Turn Cytopathology’s Crisis Into Opportunity

*(Transforming the Pap smear Cytopathology)*

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Toronto, Canada
**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.

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.

Publication Date: September 2015
STD Diagnostic Testing Market-excluding HPV testing 2011

Global Diagnostic Testing of STDs Market, Testing Volume, 2011 (in millions)

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.

<table>
<thead>
<tr>
<th>HPV assay</th>
<th>No. of data sets</th>
<th>HPV region(s) targeted [primer(s)]</th>
<th>No. of data sets proficient at:</th>
<th>Not proficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>All assays</td>
<td>118</td>
<td>L1/L2/E1/E2/E4/E6/E7</td>
<td>26 8 15 23</td>
<td>46</td>
</tr>
<tr>
<td>Linear Array (Roche)</td>
<td>17</td>
<td>L1 (PGMY)</td>
<td>8 1 1 1</td>
<td>6</td>
</tr>
<tr>
<td>innoLiPA (Innogenetics)</td>
<td>12</td>
<td>L1 (SPF10)</td>
<td>0 1 1 1</td>
<td>9</td>
</tr>
<tr>
<td>In-house LINE blot</td>
<td>10</td>
<td>L1 (GP/PGMY)</td>
<td>1 0 1 4</td>
<td>4</td>
</tr>
<tr>
<td>CLART HPV 2/3 (Genomica)</td>
<td>8</td>
<td>L1 (PGMY)</td>
<td>0 0 2 2</td>
<td>4</td>
</tr>
<tr>
<td>In-house type-specific PCR</td>
<td>6</td>
<td>L1/E6/E7</td>
<td>0 0 1 1</td>
<td>4</td>
</tr>
<tr>
<td>In-house real-time PCR</td>
<td>5</td>
<td>L1/E1/E4/E6/E7</td>
<td>0 1 0 1</td>
<td>3</td>
</tr>
<tr>
<td>In-house PCR-RFLP&quot;</td>
<td>7</td>
<td>L1/E6/E7</td>
<td>0 0 1 1</td>
<td>2</td>
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<tr>
<td>In-house PCR Luminex</td>
<td>7</td>
<td>L1 (GP)</td>
<td>0 0 1 1</td>
<td>2</td>
</tr>
<tr>
<td>In-house PCR GM-Y/CHUV</td>
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<td>L1 (PGMY)</td>
<td>1 0 1 0</td>
<td>1</td>
</tr>
<tr>
<td>In-house PCR sequencing</td>
<td>6</td>
<td>L1/E6</td>
<td>0 0 1 1</td>
<td>1</td>
</tr>
<tr>
<td>Papillocheck microarray (Greiner Bio-One)</td>
<td>4</td>
<td>E1</td>
<td>0 0 1 1</td>
<td>1</td>
</tr>
<tr>
<td>PCR Luminex (Multimetrix)</td>
<td>3</td>
<td>L1 (GP)</td>
<td>0 0 1 1</td>
<td>1</td>
</tr>
<tr>
<td>DNGene HPV genotyping LQ (Qtagen)</td>
<td>3</td>
<td>L1 (GP)</td>
<td>0 0 0 2</td>
<td>1</td>
</tr>
<tr>
<td>DNGene HPV genotyping RH (Qtagen)</td>
<td>2</td>
<td>L1 (GP)</td>
<td>0 0 1 0</td>
<td>0</td>
</tr>
<tr>
<td>Hybridra microarray</td>
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<td>L1/E6/E2/E4/E6/E7</td>
<td>0 2 3 5</td>
<td>1</td>
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<tr>
<td>DELA Line probe (Lab.Bio)</td>
<td>2</td>
<td>L1 (SPF10)</td>
<td>0 2 2 2</td>
<td>4</td>
</tr>
<tr>
<td>In-house dot blot</td>
<td>1</td>
<td>L1/E2/E4/E6</td>
<td>0 0 2 0</td>
<td>0</td>
</tr>
<tr>
<td>LCD array (Chiptron)</td>
<td>2</td>
<td>L1 (PGMY)</td>
<td>0 2 2 2</td>
<td>4</td>
</tr>
<tr>
<td>EASYChip (King Car)</td>
<td>2</td>
<td>L1/E6/E2/E4/E6/E7</td>
<td>0 2 3 5</td>
<td>1</td>
</tr>
<tr>
<td>Other commercial&quot;c&quot;</td>
<td>9</td>
<td>L1/E1/E2/E4/E6/E7</td>
<td>0 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Other in-house&quot;d&quot;</td>
<td>3</td>
<td>L1/L2/E1/E2/E4/E6/E7</td>
<td>0 0 0 0</td>
<td>0</td>
</tr>
</tbody>
</table>

SH Lee

Cytopathology-2015 | Toronto, CAN | 31.08.2015 Turn Cytopathology’s Crisis Into Opportunity | SH Lee 7
What can a cytopathologist do? Be a molecular biologist as well


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]

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.


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)
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 ↔ HPV persistent* ↔ CIN → 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


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

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

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.


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.
“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.”

30 and older (DNA with Pap). 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). 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 adjunctive HPV testing. 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.
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.

