

Turn Cytopathology's Crisis Into Opportunity

(Transforming the Pap smear Cytopathology)

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Crisis

(East meets West)

Crisis - any event that is, or is expected to lead to, an unstable and dangerous situation affecting an individual, group, community, or whole society. (1425 “turning point in a disease; 1627 “decisive moment”)

危機 occur in the 3rd century A.D., at which time, and for centuries thereafter, they convey the notion of “latent danger.”


機 machine, chance, crucial point, opportunity

The Birth of “Stress”

A direct quote from Hans Selye 1907-1982

The Closest Chinese Word to Signify Stress Is Written As Two Characters As Illustrated Below
And Can Be Translated As Crisis

CRISIS



危
機

The upper character represents: DANGER The lower character represents: OPPORTUNITY

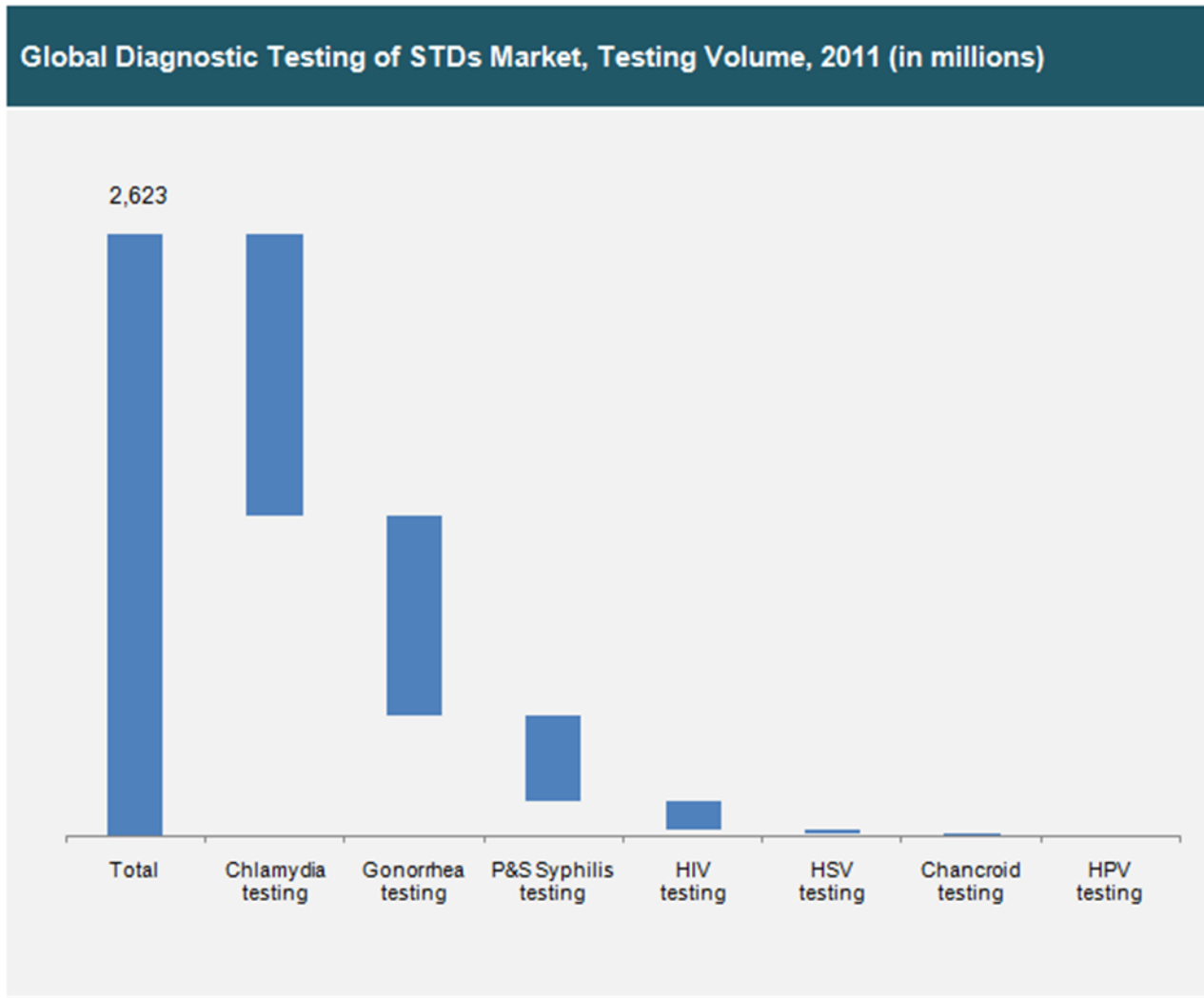
Cervical Cancer Screening Market by Test Type (PAP, HPV), Kit Type (PAP, HPV)- Global Forecast to 2020

- The global cervical cancer screening market ~ \$15 billion in 2014.
- CAGR of 7.0% to reach nearly \$22 billion by 2020.
- PAP test is the largest segment of the global market.
- However, **HPV/co-test will be the fastest-growing product segment.**
- Major players: Hologic Corporation, Becton, Dickinson and Company, Qiagen N.V., and Hoffmann-La Roche.

<http://www.marketsandmarkets.com/Market-Reports/cervical-cancer-screening-market-10110147.html>

Publication Date: September 2015

STD Diagnostic Testing Market-excluding HPV testing 2011



Source: KOL Opinions, Expert Interviews, Press Releases and TMR Analysis

CAP proficiency survey for HPV16/18 only- limited by industry



**The 2010 Global Proficiency Study of Human Papillomavirus Genotyping in Vaccinology
-WHO HPV LabNet Global Reference Laboratory.
Eklund C et al. J. Clin. Microbiol. 2012, 50(7):2289.**

Eklund et al.

TABLE 2 Proficiency of detecting HPV types tested for, by assay^a

HPV assay	No. of data sets	HPV region(s) targeted [primer(s)]	No. of data sets proficient at:				Not proficient
			100%	99-90%	89-80%	<80%	
All assays	118	L1/L2/E1/E2/E4/E6/E7	26	8	15	23	46
Linear Array (Roche)	17	L1 (PGMY)	8	1	1	1	6
InnoLiPA (Innogenetics)	12	L1 (SPF10)	0	1	1	1	9
In-house line blot	10	L1 (GP/PGMY)	1	0	1	4	4
CLART HPV 2/3 (Genomica)	8	L1 (PGMY)	0	0	2	2	4
In-house type-specific PCR	6	L1/E6/E7	0	0	1	1	4
In-house real-time PCR	5	L1/E1/E4/E6/E7	0	1	0	1	3
In-house PCR-RFLP ^b	7	L1/E6/E7	0	0	1	4	2
In-house PCR LumInex	7	L1 (GP/MGP/BSGP/PGMY)	3	0	1	1	2
In-house PGMY-CHUV	6	L1 (PGMY)	4	1	0	1	0
In-house PCR sequencing	6	L1/E6	0	0	0	5	1
Papillocheck microarray (Greiner Bio-One)	4	E1	4	0	0	0	0
PCR LumInex (Multimetrix)	3	L1 (GP)	0	0	3	0	0
Digene HPV genotyping LQ (Qiagen)	3	L1 (GP)	0	0	0	2	1
Digene HPV genotyping RH (Qiagen)	2	L1 (GP)	0	1	1	0	0
HybriBio microarray	2	L1	0	0	0	0	2
DEIA line probe (Lab.Bio)	2	L1 (SPF10)	0	0	0	0	2
In-house dot blot	2	L1	1	0	0	0	1
LCD array (Chitron)	2	L1 (PGMY)	0	0	2	0	0
EASYChIP (King Car)	2	L1	2	0	0	0	0
Other commercial ^c	9	L1	1	3	1	0	4
Other in-house ^d	3	L1/L2/E1/E2/E6	2	0	0	0	1

What can a cytopathologist do?

Be a molecular biologist **as well**

1. Lee SH. **Guidelines for the use of molecular tests for the detection and genotyping of human papilloma virus from clinical specimens.** Methods Mol Biol. 2012;903:65-101.
2. Lee SH, Vigliotti JS, Vigliotti VS, Jones W. **From Human Papillomavirus (HPV) Detection to Cervical Cancer Prevention in Clinical Practice.** Cancers (Basel). 2014 Oct 2;6(4):2072-99.

