG-FNA)
- Ultrasound-guided FNA (USG-FNA)
  - Non-palpable lesions: thyroid, thyroid bed, LNs
- Molecular thyroid cytopathology
- **Thyroid cancer – therapeutic targets**

Conventional Treatment Based on FNA Diagnosis

- **Non diagnostic** – Repeat in >3 mo. If non-dx = surgery or follow-up. If it grows = surgery
  - Cystic = repeat USG-FNA (sample the solid phase)
- **Benign** – Follow up, OH ablation, hormonal tx. If grows = repeat USG-FNA
- **Atypical** – Depends on the type of "cell atypia"
  - Repeat USG-FNA in 3 -6 mo. If "atypia" = ?surgery
  - Suspicious for HCN or MFT = surgery (lobectomy)
  - Suspicious for PTC or MC = surgery (1/2 or total)
- **Malignant** – Surgery (r/o mets or rare cancers)

Targeted Therapies in Thyroid Cancer

- WD-PTC & FC = surgery followed by I^{131}
- PD-Ca, MC = molecularly directed therapy
  - RTK inhibitors:
    - ZD6474 oral: inhibits VEGFR-2 and blocks RET TK
    - BAY 43-9006 oral: multikinase inhibitor (BRAF, VEGFR, PDGFRβ, FLT-3, c-kit kinases and RET/PTC).
    - CI-1040: MEK inhibitor

Nikiforov YE. Mod Pathol 2008;21:S37.

Modern Pathology (2008) 21, S37–S43

www.modernpathology.org

Thyroid carcinoma: molecular pathways and therapeutic targets

Yuri E Nikiforov

Department of Pathology and Laboratory Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

