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FOCUS

PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY

Companion Society of the United States and Canadian Academy of Pathology

Dedicated to Clinical Practice, Clinical Education and Clinical Research

From the Editors Desk

Vinod B. Shidham, MD, FRCPath, FIAC

Dear Colleagues:

I am delighted to organize the first issue of Focus after November, 2006. Please welcome all the publications committee members and the editorial members of the focus in executing this task. According to the tradition, we are continuing to publish two issues per year; one in June-July and the other in December-January. If you have any article, news, commentary, opinion, timely topics, etc. please send your proposal to me or any of the editorial board members.

We look forward to your proactive contribution to 'Focus'. This brief issue is released to revitalize the PSC Bulletin.

Sincerely,

Vinod B. Shidham, MD, FRCPath, FIAC

PSC President's Message

Martha Bishop Pitman, M.D.

The 17th annual PSC meeting was held in Boston, MA during the 79th annual meeting of the USCAP meeting. On behalf of the current PSC leadership and all members, I would like to thank the officers and committee chairs and members for their 2007-2009 term of service to the PSC, especially the immediate past President, Dr. Steven Raab. The officers for the 2009-2011 term include myself as President, Dr. Lester Layfield as President-elect, Dr. Eric Suba as treasurer, and Dr. David Chhieng as secretary. The Board of Directors is listed in Table 1 and Committees with charges are listed in Table 2.

The 2009 scientific program began in the afternoon with the *Cells without Borders* session moderated by Dr. Eric Suba. Dr. Rana Ramazanoglu gave a presentation on the *Cytopathology in Turkey,* and Dr. Bin Yang spoke on *Cytopathology in China.*

Following the business meeting, the PSC hosted a reception at the Harvard Club on Commonwealth Avenue. An open bar and bountiful spread of delicious food prepared participants for the scientific program that immediately followed. As the Chair of the Scientific Program Committee, I moderated the session. The topic of the session was **Where Small Biopsies Go Wrong - Mistakes Made and Lessons Learned: Pathway to Patient Safety.** The speakers included: Dr. Bruce Wenig who spoke on the *Pitfalls in the Biopsy*

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In This Issue

Link download PSC membership application:
http://www.papsociety.org/pscapp2009.pdf

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The Humanities Corner

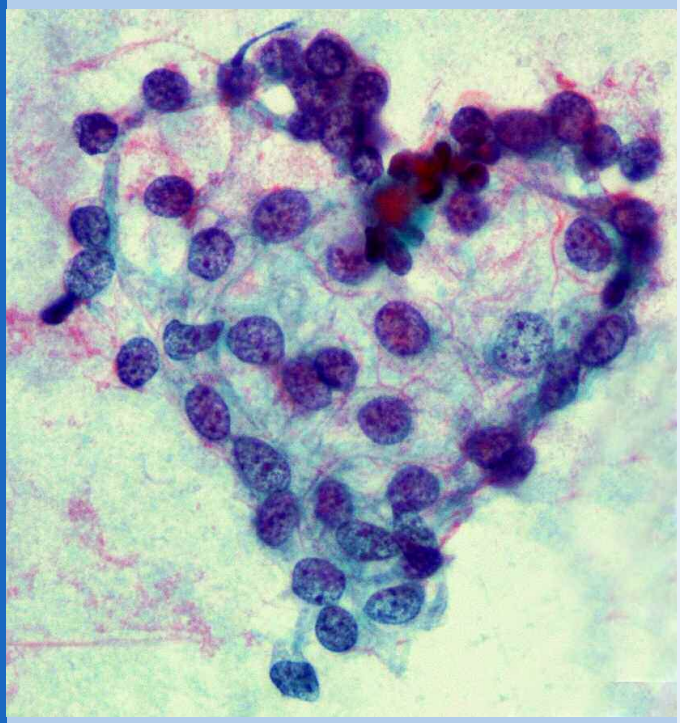
By Manon.Auger, MD, FRCP(C)
McGill University Health Center and McGill University

Of Heart-Related Matters

"The practice of medicine is an art, not a trade; a calling, not a business; a calling in which your heart will be exercised equally with your head."

from Sir William Osler, MD
in The Master-Word in Medicine, in Aequanimitas

An image for the thyroid FNA lovers!



Heart-shaped thyroid follicular cells seen in an otherwise scanty FNA of a nodule diagnosed as "Follicular lesion of Undetermined Significance" (Thyroid FNA, Pap stain, 400X)

Con't from page 1

President's Message

Diagnosis of Select Head and Neck Lesion;, Dr. Scott Kilpatrick who spoke on *The Usefulness and Limitations of FNA and Core Biopsy in the Diagnosis of Soft Tissue Tumors;* Dr. Andrea Dawson who covered *Breast Lesion Diagnosis with FNA and Core Biopsy: Challenges and Opportunities,* and Dr. Stephen Raab who finished up the session with a global overview entitled *The Future of Quality Improvement in Small Biopsy and Cytopathology Tissues.* To access the PDF of these presentations go to the USCAP web site, Education Materials tab, 2009 meeting, Companion society handouts, Papanicolaou Society.

The PSC Awards were handed out during the evening session. The **L.C. Tao Educator of the Year Award** went to Dr. Andrea Abati, and the **Yolanda Oertel Interventional Cytopathologist of the Year Award** went to Dr. Jerry Waisman. Resident Research Awards went to Dr. Fredilyn M. Lipata (1st place) and Xy Liu (2nd place). See side bar on page 6.

The PSC will sponsor a panel luncheon at the ASC meeting in Denver, CO on Sunday the 15th of November entitled "Making the Case for Cytopathologist Performed Ultrasound-Guided Thyroid FNA" moderated by Dr. David Chheing with faculty Dr. Britt-Marie Ljung and Dr. Susan Rollins.

The 2010 program for the meeting in Washington, D.C. (March 2010) is already taking shape. Our afternoon session **Cells without Borders** organized by Drs. Matt Zarka and Eric Suba will have 2-3 presentations about volunteer opportunities for cytotechnologists and pathologists in developing countries. See details on page 6. Under the leadership of Dr. Zubair Baloch, chair of the scientific program committee, the PSC will be offering a joint session with the ASC entitled **"Fine-needle aspiration of Thyroid Lesions: Beyond NCI State of the Art Thyroid FNA Conference".** This special two night session will provide expanded, multidisciplinary, in-depth coverage of this very important topic that emphasizes the role of cytology, molecular analysis and small tissue biopsy in the diagnosis and management of patients with thyroid nodules. Details on page 7.