America's medical profession – “Doc” Adams Dodge City, Kansas during the 1870's



Dr J Hinsey was the visionary to create a profession

Edward Doisy isolated the sex hormone estrone 1929

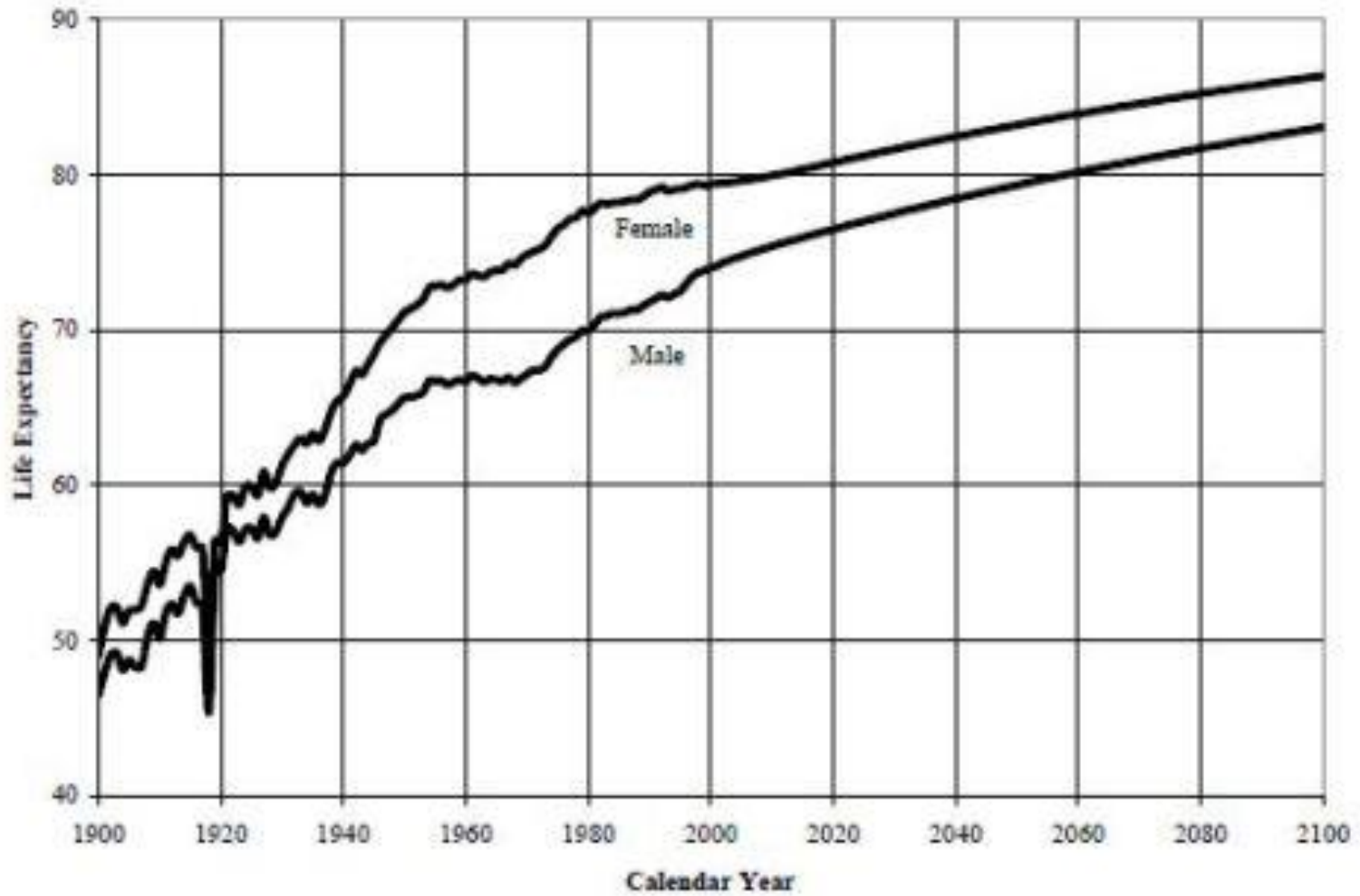
George Papanicolaou discovered the estrous cycle in guinea pigs

In 1939, the new Chair of the Department of Anatomy at Cornell, Dr Joseph C. Hinsey, strongly urged and fully supported Papanicolaou's attention to cancer detection via the vaginal smear:

“...together they outlined a program whereby the first step would be the development and establishment of its **validity**; the second phase would be to **train others** to use it; and finally an effort would be made to **educate** the medical profession and the public concerning what the method had to offer.” [1,2]

1. Erskine Carmichael. **The Pap Smear: Life of George N. Papanicolaou**. Springfield, IL; Charles C.Thomas,1973.
2. Casper MJ, Clarke AE. **Making the Pap smear into the "right tool" for the job**. Soc Stud Sci. 1998 Apr;28(2):255-90.

**Figure 2a—Life Expectancy at age 0
by Sex and Calendar Year
(Based on Period Tables)**



Cancer of the Uterus: The Vaginal Smear in Its Diagnosis.

Traut HF, Papanicolaou GN.

Cal West Med. 1943 Aug;59(2):121-2.

The **malignant epithelial cells exfoliate** from the surface of neoplastic growths, much as do normal cells. They then float downward into the **vaginal fornix**, where they accumulate and become mixed with normal cells of epithelial and blood origin, as well as with mucus, bacteria, parasites and cellular debris.

Variations in size, with lobulated, crenated, or elongated nuclei are most suggestive. If, in addition, the chromatin shows fragmentation, granulation, or displacement to one or other pole of the nucleus with one or more nucleoli, the probabilities of malignancy are great.

If, in addition, one sees numbers of **such cells in close proximity to one another** so that if, in addition, one sees numbers of such cells in close proximity to one another so that the above criteria can be established by accurate comparison, a presumptive **diagnosis of malignancy** can be made.

Cytology of CIN 1-3 (~1968-1988)

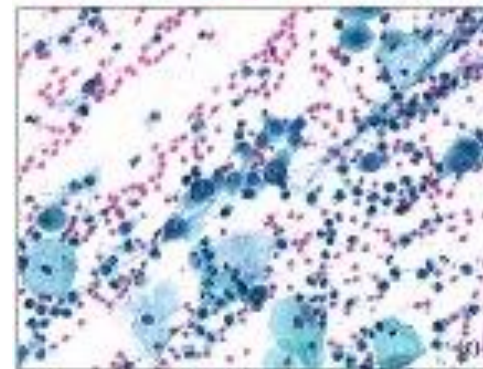
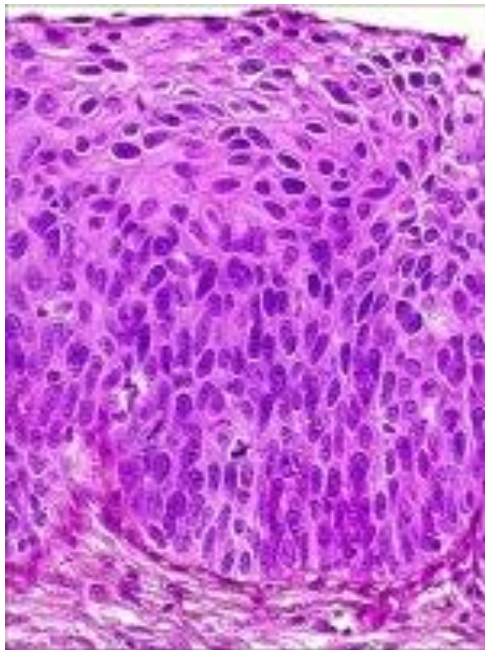
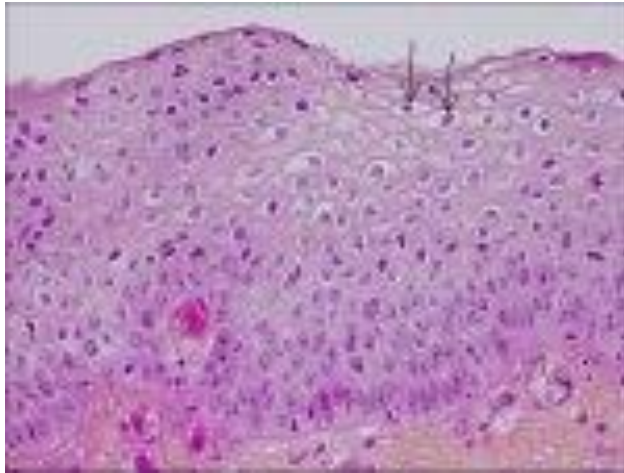


FIGURE 2.1: Cytological appearance of (a) CIN 1, (b) CIN 2, (c) CIN 3 (=20).

Uterine cervical cancer in USA

- Only cancer preventable by epithelial ablation of the transformation zone (LEEP, Cone)
- **44** cervical cancers/100,000 women in 1947
- Papanicolaou (Pap) smear widely used (cytotecs were trained at Cornell; in Tennessee , 393 intraepithelial carcinomas, of which 353 had not been suspected, and 373 invasive uterine cancers, of which 112 had not been suspected ->1956)
- **~5** cervical cancers/100,000 women, largely new immigrants and underprivileged. (now listed as a rare disease-ACS)
- Cancer deaths / 100,000 (year 2000)
 - Lung 56.5
 - Breast 27.1
 - Colorectal 20.9
 - Cervical 2.8 (**1.7**)

Conceptual Model of Cervical Carcinogenesis

INITIATION PROMOTION PROGRESSION

HPV infection \Leftrightarrow HPV persistent* \Leftrightarrow CIN \Rightarrow Invasive cancer

168 days median



E6, E7 transcription

integration, multiparity, OC use, smoking,

inflammation*, viral variant, micronutrients, host genes

*A tumor promoter- macrophage-mediated immune response causing:

cell death inhibition, genomic instability, fibroblast

activation, matrix metabolism, angiogenesis

Reported rates of spontaneous regression vary from 6-50% depending on diagnostic criteria and length of follow-up

Trimble CL, Piantadosi S, Gravitt P, et al. **Spontaneous regression of high-grade cervical dysplasia: effects of human papillomavirus type and HLA phenotype.** Clin Cancer Res. 2005 Jul 1;11(13):4717-23

Table 5. Clinical behavior of biopsy-confirmed CIN2/3

Author	Sample size (<i>n</i>)	Patient population	Time interval	Rate of regression (%)
Follen (2001)	17	CIN2/3	12 mos	50
Meyskens (1994)	48	CIN2	(21-27 mos)	27
Meyskens (1994)	35	CIN3	(21-27 mos)	31
Keefe (2001)	20	CIN2	24 mos	19
	32	CIN3	24 mos	19
Alvarez (2003)	38	CIN2/3	12 wks	32
Trimble	100	CIN2/3	15 wks	28

The Papanicolaou Test for Cervical Cancer Detection: A Triumph and a Tragedy

Leopold G. Koss, MD

JAMA. 1989;261(5):737-743.