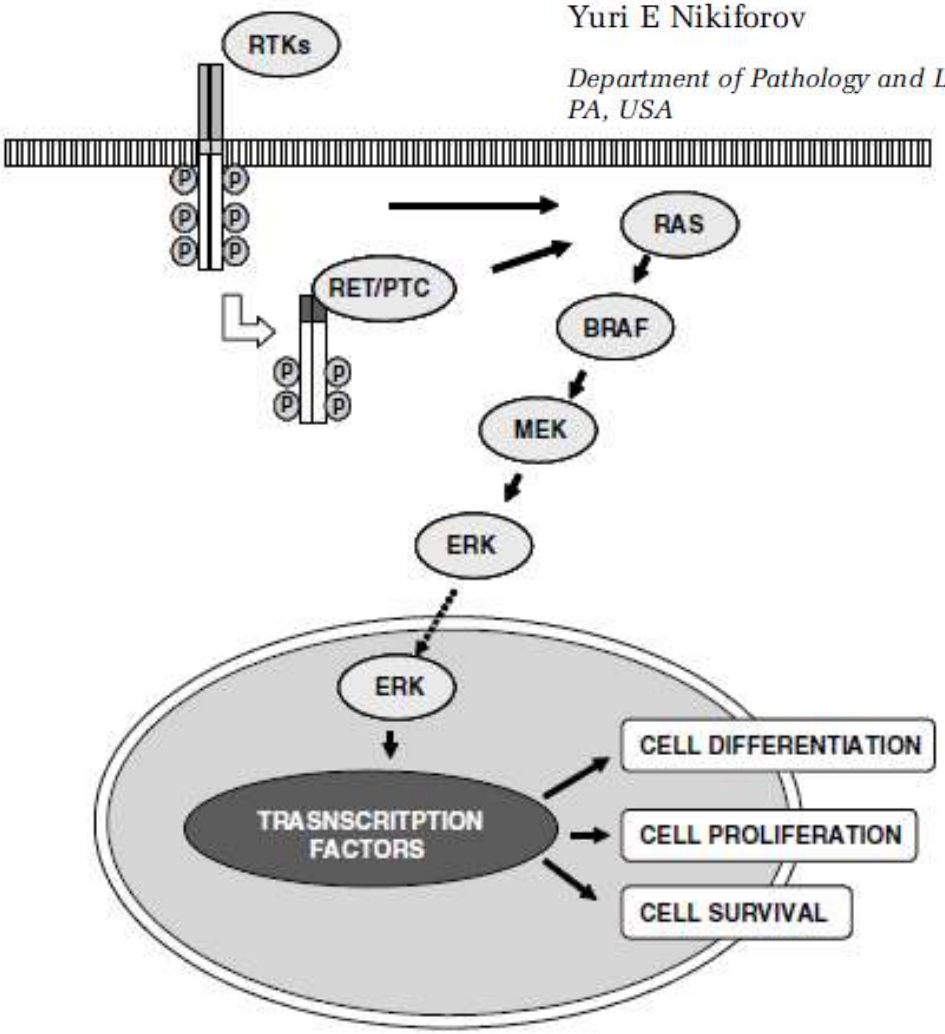


Figure 1 MAPK signaling pathway is physiologically activated by binding of growth factors to receptor tyrosine kinases (RTKs), such as RET and NTRK, resulting in receptor dimerization and activation via autophosphorylation of tyrosine residues in the intracellular domain. The activated receptor, through a series of adaptor proteins, leads to activation of RAS located at the inner face of the plasma membrane. The activated RAS binds to and recruits RAF proteins (mainly BRAF in thyroid follicular cells) to the plasma membrane. Activated BRAF phosphorylates and activate the MAPK/ERK kinase (MEK), which in turn phosphorylates and activates the extracellular signal-regulated kinase (ERK). Activated ERK translocates into the nucleus, where it regulates transcription of the genes involved in cell differentiation, proliferation, and survival. Alterations of this pathway in thyroid cancer can occur at different levels as a result of point mutation or rearrangement involving the *RET*, *RAS*, and *BRAF* genes.

Nikiforov YE. Mod Pathol 2008;21:S37.

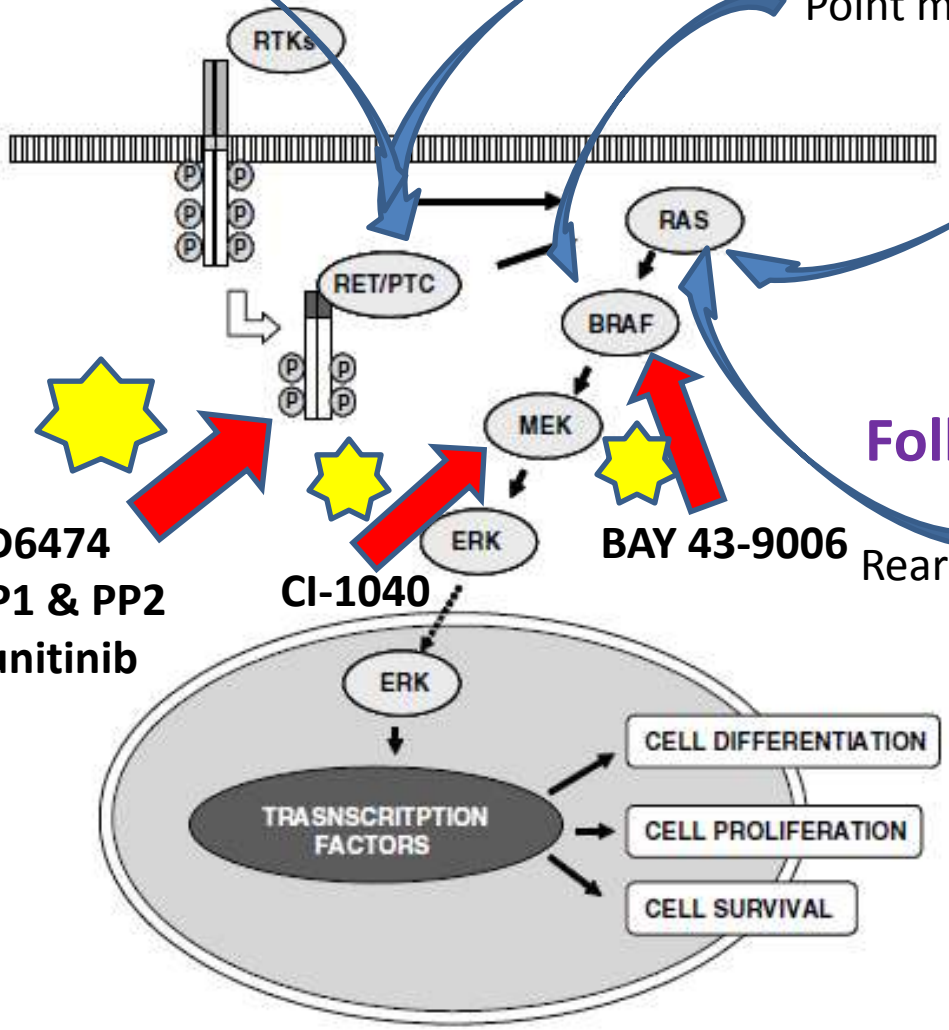
Medullary Carcinoma

Point mutations

Papillary Thyroid Carcinoma

Rearrangement

Point mutations



Follicular Carcinoma

Mutations

Rearrangement PAX8-PPARγ

Aneuploidy

ZD6474
PP1 & PP2
Sunitinib

CI-1040

BAY 43-9006

TK inhibitors

Nikiforov YE. Mod Pathol 2008;21:S37.