The future of the PSC looks bright. Our financial infrastructure is sound and our global outreach expanding. As President, I want to continue to develop our relationship with the American Society of Cytopathology, our international colleagues and expand our presence at international meetings (see sidebar on European Congress of Cytology Meeting in Lisbon, Portugal). A particular goal is to organize and formalize our support for the FNA tutorials in Africa instituted by Dr. Andrew Field from Australia. The success of the organization depends on active involvement of its members, and I invite you all to become active in the organization, attend the annual meetings and get your voice heard. I look forward to working with you all to ensure that the PSC remains a leader in the field of cytopathology, bridging the gap with surgical pathology.

Table 1: Board of Directors

Officers

President: Martha Bishop Pitman, M.D. [2009-2011]

President-Elect: Lester Layfield, M.D. [2009-2011]
Treasurer: Eric Suba, M.D. [2007-2010]; eligible for 2nd 3 year term
Secretary: David Chheing, M.D., Ph.D. [2008-2011]; eligible for 2nd 3 year term

Board of Directors: Officers above plus most immediate Past President and 6 members-at-large

Past-President: Stephen Raab, M.D. [2009-2011]

Members at Large:

Britt-Marie Ljung, M.D. [2008-2011; 1st term]
Aylin Simsir, M.D. [2007-2010; 1st term]
Tarik Elsheikh, M.D. [2007-2010; 1st term]
Andrew Field, M.D. [2009-2012; 2nd term]
Zubair Baloch, M.D. [2009-2012; 1st term]
Matt Zarka, M.D. [2009-2012; 1st term]

Table 2: Committees and Charges

Nominating Committee: Exception to the above structure. This committee is composed of the three (3) immediate past presidents of the PSC.

Stephen Raab, M.D. [2009-2015]
Andrea Abati, M.D. [2007-2013]
Kim Geisinger, M.D. [2005-2011]

Charges:

- ✓ Submit names of nominees to offices elected by the membership- President-elect, Secretary, Treasurer and Members-at-large at least ninety (90) days prior to the annual business meeting
  - o The officers of the society should have already served the PSC as a Member at Large
  - o Any member of the PSC may submit a name in nomination to the chair of the nominating committee
  - o At least two (2) names must be submitted for President-elect
  - o The ballot of names from the nominating committee shall be available by the end of September following the annual meeting; the election ballot shall be ready for email and available online by the mid-October with closure of elections by mid-November.
  - o The winner is by majority vote; a vote by the Board of Directors will decide a tie of the membership vote

Scientific Program Committee

Chair: Zubair Baloch, M.D., Ph.D. [2009-2011]
Members:
Tarik M. Elsheikh, MD
N. Paul Ohori, M.D.
Scott Boerner MD FRCPC
Guido Fadda, MD
Anjali Saqi, MD

Charges:

- ✓ Design, organize and conduct the scientific program (17th 2010 and 18th 2011) of the PSC during the Companion Meeting weekend of the USCAP
  - o Topic of the session is decided by consensus at the Annual meeting of the BOD
  - o Committee is to invite speakers and meet all deadlines as outlined by the USCAP office
  - o Chair is to communicate with the Executive Director of the USCAP about topic and speakers of the sessions
  - o If coordinating the session with the ASC, the chair and committee will work with the ASC scientific program committee and chair
  - o Chair is to act as moderator of the scientific program during his/her term
  - o Write a letter of appreciation to the faculty of the scientific session on behalf of the President and PSC membership for their time and expertise following the annual meeting (within one month)

- ✓ Create an ASC-Panel Luncheon subcommittee
  - o This group will be responsible for organizing and submitting a proposal for a panel luncheon at the annual ASC meeting

Program Development Committee

Chair: Stephen Raab, M.D.
Members:
Philippe Vielh, M.D., Ph.D.
Andrew S. Field, M.D.
Matthew Zarka, MD
Sharon Sams, M.D.

Charges:

- ✓ Raise funds to support the various programs and activities of the PSC
- ✓ Work with the treasurer to develop a foundation to support the PSC's mission
- ✓ Investigate grant opportunities to support global endeavors such as the African FNA tutorials

Table 2 (Continued)

Publication Committee

Chair and Editor: Vinod B. Shidham, MD, FRCPath, FIAC
Associate Editor- Andrew H. Fischer, M.D.,
Editorial board members-
Nancy P. Caraway, MD
Manon Auger, MD, FRCP(C)
N. Paul Ohori, M.D
Adebowale Joel Adeniran, M.D.
Santo V. Nicosia, M.D.

Charges:

- ✓ Produce a newsletter (Focus) twice a year (June and December)
  - o Focus will be available online only with a link emailed to membership
  - o Focus will be converted to PDF and archived on PSC web site
- ✓ Solicit contributions from commercial companies for advertising space in the newsletter

Web Site Committee

Chair: Dan Kurtycz, M.D.
Claire Michael, M.D.
Vincent Ko, M.D.
Stefan Pambuccian, MD
Chris Jensen, MD

Charges:

- ✓ To interact and communicate on a regular basis with the technical company hired to support the PSC web site (web master: currently Earle Barnes)
- ✓ To review, edit and approve a contract with the web master
- ✓ To receive invoices from web master for change orders to the contract and ensure timely payment for services rendered
- ✓ Work with the treasurer and web mater to create an online membership application and dues payment
- ✓ Work with the secretary and web mater to create an online election process
- ✓ To ensure that the content of the web site is up to date
  - o Officers, Board of Directors and committee members
  - o Message from the President
  - o Most recent version of bylaws (currently has 2000 version; need 2007 version)
  - o Award winners including resident research award winners with link to PDF of their winning abstract
  - o Focus newsletter archive in PDF
  - o eLearning Series with SAMs credit
  - o Thyroid atlas content
  - o Annual scientific program information
  - o Links to other cytology web sites
- ✓ Work with the secretary to develop a process of allowing conference call via Skype

Budget and Finance Committee

Chair: William Faquin, M.D., Ph.D.
Members:
Massimo Bongiovanni, MD
Jeffrey F. Krane, M.D., Ph.D.
Jill C. Ono, M.D.