Although this cancer detection system has been shown to be effective in reducing the rate of morbidity and mortality from invasive cervical cancer in appropriately screened populations, there is no evidence that the Papanicolaou test has succeeded anywhere in complete eradication of this theoretically preventable disease. It is important to inform the public about the **potential failures** of the system and the reasons for them.

Why? Imperfect sensitivity and specificity.

“Despite our desperate, eternal attempt to separate, contain, and mend, categories always leak” - Clarke AE, Casper MJ. From simple technology to complex arena: **classification of Pap smears**. Med Anthropol Q. 1996 Dec;10(4):601-23.

The Science, The Art and The Dogma in Cytopathology 2001, paving the way to an HPV industry

The 2001 Bethesda System: terminology for reporting results of cervical cytology.

Solomon D¹, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop.

Solomon D¹: Senior Investigator at the National Cancer Institute. JAMA. 2002 Apr 24;287(16):2114-9.

•The Bethesda system for reporting cervical/vaginal cytologic diagnoses:revised after the second National Cancer Institute Workshop, April,29-30, 1991. *Acta Cytol* 1993;37:115-24. (It was introduced in 1988, and revised in 1991 and 2001.)

Joël Coste, Béatrix Cochand-Priollet, Patricia de Cremoux, Catherine Le Galès, Isabelle Cartier, Vincent Molinié, Sylvain Labbé, Marie-Cécile Vacher-Lavenu, Philippe Vielh, for the French Society of Clinical Cytology Study Group
BMJ VOLUME 326 5 APRIL 2003 bmj.com

Title: Cross sectional study of conventional cervical smear, monolayer cytology, and human papillomavirus DNA testing for cervical cancer screening

Conclusions: Monolayer cytology is less reliable and more likely to give false positive and false negative results than conventional cervical smear tests for screening **for cervical cancer.**

Advances in Cervical Screening Technology

Mark H Stoler M.D.

Department of Pathology, University of Virginia Health System, Charlottesville, Virginia

Mod Pathol **2000**;13(3):275–284

“Might HPV testing be a better screening method?”

This question has been most thoroughly examined by workers in the Netherlands, who have proposed using an **extremely sensitive PCR-based** method as the first step in a cervical cancer screening program”

“The lower the prevalence of HPV in the population to be screened, the better the performance profile of an **extremely sensitive HPV screening test.**”

Austin RM. Dismantling of the U.S. cytotechnology educational infrastructure is premature and carries significant risks.

Arch Pathol Lab Med. 2008 Feb;132(2):154-8.

30 and older (DNA with Pap).³⁵ Data supporting FDA approval was significantly provided by collaborative studies co-authored by the medical director of the sole FDA-approved HPV test manufacturer and researchers in the Division of Cancer Epidemiology and Genetics of the National Cancer Institute (NCI).³⁶⁻⁴² Key leaders of a relatively new organization, the American Society of Colposcopy and Cervical Pathology (ASCCP) testified, along with leadership of the NCI group, at FDA hearings in 2000 on criteria to be used by the FDA to determine effectiveness of ad-

junctionive HPV testing.⁴³ Then, in 2001, the NCI held a consensus conference on the management of women with cervical cytologic abnormalities in Bethesda, Maryland. Bypassing the American College of Obstetricians and Gynecologists, the NCI meeting was sponsored by the ASCCP. As a major outcome of the 2001 NCI/ASCCP conference, reflex HPV DNA testing was recommended as the “preferred” method for evaluation of women with ASCUS LBC results.⁴⁴

Downstream events of the Bethesda System

- Interobserver Reproducibility of Cervical Cytologic and Histologic Interpretations Realistic Estimates From **the ASCUS-LSIL** Triage Study. **Mark H. Stoler**, MD and **Mark Schiffman**, MD, MPH. JAMA. 2001;285:1500-1505 : Conclusions Interpretive variability is substantial for all types of cervical specimens. **Histopathology of cervical biopsies is not more reproducible than monolayer cytology, and even the interpretation of LEEP results is variable.** Given the degree of **irreproducibility** that exists among well-trained pathologists, realistic performance expectations should guide use of their interpretations.
- **Castle PE, Stoler MH, Solomon D, Schiffman M.** Am J Clin Pathol. 2007 May;127(5):805-15. The relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade 2 to the quality control pathology-reviewed diagnoses: an ALTS report. In particular, we provide evidence that **CIN 2 is not a true biologic entity** but an equivocal diagnosis of precancer, representing an admixture of HPV infection and precancer.
- **Schiffman M, Wentzensen N.** From human papillomavirus to cervical cancer. Obstet Gynecol. 2010 Jul;116(1):177-85. **Future** cervical cancer prevention: prophylactic **vaccination** of adolescents against carcinogenic HPV infections; an increased role for **HPV testing**; **improvements to colposcopy** to increase sensitivity; and **reductions in the number of lifetime screens** needed for prevention.

The trend was set for the cervical screen industry before 2007

Clinical Advisor

By Nelly Edmondson Gupta

April 17, 2007

[Clinical Feature](#)

Are Pap tests in danger of being phased out?

Netherlands' Qiagen buying Digene for \$1.6 billion | Reuters

www.reuters.com/.../us-digene-qiagen-idUSN034115082007060

Reuters

Jun 3, 2007 - Qiagen NV , a maker of genetic testing equipment, said on Sunday it will acquire Digene Corp. for \$1.6 billion in cash and stock.

[Routine human papillomavirus genotyping by DNA sequencing in community hospital](#)

[laboratories.](#) Lee SH, Vigliotti VS, Vigliotti JS, Pappu S. Infect Agent Cancer. 2007 Jun 5;2:11.

The crisis was thought to affect cytotechnologists only

1. Young NA, Greening SE, Gupta P, Bibbo M, Ehya H. **The declining Pap test. An omen of extinction or an opportunity for reform?** *Acta Cytol.* 2008;52(3):277-8
2. Wilbur DC. **Perilous times for the Pap test?** CAP Today, February 2008.
Available at: www.cap.org
3. Eltoun IA, Roberson J. **Impact of expected changes in national Papanicolaou test volume on the cytotechnology labor market—an impending crisis.** *Am J Clin Pathol.* 2007;128(4):665-70.
Available at: <http://ajcp.metapress.com>
4. **Cytotechnology schools under threat.** Letter sent by 12 professional societies “to inform educators and laboratory administrators of a coming crisis in healthcare.” CAP Today, August 2008.
Available at: www.cap.org
5. Benstein B, Holladay EB, Kenwright K, Means M, Zaleski MS. **For cytotechnologists, molecular training a must.** CAP Today, August 2008.
Available at: www.cap.org

CAP Today – A Point of No Return

New pathology lab products and industry news from dozens of companies.

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Cytopathology at the tipping point



Barbara A. Crothers, DO

Daniel F.I. Kurtycz, MD

Leonard I. Bloom, SCT(ASCP)CM

May 2014—A tipping point implies a point of no return, a monumental change in the status quo, a transformation that leads to a new paradigm. Malcolm Gladwell, in *The Tipping Point: How Little Things Can*

The danger of increasing cytology screen productivity

Levi AW, et al. **Increasing cytotechnologist workload above 100 slides per day using the BD FocalPoint GS imaging system negatively affects screening performance.** Am J Clin Pathol. 2012 Dec;138(6):811-5.

Productivity was increased by decreasing the percentage of cases that underwent full manual review (from 38% to 19%) and by decreasing the time spent on each slide (from 5.5 min to 3.7 min).the false-negative fraction increased significantly, from 1% to 6.9%.

**A White Paper Commissioned by the American Society of Cytopathology
Steering Committee on the Future of Cytopathology**

Potential Strategy #1: Do Nothing: **Non-gyn** and FNA services, molecular testing and other ancillary studies may increase demand.

Potential Strategy #2: Optimize the Current Scope of Practice: Cytotechnologists performing image analysis and quantitation of immunohistochemistry; in-situ hybridization; karyotyping or chromosome analysis in cytogenetics; photographic or image acquisition and management (for reporting, conferences, research or education).

Potential Strategy #3: Expand existing Cytotechnology models using morphology skills with novel educational tools: A master degree or combining curriculum with clinical laboratory science programs may be needed.

