



Updates on Cervical Cytology for the Detection of Cancer

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- Modification in Bethesda System (TBS 2001)
- Development of Modern Cytology Practice
- Application of New Tools

Evolution of Reporting System of Cervical Smears → The Bethesda System (TBS)

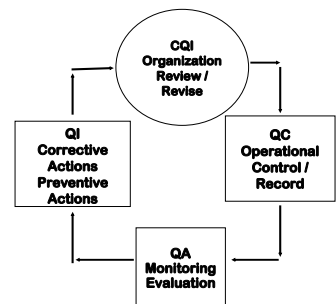


- TBS developed as result of a workshop sponsored by NCI in 1988 (with revision in 1991) to standardize terminology & reporting of cervical cytology.
- 3rd Workshop in 2001 to evaluate changes in practice of cytopathology:
 - ✓ Processing methods (Specimen types)
 - ✓ Ancillary techniques
 - ✓ Automation

Bethesda2001.cancer.gov

Accredited laboratory

- Through an educational and peer review inspection process
- Each laboratory is inspected to make sure it meets those standards and that it uses appropriate quality control and quality assurance procedures to benefit the patients it serves.



INTERNATIONAL Clinical Laboratory Accreditation Programs

- CAP (College of American Pathologists)
- NATA (National Association of Testing Authorities)
- IANZ (International Accreditation New Zealand)
- CPA (Clinical Pathology Accreditation UK Ltd)
- SINGLAS (Singapore Laboratory Accreditation Service)
- HOKLAS (Hong Kong Laboratory Accreditation Service)

New tools introduced for cervical cancer screening

- To improve on the quality of the sample: *ThinPrep**, *Autocyte** *FDA approved
**EU approved
- To increase the efficiency of screening for abnormal cells: *Papnet*, *Neopath**, *Thinprep Imaging system**
- To enhance quality control: *Papnet**, *Neopath**, *PathFinder*
- To enable detection of risk factors - such as human papilloma virus (HPV): *Digene Hybrid Capture Test**, *Roche Amplicor & Linear Array HPV Test***, *NORCHIP*, *ISH INFORM HPV probes*, *GEN-PROBE*, *PapilloCheck*, *HPV LBP Assay*, *HPV DNA Genotyping Chip*, ...
- To provide additional predictive markers: *p16 IHC & WB*, *ProExC HC test*, ... Cheung AN 2003
Cheung AN 2004

Drives to develop automated screening machine

1. To reduce human error
 - Usually only a few abnormal cells among the 30,000 - 50,000 normal cells on Pap smear → “needle-in-a-haystack” search
 - Screening errors inevitable, no matter how careful the staff is
2. Automation of slide microscopy and image analysis could allow a reduction in the manpower requirement for screening.
3. Technology exists to allow automation as both a primary screening tool and for quality control (QC) re-screening.

Advantages of automated screener

- guide the screeners to microscopic fields containing potentially abnormal cells
- improve interpretation
- reduce the labour required for manual screening
- provide a visual record of cells that have been identified as potentially abnormal ◊ expert review without microscopic re-examination of glass slides
- provide ongoing training for users with limited access to collegial consultation

Sherman 2003

Detection of HPV in clinical material

- Hybridization or Blotting
- Hybrid Capture Test
- Amplicor Test
- PCR amplification +/- Sequencing
- HPV genotyping by RMS Line Blot Assay or Linear Array Assay
- RNA assay
- HPV DNA chips

- HPV detection – Yes or No
- HPV genotyping: Identification of individual HPV genotypes



Current Position for HPV molecular testing

	HPV Detection	HPV Genotyping	Identify Multiple Infection in 1 test
Digene Hybrid Capture Test	√ (13 HR HPV)	X	X
Roche Amplicor Test	√ (13 HR HPV)	X	X
Roche HPV Linear Array	√	√	√
PCR- Sequencing	√	√	X

- Hybrid Capture Test & Roche Amplicor validated and reproducible
- ✓ cannot identify individual types
- Linear Array (EU approved) & DNA chip are suitable for genotyping
- HPV Proofer is suitable for detecting active infection
- Quality control in diagnostic settings important