Charges:

- ✓ Work with the Treasurer to ensure financial stability of the PSC
- ✓ Receive requests for budget allocations from committee chairs
- ✓ Produce a budget for the PSC Board of Director for approval at the annual business meeting
- ✓ Recommend to Board of Directors a change in membership dues if needed
- ✓ Report on the independent auditor's report of the treasurer's records

Membership Committee

Chair: Rosemary Tambouret, M.D.
Angelique W. Levi, M.D.
Beatrix Cochand-Priollet, M.D.
Jian Shen, M.D.

Charges:

- ✓ Provide new (?and old) members with a certificate of membership
- ✓ Provide Diagnostic Cytopathology with new member information to ensure delivery of the journal
- ✓ Investigate opportunities to provide group membership to developing countries
- ✓ Chair shall be the contact person on the web site link for the PSC application form
- ✓ Solicit new members globally in all categories: regular, junior, emeritus and honorary
- ✓ Review current membership roster and personally contact "lost" members if possible
- ✓ Keep an accurate and up to date record of all member's contact information (as a backup and to support the Treasurer's list) and provide that information to the web master for the membership directory

Constitution and Bylaws Committee

Chair: Kim Geisinger, M.D.
David Chhieng, MD
Simon Bergman, M.D.

Charges:

- ✓ Review the bylaws and update at least every 6 years (most recent 2006)
- ✓ To edit and amend the Bylaws when changes have been approved by membership

Table 2 (Continued)

Awards Committee

Chair: Andrea Abati, M.D.
Maureen Zakowski, M.D.
Philippe Vielh MD, PhD
William C. Faquin, M.D., Ph.D.
Lester Layfield, M.D.

Charges:

- ✓ Receive nominations from the membership at least 90 days prior to the annual meeting for
  - o L.C. Tao Educator of the Year
  - o Yolanda Oertel Interventional Cytopathologist of the Year
  - o Ensure that these awards are funded for each year
  - o Provide information to the President with contact information to ensure that a letter of congratulations and invitation to the meeting to receive the award is sent in a timely fashion
  - o Provide information to the chair of the web site committee for updating of the web site
- ✓ Investigate the creation of a new award: Lifetime Achievement Award to honor the life's work of those who have greatly contributed to the field of cytopathology
- ✓ Submit the name of the winner as decided by consensus of the committee to the President and the Board of Directors at least 60 days prior to the annual meeting
- ✓ Be responsible for the production of the plaques for these awards as well as the award plaques for the resident research awards and ensure that all plaques are at the meeting for a formal presentation at the beginning of the scientific program along with a letter of appreciation from the President with the award check

International Scientific Programs and Relations Committee

Chair: Matthew Zarka, M.D.
Eric Suba, M.D.
Martha Pitman, M.D.
Stephen Raab, MD
Tarik Elsheikh, MD
Andrew Field, MD
Phillippe Viehl, MD, PhD

Charges:

- ✓ Work with the organizers of the European Congress of Cytology on the PSC sponsored symposium at their annual meeting
- ✓ Organize the Cells without Borders afternoon session of the PSC
- ✓ Create connections with other international organizations to help recruit members and facilitate joint sessions
- ✓ Work with Dr. Field to investigate ways the PSC can sponsor and facilitate the African FNA tutorials

Research Committee

Chair: Claire Michael, M.D.
Members:
Ben Davidson, MD PhD
Stewart Knoepp, M.D. PhD
Paolo Guttuso, M.D.
Aziza Nassar, MD
Diane Kowalski, M.D.

Charges:

- ✓ Evaluate abstracts for the PSC Research Awards
  - o Chair renders all abstracts submitted by application and all Stowell-Orbison Award abstracts anonymous
  - o Committee members score abstracts based on novelty of idea, scientific and/or practical value, and effort
  - o Report results to the President with contact information and abstract title to ensure a letter of congratulations is sent and that the residents are invited to the PSC scientific session to receive the award
  - o Provide information to the web site committee chair for updating the web site
- ✓ Encourage research award applicants among cytology residents and fellows in cytology training programs

Education and Training Committee

Chair: Aylin Simsir, M.D.
Joan Cangiarella, MD
Guoping Cai, M.D.
Assistant Professor
John P. Crapanzano, M.D.
Armando Filie, MD
Anna B. Berry, M.D.
Andre L. Moreira MD, PhD

Charges:

- ✓ e-Learning initiative
  - o Produce a case of the month
  - o Finalize the on-line Thyroid Atlas
- ✓ Work with Syed Ali and Ed Cibas, editors of the Bethesda Thyroid Atlas

Standards of Practice Guidelines Committee

Chair: Britt-Marie Llung, M.D.
John Abele, M.D.
Susan Rollins, M.D
Miquel Sanchez, M.D.

Charges:

- ✓ Inform the PSC in the establishment of training and credentialing guidelines for pathologist's use of ultrasound in FNAs
- ✓ Report to the PSC progress made in this area for Focus and the web site
- ✓ Provide educational sessions (e.g. Panel luncheon at ASC meeting in 2009)

Table 2 (Continued)

Government Relations Task Force

Chair: Steve Black-Schaffer, M.D.

Charges:

- ✓ Monitor legislative and regulatory issues
- ✓ Propose areas of advocacy by the PSC
- ✓ Communicate and partner with other medical and cytopathology to advocate for issues relative to cytology organizations
- ✓ Update the PSC membership with important timely information with a brief report in the biannual Focus Newsletter

Book Series Task Force

Chair: Kim Geisinger, M.D.
David Chhieng, MD
Stephen Raab, MD

Charges:

- ✓ Edit the PSC book series on small biopsy/cytology specimens
- ✓ Organize the series and authors of the various volumes
- ✓ Be the liaison between the PSC and publisher (Cambridge University Press; Marc Strauss)
- ✓ Ensure timely productivity
- ✓ Provide a degree of uniformity to series
- ✓ Oversight of contracts to ensure that the PSC receives royalties as well as authors

AWARDS

L.C. Tao Educator of the Year Award
Dr. Andrea Abati

Yolanda Oertel Interventional Cytopathologist of the Year Award
Dr. Jerry Waisman

Resident Research Awards

1st PLACE: Dr. Fredilyn M. Lipata
F Lipata, S Mahooti, D Chhieng, P Hui.
Yale University, New Haven
Abstract: "Ki-Ras Mutation Analysis of Pancreaticobiliary Cytology Specimens with Indeterminate Diagnoses"