Potential Strategy #4: Establish a model for core skills of a cytopathology assistant: A CA is envisioned as a practitioner with increasing responsibility and an increase in independent judgment in analysis and reporting of morphologic tests.

Potential Strategy #5: Split Training for Gynecological Cytology and Non- Gynecological Cytology, creating a Non-Gynecological Expanded Practitioner: One certification would concentrate only on gynecologic cytology. The other certification would include gynecologic, non-gynecologic and fine needle aspiration cytology, as well as other ancillary skills.

Potential Strategy #6: Bachelor's Degree in Laboratory Science: Create a four-year BS with cytotechnology.

A White Paper Commissioned by the American Society of Cytopathology 5/14/2010

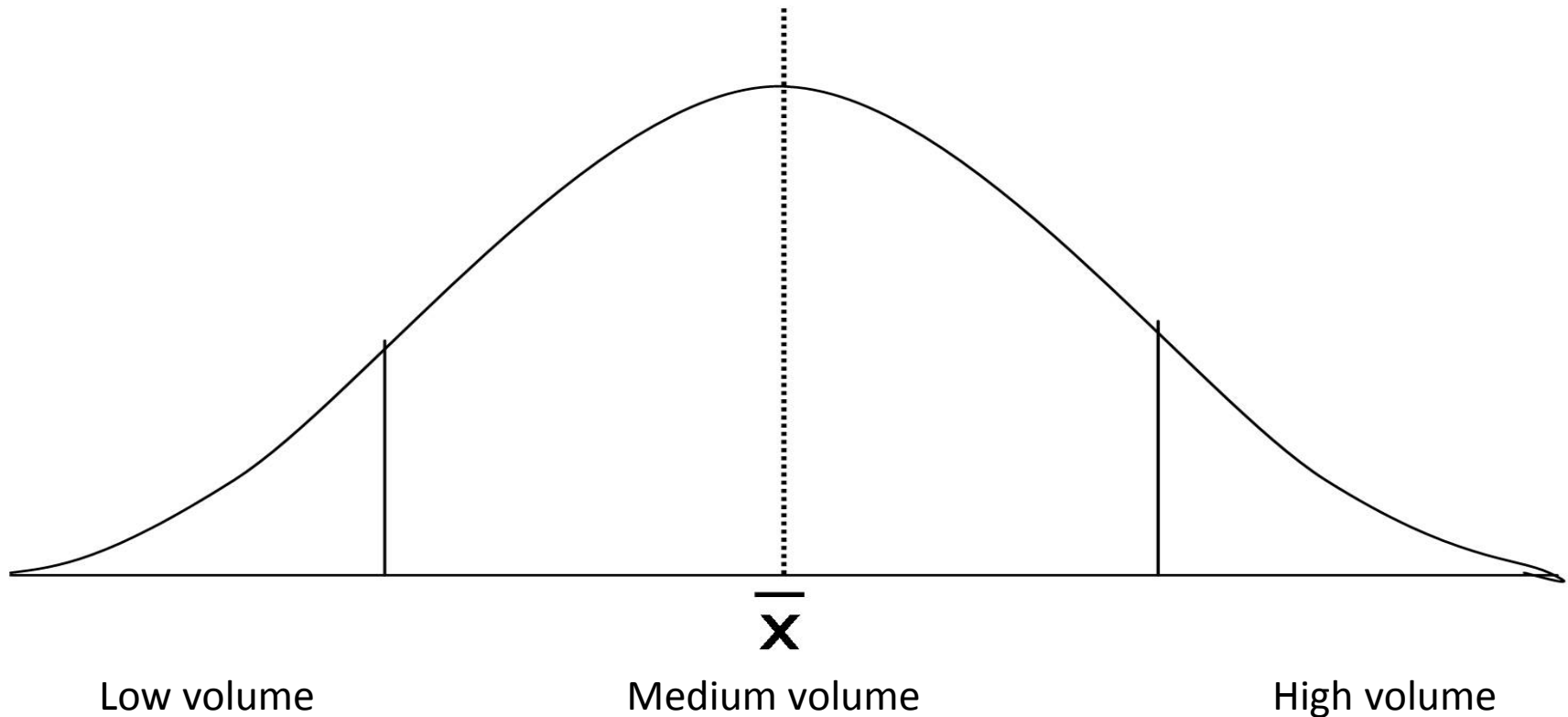
BOC Examinations Statistics

As of December 2009, the ASCP BOC has granted 15,030 Cytotechnologist (CT) and 591 Specialist in Cytotechnology (SCT) certifications.²⁷ Over the last six years, there has been a steady decline of enrollment in both the CT and SCT certification exams. The following table illustrates the CT and SCT exam statistics from January 2005-December 2009:

Exam Period	CT Exam					SCT Exam				
	Total	Pass		Fail		Total	Pass		Fail	
		N	%	N	%		N	%	N	%
Jan-Dec 2009	209	187	89%	22	11%	23	17	74%	6	26%
Jan-Dec 2008	244	210	86%	34	14%	18	14	78%	4	22%
Jan-Dec 2007	246	210	85%	36	15%	10	5	50%	5	50%
Jan-Dec 2006	266	247	93%	19	7%	19	19	100%	0	0%
Jan-Dec 2005	262	234	89%	28	11%	25	20	80%	5	20%
Jan-Dec 2004	320	273	85%	47	15%	35	31	89%	4	11%

The lack of interest in the SCT exam lies with the absence of additional professional value in terms of compensation or advancement. Comments offered by participants during Summit discussions suggest that if new roles for cytotechnologists are identified and embraced, the SCT exam might be the vehicle used to certify competency for the skills needed to fulfill these new roles.

Strategies proposed depend on availability of a high volume of anatomic pathology specimens and job shifting



Dissenting evidence is presented, from “reflex HPV test” to “reflex cytology”?

- **Dismantling of the U.S. cytotechnology educational infrastructure is premature and carries significant risks.** Arch Pathol Lab Med. 2008 Feb;132(2):154-8.
- **Austin RM, Zhao C. Is 58% sensitivity for detection of cervical intraepithelial neoplasia 3 and invasive cervical cancer optimal for cervical screening?** Cytojournal. 2014 May 22;11:14.
- **Stoler MH, Austin RM, Zhao C. Cervical cancer screening should be done by primary HPV testing with genotyping and reflex cytology for women over the age of 25 years.** J Clin Microbiol. 2015 May 6. pii: JCM.01087-15. [Epub ahead of print]:

In April of 2014 the FDA approved the use of an HPV test (the cobas HPV Test) for primary cervical cancer screening for women over the age of 25 years, without the need for a concomitant Pap test. Reaction to this decision has been mixed. Dr. Stoler explains why he favors the primary screening algorithm while Drs. Austin and Zhao explain why they prefer the co-testing approach to screening for cervical cancer.

Laboratory Economics

volume 9, No. 4, April, 2014

Insources Pap, HPV, GC, Chlamydia testing by gynecologists

WOMEN'S HEALTH CONNECTICUT OPENS LAB

On December 15, 2013, Women's Health Connecticut (Avon, CT) opened its own 11,000-square-foot laboratory in Rocky Hill, Connecticut (about 5 miles south of Hartford). Previously, Women's Health had used both national labs as well as a variety of local hospital-based labs and pathology groups.

The transition of testing to the new lab has resulted in the loss of more than 20% of Pap test volume at many hospital-based labs and pathology groups throughout the state. That's because Women's Health Connecticut employs approximately one-third of the 665 Ob/Gyn physicians practicing in the state. In fact, Women's Health Connecticut is the largest Ob/Gyn group practice in United States, employing 215 Ob/Gyns at 100 office locations throughout Connecticut. *Full details*

Google search for cytopathology meetings

-what crisis?

[Annual Scientific Meeting | American Society of Cytopathology](#)

November 13-16, **2015**. Hyatt Regency Chicago, Illinois.

[American Society of Cytopathology | Saving lives one cell at ...](#)

Basics of Billing and CPT Coding in **Cytopathology** Laboratory August 25, **2015**

[Cytopathologymeeting.org](#)

Current Issues in **Cytology**, moderated by Dr. Teresa Darragh, **focuses on cervical screening** ..no refund for those received after October 30, **2015**.

[39th European Congress of Cytology, 20 - 23 September 2015](#)

[www.cytology-iac.org/...list/377-39th-european-congress-of-cytology](#) Milano **2015**. Special ...

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[27th Annual Advances in Cytology](#)

[www.hms-cme.net/352036/](#)

Jan 15, 2015 - June 7 – 11, **2015** "Advances in **Cytology**" will provide pathologists

[USCAP Diagnostic Cytopathology 2015 Recap - YouTube](#)

Jan 29, 2015 - Uploaded by USCAP - United States & Canadian Academy of Pathology

USCAP Diagnostic **Cytopathology 2015** Recap.