Table 1 Average prevalence of mutations in thyroid cancer

Tumor type	*Prevalence (%)*
Papillary carcinoma	
BRAF	45
RET/PTC	20
RAS	10
TRK	<5
Follicular carcinoma	
RAS	45
PAX8-PPARγ	35
PIK3CA	<10
PTEN	<10
Medulllary carcinoma	
Familial forms of *RET*	>95
Sporadic *RET*	50
Poorly differentiated carcinoma	
RAS	35
β-Catenin (*CTNNB1*)	20
TP53	20
BRAF	15
Anaplastic carcinoma	
TP53	70
β-Catenin (*CTNNB1*)	65
RAS	55
BRAF	20

Nikiforov YE. Mod Pathol 2008;21:S37.

Thyroid carcinoma: molecular pathways and therapeutic targets

Yuri E Nikiforov

Department of Pathology and Laboratory Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Thyroid cancer is the most common malignant tumor of the endocrine system. The most frequent type of thyroid malignancy is papillary carcinoma. These tumors frequently have genetic alterations leading to the activation of the mitogen-activated protein kinase (MAPK) signaling pathway. Most common mutations in papillary carcinomas are point mutations of the *BRAF* and *RAS* genes and *RET/PTC* rearrangement. These genetic alterations are found in >70% of papillary carcinomas and they rarely overlap in the same tumor. Most frequent alterations in follicular carcinomas, the second most common type of thyroid malignancy, include *RAS* mutations and *PAX8-PPARγ* rearrangement. *RET* point mutations are crucial for the development of medullary thyroid carcinomas. Many of these mutations, particularly those leading to the activation of the MAPK pathway, are being actively explored as therapeutic targets for thyroid cancer. A number of compounds have been studied and showed antitumor effects in preclinical studies and are being tested in ongoing clinical trials.
Modern Pathology (2008) **21**, S37–S43; doi:10.1038/mpath.2008.10

Molecular Diagnostics of Thyroid Tumors

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• *Context.*—Thyroid cancer is the most common type of endocrine malignancy and its incidence is steadily increasing. Papillary carcinoma and follicular carcinoma are the most common types of thyroid cancer and represent those tumor types for which use of molecular markers for diagnosis and prognostication is of high clinical significance.

Objective.—To review the most common molecular alterations in thyroid cancer and their diagnostic and prognostic utility.

Data Sources.—PubMed (US National Library of Medicine)–available review articles, peer-reviewed original articles, and experience of the author.

Conclusions.—The most common molecular alterations in thyroid cancer include *BRAF* and *RAS* point mutations and *RET/PTC* and *PAX8/PPARγ* rearrangements. These nonoverlapping genetic alterations are found in more than 70% of papillary and follicular thyroid carcinomas. These molecular alterations can be detected in surgically resected samples and fine-needle aspiration samples from thyroid nodules and can be of significant diagnostic use. The diagnostic role of *BRAF* mutations has been studied most extensively, and recent studies also demonstrated a significant diagnostic utility of *RAS*, *RET/PTC*, and *PAX8/PPARγ* mutations, particularly in thyroid fine-needle aspiration samples with indeterminate cytology. In addition to the diagnostic use, *BRAF* V600E mutation can also be used for tumor prognostication, as this mutation is associated with higher rate of tumor recurrence and tumor-related mortality. The use of these and other emerging molecular markers is expected to improve significantly the accuracy of cancer diagnosis in thyroid nodules and allow more individualized surgical and postsurgical management of patients with thyroid cancer.

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COMMENTARY

The Case for Pathologist Ultrasound-guided Fine-Needle Aspiration Biopsy

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If a pathologist is strongly motivated to provide the very best care for patients, he should give careful consideration to adding USG-FNA to his practice and should do so now…..

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