2nd PLACE: Dr. Xy Liu
Xy Liu, L Hutchinson, M St.Cyr, J Garver, B Woda, EF Cosar.
UMass Memorial Medical Center, Worcester, MA
Abstract: "Integration of Morphology with Fluorescent In Situ Hybridization

2010 Cells without Borders Afternoon Session

Opportunities for volunteer pathologists and cytotechnologists in low- to moderate-income countries.
Joe Harford, Director of the NCI Office of International Affairs
http://oia.cancer.gov/about-director.shtml

Patologi Oltre Frontiera ('Pathologists Without Borders'): The Italian Volunteer Efforts in Cuba, Kosovo, Madagascar, Palestine,Tanzania, and Zambia
Chiara Sugrue, Island Jewish Medical Center
http://www.patologioltrefrontiera.it/

This companion meeting will represent the first joint session with the American Society of Cytopathology. The PSC and ASC will co-sponsor a joint program covering two nights, the PSC at 7:00pm on Saturday, March 20, 2010, and the ASC at 7:30 PM on Sun. March 21, 2010. The program will be co-moderated by the chairs of the PSC and ASC scientific program committees, Dr. Zubair Baloch and Dr. Ritu Nayar. An overview of the program is as follows:

"Fine-needle aspiration of Thyroid Lesions: Beyond NCI State of the Art Thyroid FNA Conference".

Saturday, March 20, 2010: PSC

7:00-7:15	Introduction of program and panelists	Dr. Zubair Baloch
7:15-7:45	Endocrinologist View of role of FNA in the management of Thyroid Lesions	Dr. Susan Mandel
7:45-8:15	Molecular "Reflex" Testing of Thyroid FNA specimens.	Dr. Yuri Nikiforov
8:15-8:40	Questions and Answer session	
8:40-9:00	Case 1 - Presentation	Dr. Anjali Saqi
9:00-9:20	Case 2 - Presentation	Dr. Melina Flanagan
9:20-9:40	Case 3 - Presentation	Dr. Scott Boerner
9:40-10:00	Question and Answer session	

Sunday, March 21, 2010: ASC

7:30-7:40	Introduction of program and panelists	Dr. Ritu Nayar
7:40-8:15	Experience with the Thyroid Bethesda Reporting System in Practice. Effect of various Preparatory Techniques on cytomorphology of thyroid FNA	Dr. Edmund Cibas
8:15- 8:40	How much is enough to histologically diagnose Papillary thyroid carcinoma in less than classic cases?	Dr. Virginia LiVolsi
8:40- 8:50	Questions	
8:45- 9:30	Case Presentations	
	1. 8:45-9:00 Case 1	Dr. Edward Stelow
	2. 9:00-9:15 Case 2	Dr. Yun Gong
	3. 9:15-9:30 Case 3	Dr. Michael Thrall

European Congress of Cytopathology: PSC sponsored Satellite Symposium
http://www.cytologylisboa2009.com

SATELLITE SYMPOSIUM EFCS-PSC

EUS of the Mediastinum and Pancreas: Clinical and Cytological Features of Pre-Operative Diagnosis

Chairs: Martha Pitman, USA and Marleen Praet, Belgium

Speakers:

16h00-16h05 Introduction

M. Pitman, USA

16h05-16h30 The role of EUS in the diagnosis and staging of pancreatic malignancy

W. Brugge, USA

16h30-16h55 Implementation of endoscopic ultrasound with real time guided fine needle aspiratons in pulmonary medicine: The crucial role is for the pathologist!

M. Praet, Belgium

16h55-17h30 Endoscopic Ultrasound Guided Fine Needle Aspiration biopsy of the pancreas: A morphological and multimodal approach to the diagnosis of solid and cystic mass lesions

M. Pitman, USA

Call for Nominations for Awards

Every year PSC presents two awards to the cytopathologists in recognition of their work at our annual meeting.

The awards committee is now accepting nominations for these awards to be presented at the 2010 Companion Meeting at USCAP.

The two awards are:

The Educator of the Year Award is presented to a Pathologist in recognition of his/her meritorious service, and contributions to the field of cytopathology education. The award is presented during the Papanicolaou Society of Cytopathology Companion Meeting held during the annual meeting of the United States and Canadian Academy of Pathology. The award also includes $1,000.00 made possible by the generosity of Dr.
L.C. Tao.

Nominees for this award should have demonstrated significant contributions to the field of cytopathology education, including but not limited to: active participation in, or development of, exemplary training programs and educational activities at the state, regional and national levels. Any member of the Society may submit nominations to the Award Committee for consideration.

The Yolanda Oertel Interventional Cytopathologist Award is an annual award started to acknowledge the contribution of pathologists to the fine needle aspiration service. The award recognizes those who promote the use of fine needle aspiration, and encourage other pathologists to utilize fine needle aspiration.

Any cytopathologist promoting or utilizing the fine needle aspiration service is eligible for the award.

Please nominate for one or both of these awards and submit:

CV of the nominee
A photograph of the nominee
A statement regarding the accomplishments of the nominee

The above information will be distributed to the Awards Committee who will decide upon the winners. The winners will then be approved by the Executive Board and Officers of the Society.

Molecular Diagnostics in Cytology: Bridging Cyto-Morphology with Molecules

Sameer S. Talwalkar, MD[1], Marina Nikiforova, MD[2], Andrew H. Fischer[4], N. Paul Ohori, MD[3]

Divisions of Molecular Diagnostics[1]; Molecular Anatomic Pathology[2], and Anatomic Pathology[3], Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania. University of Massachusetts[4].

Few disciplines in clinical and laboratory medicine have progressed as rapidly as molecular diagnostics in the last decade. The completion of the Human Genome Project opens many opportunities, and in parallel, molecular diagnostic methods have become more affordable, less labor-intensive, and less complex. Increasing numbers of academic medical centers and commercial laboratories have begun integrating molecular testing methods into their labs, and penetration is now evident in a number of community hospital laboratories. The advent of commercial in-vitro diagnostics (IVD), analyte specific reagent (ASR) and research use only (RUO) kits has facilitated the transfer of moderate to higher volume tests to individual laboratories, and new offerings are constantly under development. Needless to say, the realm of molecular diagnostics has also spread in areas of surgical pathology and cytology. Here we briefly review the commonly utilized molecular technologies, most of which have application in cytology, and then briefly discuss their specific applications for diagnosis and prognosis in gynecologic and non-gynecologic cytology.