Causes of the Pap smear cytopathology crisis

- Intrinsic defects of Pap smears to meet the expectation as the ultimate screening tool
- Newly gained knowledge of the viral etiology in cervical carcinogenesis demands changes
- Creation of the HPV industry and marginalization of pathology as a profession
- Fragmentation of leadership in the profession
- Lack of innovative spirit and willingness to take risk

The Future of Cytopathology

- Reading **reflex Pap cytology**. Questionable value if high-risk HPV is triage to colposcopic biopsies-good business, poor health care policy.
- Reading **FNAs of solid tumors**. Does not have enough volumes and needs in community hospitals.
- Science-based **molecular cytopathology**. Using HPV, GC, Chlamydia and Pap smear as the core tests of a DNA sequencing-based laboratory-revolutionary, good patient care and financially self-sustainable.

Bring DNA sequencing to hospital labs

1. Peacock S. **Health care: Bring microbial sequencing to hospitals.** Nature 2014; 509(7502):557-9.
2. Peacock SJ, Weinstock GM. **Microbial sequencing to improve individual and population health.** Genome Med. 2014; 6:103. An Editorial.
3. Lee SH, Vigliotti VS, Vigliotti JS, Pappu S. **Routine human papillomavirus genotyping by DNA sequencing in community hospital laboratories.** Infect Agent Cancer. 2007 Jun 5;2:11

Ebola, Dengue fever, Lyme disease:

The growing economic cost of **infectious diseases**

http://www.nsf.gov/discoveries/disc_summ.jsp?cntn_id=133576

Sensitivity and Specificity of all NAATs are questionable!!

The Opportunity

In the U.S.A.

1. *Chlamydia trachomatis*
2. *Neisseria gonorrhoeae*
3. Human papillomaviruses
4. **Lyme disease (borrelioses)**

In west African countries

1. Malaria?
2. Lassa fever?
3. Cholera?
4. **Ebola? – big consequence**

HPV DNA test promotes unnecessary cervical biopsies in US >\$ 10 billion per year

(Sin Hang Lee testified at the FDA transparency meeting on June 24, 2009, Washington, DC)

- **More than 95% of referrals to colposcopy for diagnostic workup are false positive and/or potentially excessive (*unnecessary*). Screening with combined cytologic and HPV testing, regardless of patient age, leads to the highest number of excessive colposcopic referrals. [Stout NK et al. Department of Health Policy and Management, Harvard School of Public Health. 2008]**
- **The estimated 1992 annual cost of the overused colposcopic biopsy was at \$6 billion. The number of colposcopic biopsies increased markedly since.** [[Lousuebsakul V et al. *Is colposcopic biopsy overused among women with a cytological diagnosis of atypical squamous cells of undetermined significance \(ASCUS\)?* J Women's Health \(Larchmt\). 2003; 12:553-9.](#)]
- **Since 2003, more ASCUS diagnoses have been made by pathologists after the HPV DNA test was approved for triage to 4-quadrant cervical biopsies. Now the unnecessary biopsies may cost more than \$10 billion in 2009.**
- **Cost due to psychological and physical trauma to patients not counted.**
- **Cost for complications, such as excessive bleeding and infections not counted.**
- **Cost of loss of work days of the patients not counted.**

Evaluation of HPV-16 and HPV-18 Genotyping for the Triage of Women With High-Risk HPV+ Cytology- Negative Results

Thomas C. Wright Jr, MD, Mark H. Stoler, MD, Abha Sharma, PhD, Guili Zhang, PhD, Catherine Behrens, MD, PhD, Teresa L. Wright, MD and the ATHENA (Addressing THE Need for Advanced HPV Diagnostics) Study Group
Am J Clin Pathol 2011;136:578-586

Conclusion

These analyses validate the 2006 American Society of Colposcopy and Cervical Pathology guidelines for HPV-16/HPV-18 genotyping, which recommend referral to **colposcopy of HPV-16/HPV-18+ women with negative cytology.**

Current commercial HPV test kits may not be sensitive enough or comprehensive enough to detect all carcinogenic HPV genotypes

- Eight percent (**8%**) of patients with cervical squamous cell carcinoma were found to be HPV-negative within 30 months preceding the histological diagnosis of a cervical squamous cell carcinoma [1]; and **12.6%** of histologically confirmed cases of cervical carcinoma were HPV-negative [2].
- Based on the analysis of large-scale data from 17 European countries, laboratories using the PCR-based SPF10-LiPA25 test kit did not find HPV DNA in **8.2% of histologically** proven invasive cervical cancers [3]; and no HPV DNA was detected in **13%** (1234/9486) of confirmed cases of squamous cell carcinoma [4].

1. Farnsworth A. Acta Cytol. 2011, 55, 307–312.
2. Poljak M, et al. ; Acta Dermatovenerol. Alp. Panon. Adriat. 2009, 18, 94–103.
3. Tjalma WA, et al. Int. J. Cancer 2013, 132, 854–867.
4. De Sanjose S, et al. Lancet Oncol. 2010, 11, 1048–1056.

Undisputable facts facing the cytopathologists

- Pap smear cytology has a **higher specificity** for invasive cancer and carcinoma in situ (CIN3, severe dysplasia) than HPV tests.
- Pap smear cytology has a **lower sensitivity** in detecting CIN2/3 lesions, compared to current commercial HPV test kits.
- The value of commercial **HPV test** kits for screening **invasive** cancers is unknown.
- Commercial HPV tests designed to be **not “too sensitive”** may be negative in invasive cancer or carcinoma in situ with low HPV copy number (~1) per cancer cell.
- Commercial HPV tests based on hybridization may not provide **accurate genotyping** for persistent infection follow-ups.

Cervical Cancer Screening Rates in the United States

Solomon D et al. CA Cancer J Clin 2007;57:105–111

An estimated **65.6 million** Pap tests performed in 2003.

Full compliance with ACS guidelines would approximately halve the total number of tests to **34 million**. –This prediction of declining Pap tests was amid the news about a booming HPV industry, for examples:

[News in brief, June 07, 2007 - PMLiVE](#)

Jun 7, 2007 - Financial news. **Qiagen acquires Digene** for USD 1.6bn. **Qiagen**, a Dutch manufacturer of genetic testing equipment, has revealed it will **buy** ...

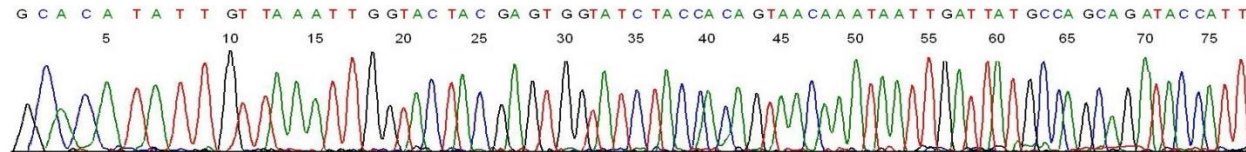
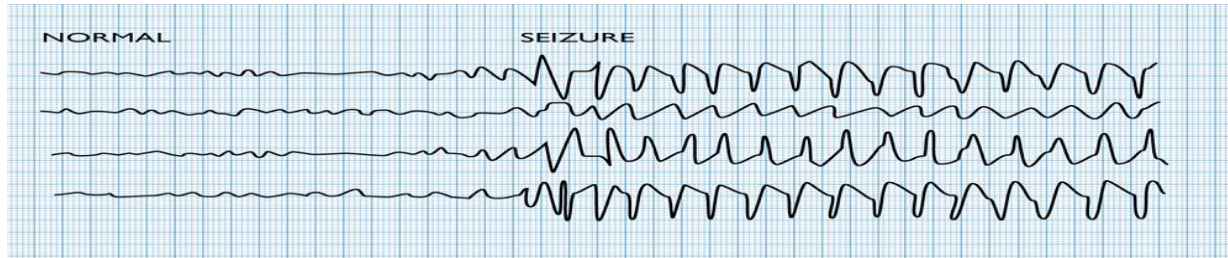
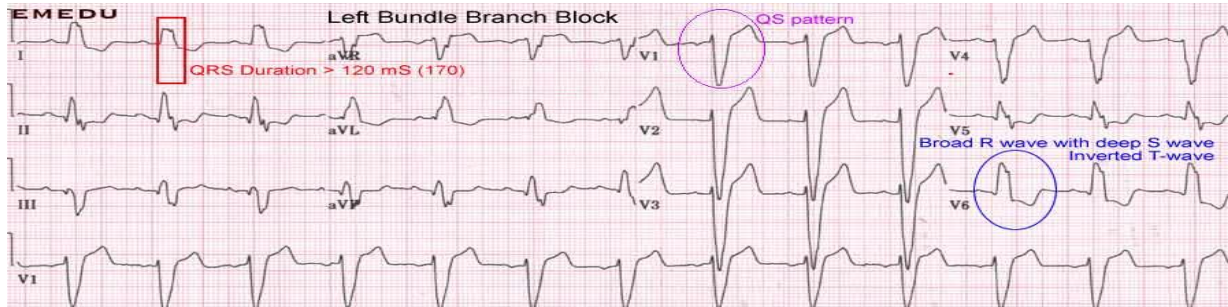
[Hologic to buy Third Wave for \\$580 million | Reuters](#)

Jun 9, 2008 - O) said on Monday it would **buy** Third Wave Technologies Inc TWTI. ... **Hologic** estimated the **HPV testing** market is currently worth \$200 million ...

Appenzeller T. Democratizing the DNA sequence. Science 1990; 247:1030-2.