The crisis was thought to affect cytotechnologists only


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


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%.
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.
BOC Examinations Statistics

As of December 2009, the ASCP BOC has granted 15,030 Cytotechnologist (CT) and 591 Specialist in Cytotechnology (SCT) certifications. 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:

<table>
<thead>
<tr>
<th>Exam Period</th>
<th>Total</th>
<th>CT Exam</th>
<th></th>
<th></th>
<th>SCT Exam</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pass</td>
<td>Fail</td>
<td></td>
<td>Pass</td>
<td>Fail</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Jan-Dec 2009</td>
<td>209</td>
<td>187</td>
<td>22</td>
<td>11%</td>
<td>23</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>Jan-Dec 2008</td>
<td>244</td>
<td>210</td>
<td>34</td>
<td>14%</td>
<td>18</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Jan-Dec 2007</td>
<td>246</td>
<td>210</td>
<td>36</td>
<td>15%</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Jan-Dec 2006</td>
<td>266</td>
<td>247</td>
<td>19</td>
<td>7%</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Jan-Dec 2005</td>
<td>262</td>
<td>234</td>
<td>28</td>
<td>11%</td>
<td>25</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Jan-Dec 2004</td>
<td>320</td>
<td>273</td>
<td>47</td>
<td>15%</td>
<td>35</td>
<td>31</td>
<td>4</td>
</tr>
</tbody>
</table>

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”? 


• 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.
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.
Google search for cytopathology meetings -what crisis?

Annual Scientific Meeting | American Society of Cytopathology

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 ...

International Academy of Cytology
The International Academy of Cytology is a scientific, non-profit organization of ... 39th European Congress of Cytology, 20 - 23 September 2015 · more ...

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

USCAPDiagnostic 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


Ebola, Dengue fever, Lyme disease: The growing economic cost of infectious diseases


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 colposopic 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

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].

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.
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 ...
Evidence-based Diagnosis of Left Bundle Branch Block, Seizure and HPV-18 by EKG, EEG and DNA Sequencing
Heminested or same-nested PCR for detection and sequencing template preparation in Chlamydia trachomatis, gonorrheal, HPV, Lyme and related borrelia infections

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


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.

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DEPARTMENT OF MOLECULAR DIAGNOSTICS

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2088 BRIDGEPORT AVENUE, MILFORD, CT. 06460  Phone #: (203) 876-4254  Fax #: (203) 876-4548

Patient: 
Collected:  12/07
Received:  12/07
Submitting Dr.:  PAGE, SALVATORE A
Other Doctor:  
Specimen #:  M07-

Molecular Diagnostics Report

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

SPECIMEN RECEIVED

(Cytoc) Cytospecimen for Chlamydia trachomatis analysis
(Cytoc) Cytospecimen for Neisseria gonorrhoeae analysis
(Cytoc) Cytospecimen 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 18, 16, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82, 26, 53 & 66 are considered “high-risk” [NEJM 2003;349:515-27]. 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 10. The same methodology is used to detect and validate the species-specific Chlamydia trachomatis cryptic plasmid DNA and the Neisseria gonorrhoeae opa gene DNA. 11 If the specimen is positive for any of these agents, a table 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.
*Positive (HIGH) = Total target DNA in the cervicovaginal specimen submitted in a 20 mL Cytocentral container or in a 10 mL SurePath container is substantially equal to or exceeds the equivalent extracted from 2x10^4 elementary bodies of C. trachomatis or 2x10^3 bacteria of N. gonorrhoeae, respectively. This amount of target nucleic acids is substantially equivalent to the detection threshold of a non-amplified Chlamydia trachomatis/Walvania 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 Cytocentral container or in a 10 mL SurePath container is below the equivalent extracted from 2x10^4 elementary bodies of C. trachomatis or 2x10^3 bacteria of N. gonorrhoeae, respectively. Its significance must be evaluated in the context of other clinical 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 material collected.

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

Signed by: Sin Hang Lee, M.D. Pathologist
Sign Date: 01/07/08
M07-76 CHL

Chlamydia trachomatis strain J/JW-16 cryptic plasmid ORF2
gene
partial cds
Length: 388

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

Query 1
| TGGATAAAAACACGCTTTTGGTTGTTCTCCCTTGGTAAATTCTGGCAGCTCAGTAATCTTTGGA |
|-------------------|------------------------------|
| 60                | 129                          |

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

Reviewed by: Sin Hang Lee, M.D. Date:
Human papillomavirus type 58 isolate DF03 major capsid protein
Li gene, partial cds
Length=609

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Strand=Plus/Minus

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Subject 50
TACGAGTTGATCAACCAACGGTAAACAAAATAACTGATTGCCCCCCAGCAAATG 1

Testing Performed at: Milford Medical Laboratory State License: CL-0562 | CLIA - 88: 07D0670185
Reviewed by: Sin Hang Lee, M.D. ___________________________ Date:________________________
Neisseria gonorrhoeae diagnosis by opacity genes analysis


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.
Zaire ebolavirus strain ZEBOV/Homo sapiens-tc/COD/Mayinga_57935/1976, complete genome

Sequence ID: gb|KR063671.1| Length: 18957 Number of Matches: 1

Related Information

Range 1: 13326 to 13620

Alignment statistics for match #1

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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

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