Technologies:

a) In-situ Hybridization (Morphology-based microscopic diagnostics)

In-situ hybridization (ISH) involves the use of a single-stranded DNA or RNA probe to locate a gene or mRNA molecule in a cell or tissue. Like PCR, ISH (Figure 1) begins with denaturation of double-stranded nucleic acid. This is followed by hybridization with a labeled probe specific to the target. After several washes to remove the unbound probe, the specifically bound probe is then interpreted by fluorescent or a bright field microscope. The advantages are that this technique can be performed on a cell block or smear to allow correlation with morphology, it has a high specificity, and is less technically challenging and relatively less labor-intensive compared to other molecular methods. An advantage of this method with respect to HPV detection in cervical smears is that it allows differentiation between episomal (diffused) versus integrated (punctate) HPV DNA patterns. Integrated HPV is strongly associated with a high grade lesion whereas episomal HPV is associated with transient or low-grade lesions.

b) Hybrid Capture®

A seminal advancement for cytology was the development of molecular testing for HPV. This is not only more sensitive than the conventional Pap smears but also provides valuable clinical information on the high-risk HPV genotypes which cannot be attained by cyto-morphologic or non-molecular methods alone. Although there are several methods for detecting HPV, the HPV Digene® assay was one of the earliest methods available. This assay is based on the Hybrid Capture Technology®, a patented method that essentially involves hybridizing an RNA probe to a target DNA sequence of high risk HPV types, thereby creating RNA:DNA hybrids. These hybrids are then captured onto a solid phase coated with universal capture antibodies (specific to RNA:DNA hybrids), washed, then incubated with additional alkaline phosphatase-conjugated antibody against RNA:DNA hybrids. The bound alkaline phosphatase is detected with a chemiluminescent dioxetane substrate. Upon cleavage by alkaline phosphatase, the substrate produces light that is measured on a luminometer in Relative Light Units (RLUs). The RLUs are directly proportional to the viral load. The hybrid capture technique can use multiple specific RNA probes for different targets, providing information on multiple HPV genotypes. The specimen requirements for this assay are flexible, but liquid cytology fixatives, such as ThinPrep® is a preferable source.

c) Invader® Technology

The hybrid capture technique uses antibodies conjugated to alkaline phosphatase to amplify the signal from the captured target DNA. Invader Technology® is a new method for amplifying target DNAs. The principle of the Invader Technology® lies in a single-strand DNA nuclease derived from thermophilic Archaea bacteria. The nuclease (dubbed "Cleavase®") specifically recognizes free flaps of single stranded DNA which occur when overlapping oligonucleotides are hybridized to a single stranded DNA target template. The enzyme performs a natural function since overlapping flaps occur normally during DNA synthesis. The site of cleavage occurs at a precise place on the free-flap of single stranded DNA in the overlapping oligonucleotide. The left side of Figure 2 shows the two oligos, provided in vast excess, for detecting the "genotype specific target". One oligo is called the "invader oligo" that does not form a flap because it is entirely complementary to the target DNA throughout its length. The second oligo is called the "probe" in the left side of Figure 2 and it bears an extension (flap) without complementarity to the DNA template. By conducting the enzymatic reaction with the thermostable Cleavase at a single (non-cycling) elevated temperature at about the melting point of duplex DNA, the probe oligos melt off and re-anneal to the template. Amplification is achieved because each time the probe anneals, its flap is released by Cleavase. Occurring simultaneously is a second set of Cleavase reactions, this time with a fluorescent resonance energy transfer cassette ("FRET") oligonucleotide probe that hybridizes specifically to the accumulating flap oligos (left lower part of Figure 2) . Cleavase recognizes the resulting overlapping hybridization between the cleaved flap, and it cleaves a fluorescence quencher on the FRET cassette (designated as a light purple "Q" in the lower part of Figure 2). When the quenching signal is cleaved, the FRET

cassette emits detectable fluorescence. This second Cleavase reaction, in which the quencher is released from the FRET cassette, results in a further amplification of the original signal. The result is a highly sensitive detection of specific target sequences. Multiple reactions can take place simultaneously using specific probes, invader oligos and FRET cassettes with different fluorescence emissions (right side of Figure 2), allowing multiple targets to be specifically identified. The Invader technology has emerged as an FDA-approved method for detecting HPV in cervical samples.

d) Real-Time PCR

The advent of real-time PCR has revolutionarized the field of molecular diagnostics. Not only does it permit rapid and quantitative assessment of targets over a wide dynamic range, but also has a distinct advantage of being a 'closed-system' application, thereby greatly reducing the risk of contamination and virtually eliminating the need for separate pre and post-amplification rooms. Real-time PCR can be performed using: a) double-stranded DNA dyes such as SYBR green or b) fluorescent reporter probes, most commonly hybridization probes and hydrolysis (TaqMan®) probes. The major disadvantage of using the dyes is that they bind to all the dsDNA products, including non-specific PCR products such as primers and dimers. This can interfere with signal interpretation, especially in assays where target quantification is desired. The probe-based method is the most accurate and reliable. A distinct advantage of using hybridization probes is the ability to perform melting curve analysis. This can also be exploited as a technique for mutation scanning, a rapid method to identify mutations that can later be confirmed by sequencing. Figure 3 shows the altered melting of a hybridization probe with a mismatch for the base change resulting in the constitutively active BRAF V600E mutation.

e) Sequencing

The availability of DNA sequencing assays has expanded dramatically as a result of the Human Genome Project, along with the development of capillary electrophoresis-based methods to obtain results. Most molecular laboratories still rely on dideoxynucleotide chain termination (Sanger) sequencing for clinical diagnostics. Most of the dideoxy sequencing is now performed on automated platforms which use the dye-terminator method, labelling the chain-terminator ddNTPs, thereby permitting sequencing in a single reaction, rather than four separate reactions as originally designed. Not far behind is pyrosequencing, which has gained an increasing presence in some clinical laboratories. Although the higher cost and smaller sequence reads are the major prohibitive factors, pyrosequencing offers advantages in ease of use and the ability to detect smaller percentages of mutation-bearing cells, which is especially relevant to cytologic specimens.