Evidence-based Diagnosis of Left Bundle Branch Block, Seizure and HPV-18 by EKG, EEG and DNA Sequencing



Human papillomavirus type 18 isolate Q:04924, complete genome
Sequence ID: [gb:EF202155.1](#) Length: 7824

Score	Expect	Identities	Gaps	Strand
143 bits(77)	2e-31	77/77(100%)	0/77(0%)	Plus/Minus
Query 1		GCACATATGTTAAATGGTACTACGACTGGTATCTACACAGTAACAATTAATGATTA		60
Sbjct 6623		GCACATATGTTAAATGGTACTACGACTGGTATCTACACAGTAACAATTAATGATTA		6564
Query 61		TGCCAGCAGATACCATT		77
Sbjct 6563		TGCCAGCAGATACCATT		6547

Heminested or same-nested PCR for detection and sequencing template preparation in Chlamydia trachomatis, gonorrhoea, HPV, Lyme and related borrelia infections

1. Lee SH, Vigliotti JS, Vigliotti VS, Jones W. [From Human Papillomavirus \(HPV\) Detection to Cervical Cancer Prevention in Clinical Practice](#). Cancers (Basel). 2014 Oct 2;6(4):2072-99.
2. Lee SH, Vigliotti JS, Vigliotti VS, Jones W, Moorcroft TA, Lantsman K. [DNA sequencing diagnosis of off-season spirochetemia with low bacterial density in Borrelia burgdorferi and Borrelia miyamotoi infections](#). Int J Mol Sci. 2014 Jun 25;15(7):11364-86.
3. Lee SH, Vigliotti JS, Vigliotti VS, Jones W, Shearer DM. [Detection of borreliae in archived sera from patients with clinically suspect Lyme disease](#). Int J Mol Sci. 2014 Mar 11;15(3):4284-98.
4. Lee SH. Guidelines for the use of molecular tests for the detection and genotyping of human papilloma virus from clinical specimens. [Methods Mol Biol](#). 2012;903:65-101.
5. Lee SH, Vigliotti VS, Vigliotti JS, Jones W, Williams J, Walshon J. [Early Lyme disease with spirochetemia - diagnosed by DNA sequencing](#). BMC Res Notes. 2010 Nov 1;3:273.
6. Lee SH, Vigliotti VS, Vigliotti JS, Jones W, Pappu S. [Increased sensitivity and specificity of Borrelia burgdorferi 16S ribosomal DNA detection](#). Am J Clin Pathol. 2010 Apr;133(4):569-76.
7. Lee SH, Vigliotti VS, Pappu S. [Signature sequence validation of human papillomavirus type 16 \(HPV-16\) in clinical specimens](#). J Clin Pathol. 2010 Mar;63(3):235-9.
8. Lee SH, Vigliotti VS, Vigliotti JS, Pappu S. [Validation of human papillomavirus genotyping by signature DNA sequence analysis](#). BMC Clin Pathol. 2009 May 22;9:3.
9. Lee SH, Vigliotti VS, Pappu S. [Molecular tests for human papillomavirus \(HPV\), Chlamydia trachomatis and Neisseria gonorrhoeae in liquid-based cytology specimen](#). BMC Womens Health. 2009 Apr 9;9:8.
10. Lee SH, Vigliotti VS, Pappu S. [HPV infection among women in a representative rural and suburban population of the USA](#). Int J Gynaecol Obstet. 2009 Jun;105(3):210-4.
11. Lee SH, Vigliotti VS, Pappu S. [DNA sequencing validation of Chlamydia trachomatis and Neisseria gonorrhoeae nucleic acid tests](#). Am J Clin Pathol. 2008 Jun;129(6):852-9.
12. Lee SH, Vigliotti VS, Vigliotti JS, Pappu S. [Routine human papillomavirus genotyping by DNA sequencing in community hospital laboratories](#). Infect Agent Cancer. 2007 Jun 5;2:11.

The science of PCR amplification followed by Sanger sequencing for HPV detection and genotyping

The HPV consensus primers MY09 and MY11 and GP5+ and GP6+ were used in **a nested**, single-tube **PCR** assay.

Typing of HPV DNA. The fragment containing GP5+ and GP6+ was used as template DNA with the Big Dye Terminator **Cycle Sequencing**. Wallin, K.L.; Wiklund, F.; Angstrom, T.; Bergman, F.; Stendahl, U.; Wadell, G.; Hallmans, G.; Dillner, J. **Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer.** N. Engl. J. Med. 1999, 341, 1633–1638.

Lee SH, Vigliotti VS, Vigliotti JS, Pappu S. **Routine human papillomavirus genotyping by DNA sequencing in community hospital laboratories.** Infect Agent Cancer. 2007 Jun 5;2:11.

Sequencing was four times more likely to identify the viral type in positive samples than TS-PCR

Carvalho Nde O, del Castillo DM, Perone C, Januário JN, Melo VH, Brasileiro Filho G. **Comparison of HPV genotyping by type-specific PCR and sequencing.** Mem Inst Oswaldo Cruz. 2010 Feb;105(1):73-8.

The high-risk HC2 test (Digene/Qiagen) detected only **57.6% (388/674) of the high-risk** HPV isolates in clinical specimens, **misclassified 46.8% (88/188) of the low-risk** HPV isolates as high-risk genotypes, and **misclassified 27.4% (180/657) of the negative** samples as being infected by high-risk HPV. Ge S, Gong B, Cai X, Yang X, Gan X, Tong X, Li H, Zhu M, Yang F, Zhou H, Hong G. **Prevent cervical cancer by screening with reliable human papillomavirus detection and genotyping.** Cancer Med. 2012 Aug;1(1):59-67.

MILFORD MEDICAL LABORATORY, INC.

DEPARTMENT OF MOLECULAR DIAGNOSTICS

SIN HANG LEE, M.D., F.R.C.P. (C), F.C.A.P., DIRECTOR

2068 BRIDGEPORT AVENUE MILFORD, CT. 06460 Phone #: (203) 876-4254 Fax #: (203) 876-4548

Patient: [REDACTED]
Collected: 12/ [REDACTED] /07
Received: 12/ [REDACTED] /07
Submitting Dr.: PACE, SALVATORE A
Other Doctor:

Specimen #: M07-[REDACTED]6
Unit #: M00054-[REDACTED]
Acct #: V0008245-[REDACTED]
DOB: 05/[REDACTED]/1977
Pt. Age / Sex: 31 / F
Location: LAB
Copy for: M07-[REDACTED]6

MOLECULAR DIAGNOSTICS REPORT

Nucleic Acid Amplification (NAA) *Chlamydia/Gonococcus* Detection and Human Papillomavirus genotyping – all positive results validated by DNA Sequencing

SPECIMEN RECEIVED

(Cytc) Cyto specimen for Chlamydia trachomatis analysis
(Cytc) Cyto specimen for Neisseria gonorrhoeae analysis
(Cytc) Cyto specimen for Human papillomavirus analysis
DNA SEQUENCING - MOLECULAR DIAGNOSTICS

MOLECULAR DIAGNOSIS

Specimen Adequacy: Satisfactory for evaluation.

Chlamydia trachomatis: POSITIVE (HIGH) - DNA equivalent to 2 million organisms or more*.
See signature DNA sequence with on-line BLAST.

Neisseria gonorrhoeae: NEGATIVE - No gonococcal opa genes detected by nested PCR.

Human papillomavirus: POSITIVE for HPV.
Genotype(s): 58
(See DNA sequence with on-line BLAST algorithm report)
HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 73, 82, 26,
53 & 66 are considered "high-risk" [NEJM 2003;348:518-27]. However,
CIN3 has been reported in persistent infection with other genotypes.

Notes to health care professionals

We use polymerase chain reaction (PCR) amplification followed by direct DNA sequencing of the nested PCR amplicons to analyze the type-specific hypervariable region of the L1 gene for HPV genotyping. The same methodology is used to detect and validate the species-specific *Chlamydia trachomatis* cryptic plasmid DNA and the *Neisseria gonorrhoeae* opa gene DNA. If the specimen is positive for any of these agents, a tracing representing the consensus DNA signature sequence of the infectious agent with its BLAST algorithm will be attached to the original hard copy of this report.

A positive nested PCR result validated by DNA sequencing provides unequivocal evidence for the presence of a molecular genetic marker for the infectious agent in the specimen submitted. However, this molecular technology is extremely sensitive, capable of detecting a single copy of target DNA which may not have any direct relevance to the symptoms or clinical presentation of the patient. To assist clinical management, one of the two levels of positivity on *C. trachomatis* and *N. gonorrhoeae* DNA detection is reported when the positive result is finally validated by DNA sequencing.

MILFORD MEDICAL LABORATORY, INC.