Application of HPV DNA test

- Primary screening
- Triage of ASCUS
- Determine risk in persistent disease

Cheung AN 2007

p16INK4a is a promising surrogate biomarker of high-risk HPV and CIN

- *p16INK4a* protein maintains tumour suppressor retinoblastoma gene in the phosphorylated active state → blocks the cell cycle progression → ↓ cell proliferation
- HPV_s, in particular the high-risk HPV_s, can inactivate *p16INK4a* → enhance cell cycle progression, yet paradoxically lead to *p16INK4a* overexpression in the dysplastic epithelium.
- *p16INK4a* overexpression correlated with degree of cervical dysplasia in histological sections and in liquid-based cervical cytology.
- *p16* immunostaining on liquid based cytology reported to have a higher positive predictive value than reflex HPV testing.

Sano et al 1998
Keating et al 2001
Klaes 2002
Tringler B 2004
Murphy N J 2006
Kalof AN 2006
Baak et al 2006

p16INK4a – Benefit & Limitations

- p16INK4a allows identification of HGCIN as a biomarker for ASCUS +/- LSIL with good sensitivity and specificity
- Standardization of p16INK4a scoring and reporting is necessary:
 - ✓ subcellular localization
 - ✓ proportion of epithelial cells positive
 - ✓ distribution within the epithelium
- Negative staining for p16INK4a does not exclude the presence of CIN
- Utility of p16INK4a as a screening method for CIN has not yet been established
- Its use should be restricted to lesions that are morphologically suspicious for CIN.

Wentzensen 2006
Kalof & Cooper et al. 2006

Ki-67 (MIB1) immunocytochemistry & Cervical Cytology

- Normal cells and koilocytes showed inconspicuous immunoreactivity Vs strongly immunoreactive nuclei were found in cancer cells and HSIL (P<0.0001).
- Neoplastic squamous and glandular cells could be easily identified based on strong Ki-67 immunoreactivity in the context of abnormal nuclear morphology.

Cytological Diagnosis	No of Cases	Ki-67		
		Negative	Inconclusive	Positive
Negative	23	23	0	0
LSIL	13	13	0	0
HSIL	10	0	2	8
SCC	6	0	1	5
Cx AdenoCA	3	0	0	3
Cx AdenoSq CA	2	0	0	2
Em AdenoCA	3	0	1	2

Cheung et al. 2004

Ancillary Techniques

- Methylation analysis
- Telomerase activity
- In Situ Hybridization: ploidy, telomerase

Cheung, 2007
Gustafson 2004
Bianchi et al, 2002
Fetsch et al, 2002
Maitra et al, 2002
Polett et al, 2000
Leung & Bedard 1999
Florentine et al, 1997

Detection of 3q gain & amplification of *TERC* in Pap smears can assist in identifying LSIL with high progression risk

- CIN1/CIN2 that progressed to CIN3 (progressors) Vs CIN1/CIN2 that regressed spontaneously (regressors) Vs normal Pap smears from women who subsequently developed CIN3 or Cx CA.
- Progressors displayed a gain of 3q Vs NO regressors showed such this genetic aberration.
- 3q gain is required for transition from CIN1/CIN2 to CIN3 and that it predicts progression.
- 3q gain found in 33% of normal Pap smears from women who shortly developed CIN3 or Cx CA.
- Sensitivity for predicting CIN1/CIN2 to CIN3: 100%
- Specificity (prediction of regression): 70%.
- ? Decrease false-negative cytological screenings.

Heselmeyer-Haddad 2005

Chromosome In Situ Hybridization in Cervical Cytology

- Normal squamous and glandular cells showed diploid pattern.
- ↑ in chromosomes 11 and 16 (aneusomies) in HSIL and CA.
- Neoplastic squamous and glandular cells easily identified based on nuclear aneusomy in the context of abnormal nuclear morphology.

Cheung et al. 2004

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- Pathologists should bear in mind that no marker is specific and interpretation of any findings derived from adjunct laboratory techniques must be employed in conjunction with the morphologic and clinical findings.
- The importance of quality control need to be highlighted.