Applications:

The applications of molecular diagnostics in cytopathology can be broadly divided into gynecologic and non-gynecologic cytology. The gynecologic applications are largely, but not entirely, restricted to screening and diagnosis of HPV, and detecting high grade SIL and invasive carcinomas. The non-gynecologic applications include many solid tumors on which a cell block can be made. FNA specimens from thyroid and pancreatic cysts can be submitted directly for molecular testing, which is a routine practice in our medical center. Since the yield from cytology specimens is not as high as compared to resection specimens, maintaining cellular and nucleic acid integrity is extremely crucial for the validity of the results. Commercially available nucleic acid preservative solutions are an excellent way to transport fluid specimens to the laboratory. Recent unpublished results have shown integrity of RNA through paraffin embedding in cell blocks, if formalin is not used, and if the dehydration step of paraffin-embedding takes place within about 30 minutes (Fischer, manuscript in preparation). As far as the quantity of the specimen is concerned, the goal is to triage sufficient number of representative cells for molecular studies, without compromising the specimen for morphologic evaluation. A usual practice followed at most institutions is to use the first pass during an FNA procedure for preparing a direct smear for cytology and the subsequent passes and / or the needle washing can be collected into a tube containing nucleic acids preservative solution. The minimum amount of nucleic acid used for real-time PCR and RT-PCR testing can be 5-50 ng RNA per reaction—well within the capacity of FNA to procure RNA (2).

Gynecologic Cytology

a) CIN assessment

While there are many methods for detecting HPV, HPV infection by itself is associated with a normal pap test in over 50% of cases, as reviewed in the 2006 ASCCP guidelines. Detecting CIN 1, or especially CIN 2/3 and invasive carcinoma is the object of oncoFISH®: a qualitative FISH test for determining the acquisition of aneuploidy of 3q26 (1). Gain of the 3q region appears to predict progression from CIN1/2 to CIN3 and invasive carcinoma. The clinical utility and validity may be strengthened as more studies are published using this test.

Non-Gynecologic Cytology

a) Thyroid

One of the important contributions of molecular diagnostics in cytology is for the pre-operative diagnosis of thyroid nodules. Recent data (2) has shown that identification of any of the following mutations: BRAF, N and HRAS, RET/PTC or PAX8/PPARã is a strong indicator of malignancy and therefore, testing for these mutations improves the accuracy of the cytologic diagnosis, particularly samples with indeterminate cytology. Detection of BRAF mutation and RET/PTC and PAX8/PPARã rearrangements in FNA samples has a 100% positive predictive value for thyroid cancer (2). Interestingly, the RAS mutations also appear to be of high diagnostic value. The detection of RAS mutation has an 88% probability of malignancy, including a 63% probability of a papillary carcinoma and a 25% probability of a follicular carcinoma (2). The high risk of malignancy seems to provide justification for recommending surgery to all patients with mutation-positive nodules. Overall, 62% of malignant thyroid lesions had a mutation detected in FNA samples, and therefore Cytology still complements

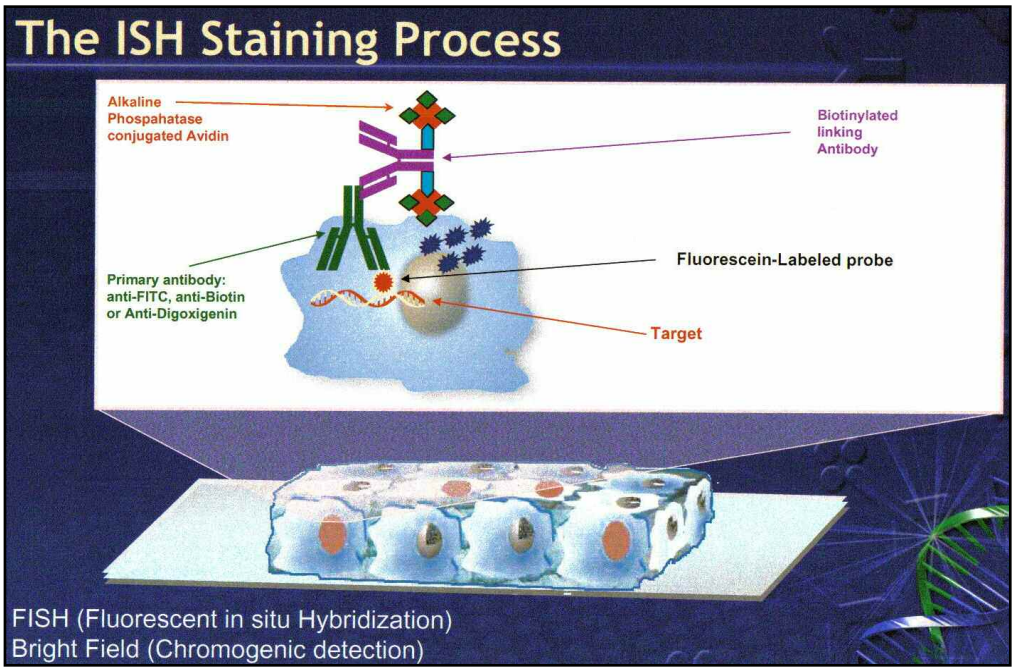


Figure 1: Principle of in-situ hybridization that can be used with fluorescent (FISH) or chromogenic (CISH) methods. *(Courtesy of E. Blair Holladay, PhD, SCT(ASCP), Medical University of South Carolina, Charleston, SC, USA)*