DEPARTMENT OF MOLECULAR DIAGNOSTICS

2068 BRIDGEPORT AVENUE MILFORD, CT, 06460 Phone #: (203) 876-4254 Fax #: (203) 876-4548

Patient: [REDACTED]

Specimen #: M07- [REDACTED] 6

***Positive (HIGH)** = Total target DNA in the cervicovaginal specimen submitted in a 20 mL Cytoc container or in a 10 mL Surepath container is substantially equal to or exceeds the equivalent extracted from 2×10^7 elementary bodies of *C. trachomatis* or 2×10^7 bacteria of *N. gonorrhoeae*, respectively. This amount of target nucleic acids is substantially equivalent to the detection threshold of a non-amplified *Chlamydia trachomatis/Neisseria gonorrhoeae* nucleic acid screen test (Gen-Probe® PACE 2C System CT/NG) which was initially developed by comparison with the standard bacteriological culture results.

***Positive (LOW)** = Total target DNA in the cervicovaginal specimen submitted in a 20 mL Cytoc container or in a 10 mL Surepath container is below the equivalent extracted from 2×10^7 elementary bodies of *C. trachomatis* or 2×10^7 bacteria of *N. gonorrhoeae*, respectively. Its significance must be evaluated in the context of other clinical y relevant information since a single copy of non-viable target DNA which may not be clinically relevant can cause low copy positive results during nested PCR amplification.

If the clinical significance of a molecular diagnostic test positive for a sexually transmitted infection is not clear, you may consider submitting another specimen for a repeat test to confirm the persistent presence of the molecular marker of the infectious agent detected in the first sample.

One **Negative** nested PCR result does not rule out the presence of sexually transmitted infectious agents because the number of target DNA molecules derived from the microbes or virus in the liquid-based Pap cytology specimen might be below the threshold of detection or because there were PCR inhibitors in the clinical materials collected. If a negative result does not fit with the clinical impression, a new specimen may be necessary.

Result uncertain means that a nested PCR product was generated, suggestive of a positive test result. However, it could not be validated by automated DNA sequencing due to the presence of a sequencing inhibitor.

Unsatisfactory specimen means that there is inadequate DNA or a PCR inhibitor in the clinical material. These nested PCR/direct DNA sequencing tests using analytic specific reagents and performed at Milford Medical Laboratories are approved by the State of Connecticut Department of Health and CMS (under "CLIA 88") as high complexity tests. The tests are used for clinical purposes, and should not be regarded as investigational or for research. These tests were developed and their performance characteristics determined by Milford Medical Laboratories, and they have not been cleared or approved by the U.S. Food and Drug Administration.

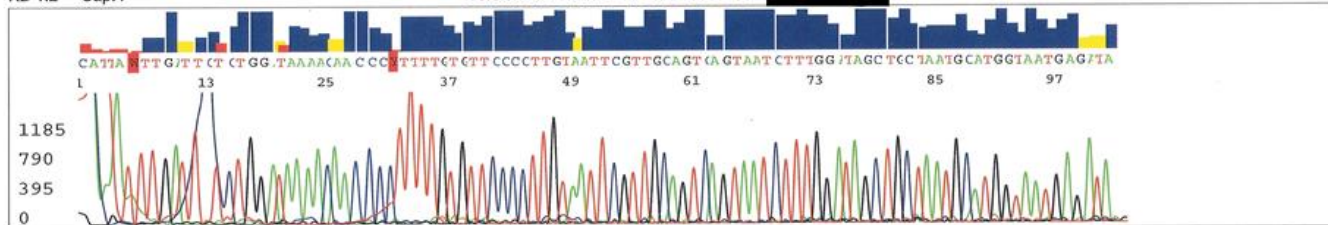
References

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2. Speich N, Schmitt C, Bollmann R, Bollmann M. Human papillomavirus (HPV) study of 2918 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. *J Med Microbiol* 2004;53:125-8.
3. Lee SH, Vigliotti VS, Vigliotti JS, Pappo S. Routine human papillomavirus genotyping by DNA sequencing in community hospital laboratories. *Infect Agent Cancer*. 2007;3:11.
4. Lee SH, Vigliotti VS, Pappo S. DNA Sequencing Validation of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Nucleic Acid Tests. *Am J Clin Pathol*. 2008;129: 852-859.
5. www.ccr.gov/07m/07p/07s/07L/07L01

Signed by: Sin Hang Lee, M.D. Pathologist

(electronic signature)

Sign Date: 01/07/08



M07-██████6 CHL ██████████

> [gi|73543930|gb|DQ063733.1](#) Chlamydia trachomatis strain J/UW-36 cryptic plasmid ORF2
gene,
partial cds
Length=388

Score = 116 bits (60), Expect = 1e-23
Identities = 60/60 (100%), Gaps = 0/60 (0%)
Strand=Plus/Minus

```
Query 1   TGGATAAAACAACCCCTTTTGTGTTCCCCCTTGTAATTCGTTGCAGTCAGTAATCTTTGGA   60
          |||
Sbjct 188 TGGATAAAACAACCCCTTTTGTGTTCCCCCTTGTAATTCGTTGCAGTCAGTAATCTTTGGA   129
```

Testing Performed at: **Milford Medical Laboratory** State License: CL-0502 ; CLIA - 88: 07D0670185

Reviewed by: **Sin Hang Lee, M.D.** _____ Date: _____

M07-█████6 (HPV) ██████████

> gi|90109956|gb|DQ431189.1 Human papillomavirus type 58 isolate DF03 major capsid protein
L1 gene, partial cds
Length=409

Score = 99.6 bits (50), Expect = 7e-19
Identities = 50/50 (100%), Gaps = 0/50 (0%)
Strand=Plus/Minus

```
Query 1  TACGAGTGGTATCAACCAACCGTAACAAATAACTGATTGCCCCAGCAAATG 50
          |||
Sbjct 50  TACGAGTGGTATCAACCAACCGTAACAAATAACTGATTGCCCCAGCAAATG 1
```

Testing Performed at: **Milford Medical Laboratory** State License: CL-0502 ; CLIA - 68: 07D0670186

Reviewed by: **Sin Hang Lee, M.D.** _____ Date: _____

Neisseria gonorrhoeae diagnosis by opacity genes analysis

Lee SH, Vigliotti VS, Pappu S. [DNA sequencing validation of Chlamydia trachomatis and Neisseria gonorrhoeae nucleic acid tests.](#) Am J Clin Pathol. 2008 Jun;129(6):852-9.

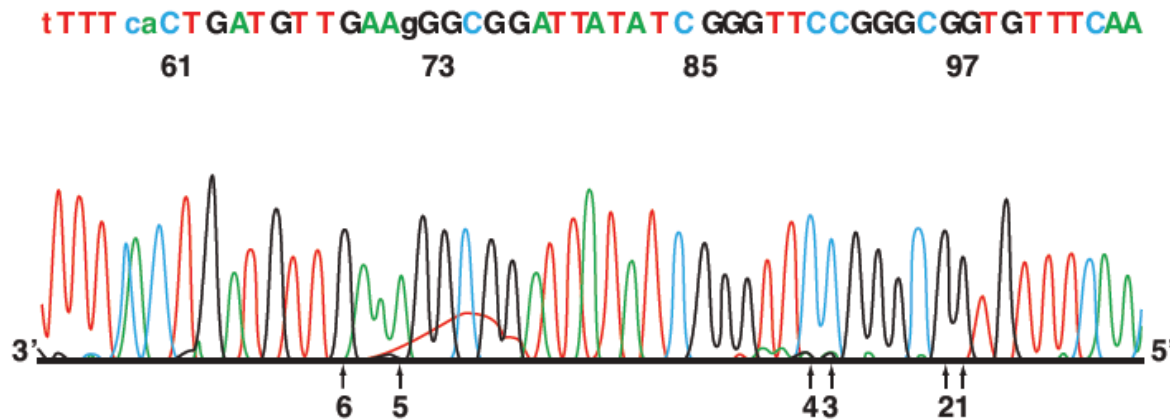
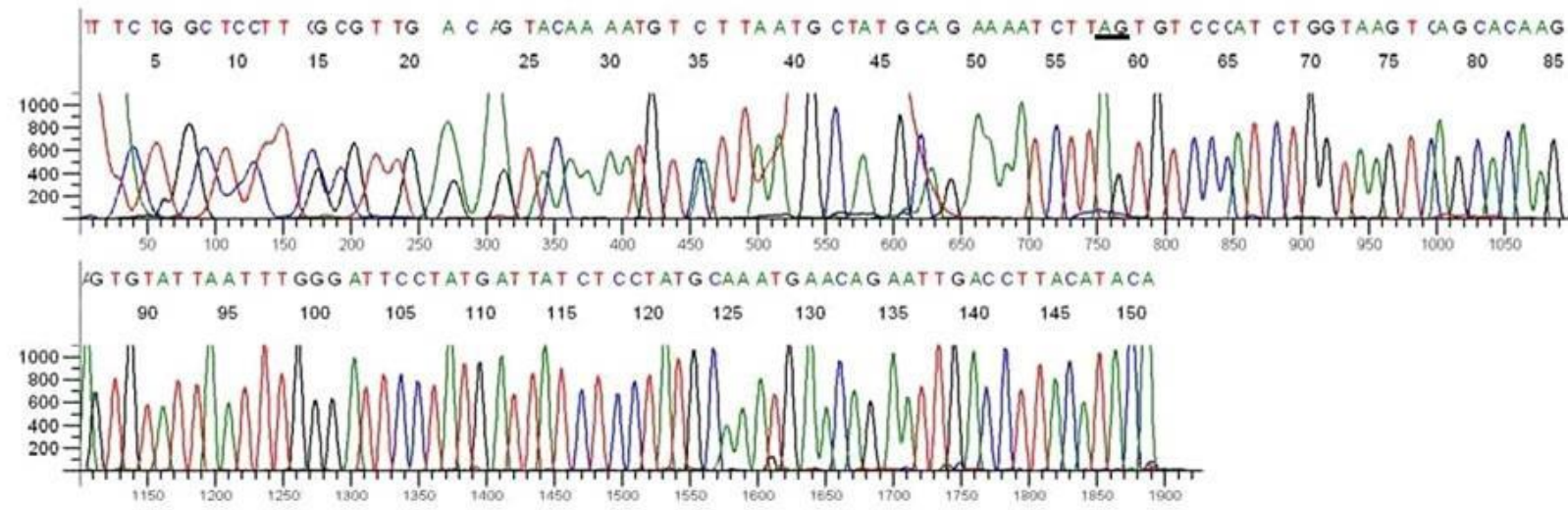
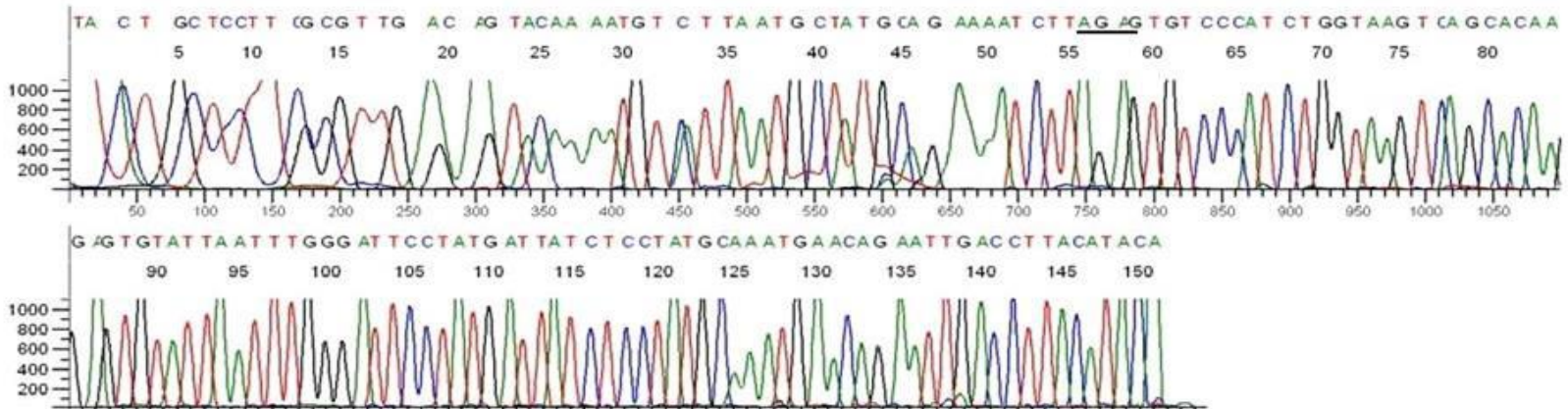


Image 3 Electropherogram of signature sequences for the *Neisseria gonorrhoeae opa* gene DNA. Generated by the ABI 3130 4-capillary genetic analyzer. Template, polymerase chain reaction (PCR) product of an endocervical sample by gonococcal *opa* HiFi nested PCR primary pair; sequencing primer, gonococcal *opa* HiFi DNA sequencing primer.

**BRCA1 sequencing electropherogram (Pap smear cells)-
no 185delAG mutation (upper) and with 185delAG mutation**



Democratization of Sanger sequencing (1) for Ebola diagnosis



Is it Ebola? Malaria? Or MERS?



Perform a same-nested PCR at the site of outbreak in the shade under a tree, then the answer may become obvious.

Democratization of Sanger sequencing (2) for Ebola diagnosis

The non-infectious nested PCR amplicons can be safely transported to a **regional laboratory for Sanger sequencing**





[GenBankGraphics](#) Next Previous [Descriptions](#)

Zaire ebolavirus strain ZEBOV/Homo sapiens-tc/COD/Mayinga_57935/1976, complete genome
Sequence ID: [gb|KR063671.1](#)|Length: 18957Number of Matches: 1

Related Information

Range 1: 13326 to 13620[GenBankGraphics](#) Next Match Previous Match [First Match](#)

Alignment statistics for match #1

	Score	Expect	Identities	Gaps	Strand	Frame
	545 bits (295)	1e-151	295/295 (100%)	0/295 (0%)	Plus/Plus	
Features:						
Query	1		GATGGTCTTGCTAAAGCATTTCCCTAGCAATATGATGGTAGTTACGGAACGTGAGCAAAAA			60
Sbjct	13326		GATGGTCTTGCTAAAGCATTTCCCTAGCAATATGATGGTAGTTACGGAACGTGAGCAAAAA			13385
Query	61		GAAAGCTTATTGCATCAAGCATCATGGCACCACACAAGTGATGATTTTGGTGAACATGCC			120
Sbjct	13386		GAAAGCTTATTGCATCAAGCATCATGGCACCACACAAGTGATGATTTTGGTGAACATGCC			13445
Query	121		ACAGTTAGAGGGAGTAGCTTTGTAAGTATTTAGAGAAATACAATCTTGCATTTAGATAT			180
Sbjct	13446		ACAGTTAGAGGGAGTAGCTTTGTAAGTATTTAGAGAAATACAATCTTGCATTTAGATAT			13505
Query	181		GAGTTTACAGCACCTTTTATAGAATATTGCAACCGTTGCTATGGTGTTAAGAATGTTTTT			240
Sbjct	13506		GAGTTTACAGCACCTTTTATAGAATATTGCAACCGTTGCTATGGTGTTAAGAATGTTTTT			13565
Query	241		AATTGGATGCATTATACAATCCCACAGTGTTATATGCATGTCAGTGATTATTATA	295		
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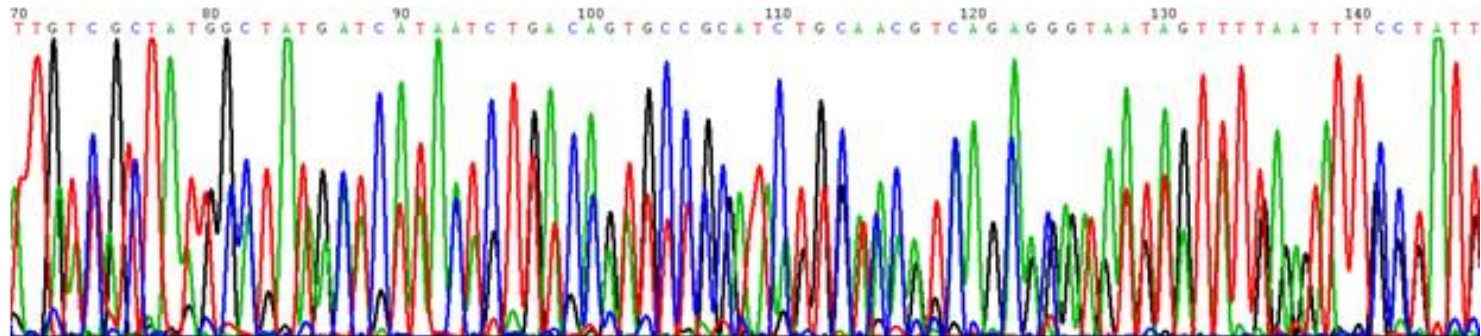
A novel low temperature PCR makes it possible

* **Low temperature PCR** - the gateway to diagnostic Sanger sequencing for infectious diseases: **minimize *Taq* errors**

- A **HiFi** polymerase(s) for **60-120 cycles** of precision PCR amplification
- Chemical-assisted denaturing at **85°C**, instead of 94-95°C
- Chemical stabilizers for enzymes (**polymerases, ribonuclease inhibitor and reverse transcriptase**) and **dNTPs**
- Melting chemicals to **reduce mispriming**
- Wide-ranged PCR master mix **stored at 4-40°C** for clinical diagnostics
- **No pipet transferring** of post-PCR products to reduce contamination
- **No post-PCR purifications** before sequencing
- Use crude sample for primary PCR
- Adjust annealing temperature (40-50°C) for PCR stringency

* *Hong G, Lee SH, Ge S, Zhou S. A Novel Low Temperature PCR Assured High-Fidelity DNA Amplification. International Journal of Molecular Sciences. 2013; 14:12853-12862.*

Sequencing electropherogram of Taq PCR products after 60 cycles of target amplification in the presence of human genomic DNA



The Final Message

- **Cyto-histopathology is a diagnostic art in medical practice based on science; it takes years to master.**
- **HPV detection and genotyping is straightforward science, following the laws of physics; it takes a few weeks to master.**
- **Cytopathologists and cytologists are in position to play a major role in molecular personalized medicine, especially in optimizing women's health care because they are in the position to direct a multi-billion health care industry at the gateway to colposcopic biopsies.**

Pap cytopathology and HPV assay can be the center of molecular personalized medicine