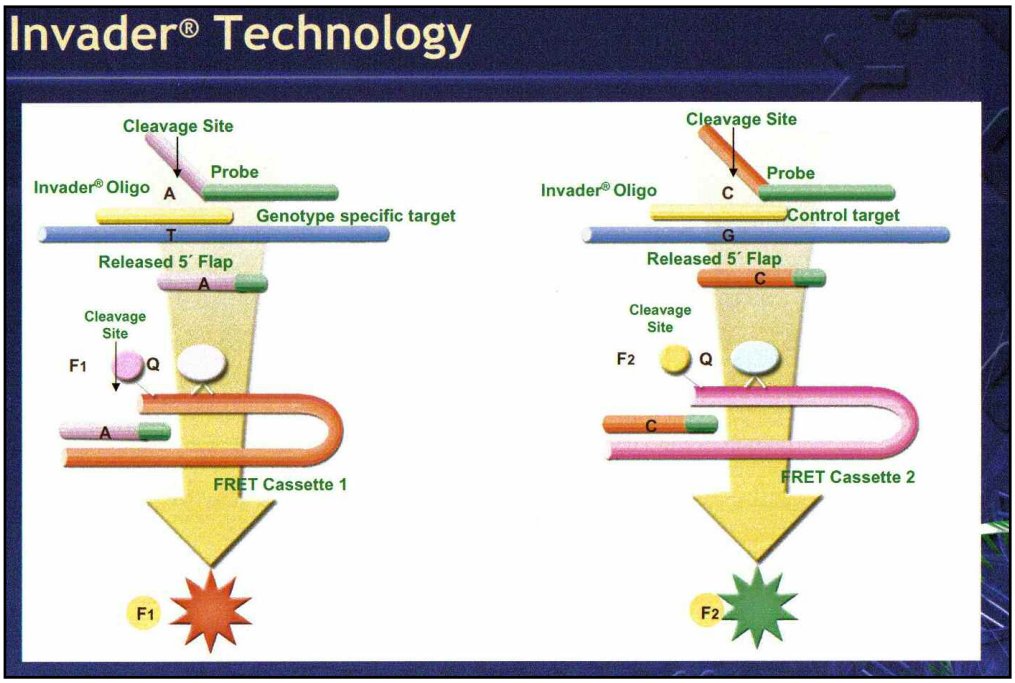


Figure 2: Principle of Invader® technology. *(Courtesy of E. Blair Holladay, PhD, SCT(ASCP), Medical University of South Carolina, Charleston, SC, USA)*

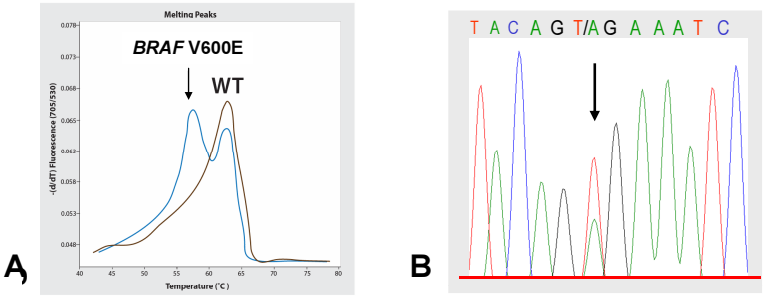


Figure 3: Mutation scanning by (a) real-time PCR with melt curve analysis revealing the mutant allele showing a lower melting temperature compared to the wild type allele and by (b) di-deoxy nucleotide chain termination (Sanger) sequencing showing a heterozygous nucleotide change from a T to A.

molecular testing. Further studies will likely define particular scenarios in which molecular testing is most likely to be helpful, for example as an adjunct for an atypical thyroid FNA. It is also possible that the preoperative detection of mutations may provide helpful prognostic information to refine patient management. This may be particularly true for the BRAF mutation, which is considered an independent marker of aggressiveness of papillary thyroid carcinomas and correlates with the frequency of lymph node metastases (3, 4). It seems likely in the future that patients with BRAF mutations and non-malignant cytology diagnoses should undergo total thyroidectomy, eliminating the need for intraoperative pathology consultation or completion thyroidectomy, ultimately reducing costs and morbidity.

Another potential test that can be performed on thyroid FNA specimens is the analysis of Telomerase activity. Telomerase activity and hTERT gene expression are noted to be elevated in malignant lesions and therefore are useful in differentiating benign from malignant lesions. The major drawback for this test, however, is the lack of specificity, since inflammatory conditions such as lymphocytic thyroiditis and up to 40% of benign lesions, especially follicular adenomas also reveal an elevated Telomerase activity.

b) Pancreas

KRAS is activated by point mutations in 75% and 90% of pancreatic adenocarcinomas, with most mutations localized to codon 12. Mutations of KRAS oncogene have been most frequently investigated using FNA specimens as a possible adjunct to conventional cytology for making a definite diagnosis of pancreatic adenocarcinoma. Another useful specimen source is pancreatic cyst fluid. Pancreatic cyst fluid contains enough DNA to allow mutational analysis. A first-hit KRAS mutation followed by allelic loss is most predictive of the presence of malignancy in a pancreatic cyst (5) and can therefore help in differentiating benign from malignant cysts.

c) Lung

Although cell blocks from FNA specimens are commonly used, sputum cytology, bronchoalveolar lavage, or even circulating tumor cells can be used for molecular analysis. The most common assessment is for KRAS codon 12 and 13 and EGFR exons 19 and 21 mutations, the presence of which can modulate response to treatment and may serve as criteria for selection to receive targeted therapy. There are also reports indicating the utility of assessing EGFR gene amplification (6) or EGFR mutations (7) for selecting patients for EGFR inhibitor therapy. EGFR mutations can be detected in scant circulating tumor cells.

d) Genitourinary

Tests such as UroVision® that identify aneuploidies of chromosomes 3, 7, 9p21, and 17 are widely used in clinical practise for the diagnosis of urothelial carcinomas. MN/CA9 gene expression by RT-PCR in FNA samples of kidney has been investigated and found to be useful and reliable marker for renal cell carcinomas with a high specificity (8). Recent developments also include analysis of microsatellite and telomerase activity in these tumors. These tests, still at a research stage, can be performed on cytology specimens.

In conclusion, there is an excellent potential of using material procured from FNA or other cytology sample for almost any type of molecular test. Many of the tests that we discussed are performed in major academic centers and commercial laboratories. Some of the tests have potential for clinical use in the near future. Therefore, with molecular tests becoming a part of a routine diagnostic work-up, the cytopathologist plays a key role in coordinating the testing events with the molecular pathology group to facilitate better patient care.

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Inadequate fine needle aspiration biopsy samples: Pathologists versus other specialists

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Abstract

Background: Fine needle aspiration biopsy (FNAB) is a simple, sensitive, quick and inexpensive method in which operator experience is essential for obtaining the best results.

Methods: A descriptive study in which the aspiration biopsy cases of the Pathology and Cytopathology Service of the University Hospital of the UANL (2003-2005) were analyzed. These were divided into three study groups: Group 1, FNAB performed by a pathologist; Group 2, FNAB performed by specialists who are not pathologists, Group 3, FNAB guided by an imaging study with immediate evaluation by a pathologist. The samples were classified as adequate and inadequate for diagnosis, the organ, the size and characteristics of the lesions were taken into consideration.

Results: A total of 1905 FNAB were included. In Group 1: 1347 were performed of which 1242 (92.2%) were adequate and 105 (7.7%) were inadequate. Of the 237 from Group 2, 178 were adequate (75.1%) and 59 inadequate (24.8%); in Group 3 there were 321 of which 283 (88.1%) were adequate and 38 (11.8%) inadequate. A statistically significant difference was found between FNAB performed by Group 1 ($p< 0.001$) and the other groups. A multivariate analysis was done where the organ punctured, the study groups, the size and characteristics of the lesion by study group were compared, finding that the most important variable was the person who performed the procedure.

Conclusion: The experience and training of the person performing the aspiration biopsy, as well as immediate evaluation of the material when it is guided, substantially reduces the number of inadequate samples, improving the sensitivity of the method as well as reducing the need for open biopsies to reach a diagnosis.

NEWS

The PSC will sponsor a panel luncheon at the ASC meeting in Denver, CO
On Sunday the 15th of November, 2009 this timely panel luncheon entitled "Making the Case for Cytopathologist Performed Ultrasound-Guided Thyroid FNA" is moderated by Dr. David Chheing with faculty Dr. Britt-Marie Ljung and Dr. Susan Rollins.
http://www.cytopathology.org/website/article.asp?id=2169

March 2010 PSC Scientific Program: USCAP Meeting, Washington, DC
This companion meeting will represent the first joint session with the American Society of Cytopathology. *The PSC and ASC will co-sponsor* a joint program covering two nights, the PSC at 7:00pm on Sat. March 20, 2010, and the ASC at 7:30 PM on Sun. March 21, 2010. The program will be co-moderated by the chairs of the PSC and ASC scientific program committees, Dr. Zubair Baloch and Dr. Ritu Nayar.
Fine-needle aspiration of Thyroid Lesions: Beyond NCI State of the Art Thyroid FNA Conference.- Saturday, March 20, 2010.
http://www.uscap.org/

The PSC will co-sponsor a symposium at the European Congress of Cytology in Lisbon, Portugal.
EUS of the Mediastinum and Pancreas: Clinical and Cytological Features of
Pre-Operative Diagnosis Chairs: Martha Pitman, USA and Marleen Praet, Belgium
http://www.cytologylisboa2009.com

The 17th International Congress of Cytology will take place in Edinburgh, Scotland, 16.-20. May, 2010.
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Test group biases and ethical concerns mar New England Journal of Medicine articles promoting HPV screening for cervical cancer in rural India. R Marshall Austin, Chengquan Zhao *CytoJournal* 2009, 6:12 (16 July 2009)
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Joe Harford, Director of the NCI Office of International Affairs
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Patologi Oltre Frontiera ('Pathologists Without Borders'): The Italian Volunteer Efforts in Cuba, Kosovo, Madagascar, Palestine,Tanzania, and Zambia
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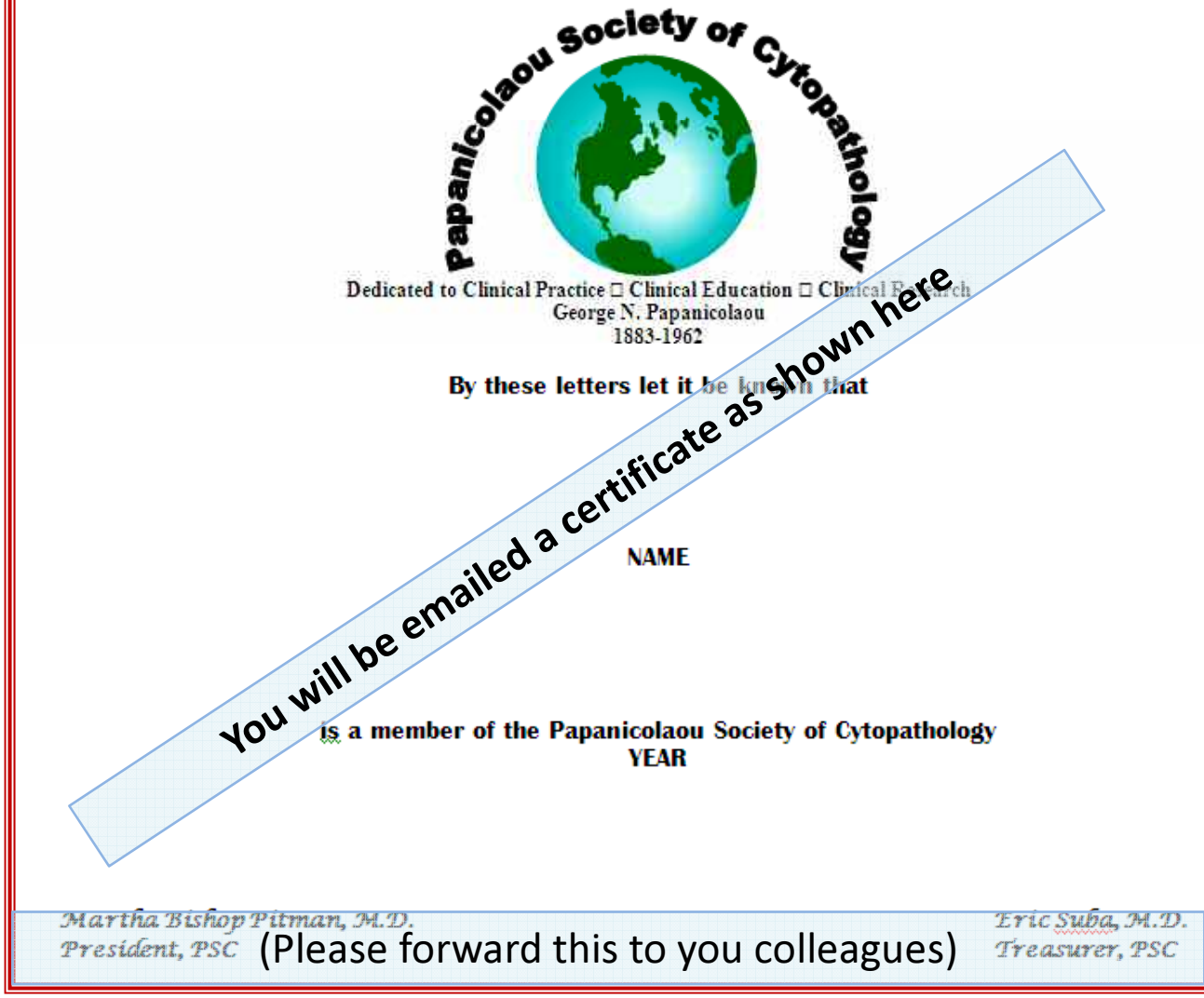

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