Conventional versus liquid based cytology

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Introduction
Overview

• Cytology in general
  • Uses
  • Advantages
  • Disadvantages

• Cervical cytology
  • History
  • HPV and cervical cancer
  • Liquid based versus conventional smears
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What is cytology?

- Cytology is the study of cells under the microscope in order to diagnose diseases.
- Details of the cells: nucleus, cytoplasm, background etc. without architecture of the tissue.
- Cytopathology is best used as one of three tools, the second and third being the physical exam and medical imaging.
- Cytology can be used to diagnose a condition and spare a patient a more invasive technique to obtain a larger specimen (biopsy, surgery).
In the lab

• Macroscopic evaluation of the material: how many slides or testtubes, what does the material looks like (bloody, mucinous, colorless ect)
Centrifuging

• For received fluids to separate the cells from the fluid and get a higher yield of representative slides
Slightly different for thinprep/lbc

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**The ThinPrep Sample Preparation Process**

1. Dispersion
   - The ThinPrep Pap test filter rotates within the sample vial, creating currents in the fluid that are strong enough to separate debris and disperse mucus, but gentle enough to have no adverse effect on cell appearance.

2. Cell Collection
   - A gentle vacuum is created within the ThinPrep Pap test filter, which collects cells on the exterior surface of the membrane. Cell collection is controlled by the ThinPrep 5000 processor’s software that monitors the rate of flow through the ThinPrep Pap test filter.

3. Cell Transfer
   - After the cells are collected on the membrane, the ThinPrep Pap test filter is inverted and gently pressed against the ThinPrep microscope slide. Natural attraction and slight positive air pressure cause the cells to adhere to the ThinPrep microscope slide resulting in an even distribution of cells in a defined circular area.
Transfer of cells on to slides

• Cytospin
• Machine based
• Pipet
• Direct transfer after FNA or scraping
Staining of cells by one of several techniques

• Romanowsky type stains (for air dried slides)
  • Wright’s stain
  • Giemsa stain
  • Wright’s Giemsa stain
  • May Grunwald Giemsa stain
  • Diff-Quik stain

• Papanicolaou stains (for immediate fixated slides)
Diff quick methode

- Nuclear and nucleolar features less well preserved
- Better for cytoplasmic features
Pananicolau stain

**Principles**

- **Stains include:**
  - **Nuclear staining:** Hematoxylin
  - **Two cytoplasmic counter staining:**
    - **Orange G (OG)-6, OG-5 and OG-8** is an acidic dye, stains keratin a bright, intense orange.
    - **Eosin Azure (EA), EA-36, AE-50 and EA-65** including three stains
      - Eosin Y
      - Light Green
      - Bismarck brown Y
Principles

• Hydration and dehydration:
  – Hydration prepares the cell sample for uptake of the nuclear dye;
  – dehydration prepares the cell sample for uptake of the counterstains.

• Dehydration and clearing solutions result in cellular transparency and prepare the cell sample for the final steps
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Subclassification of cytology

1. Exfoliative cytology: spontaneously cells in body fluids
   • Urine
   • CSF
   • Sputum
   • Effusions in body cavities (pleura, pericardium, peritoneum)
Subclassification of cytology

2. Abrasive cytology: dislodges cells from body surfaces
   • Imprint
   • Scraping (cervix)
   • Endoscopic brushing of mucosal surfaces
   • Washing (lavage) of mucosal or serosal surfaces
   • Swab
Subclassification of cytology

3. Fine needle aspiration cytology: FN, FNA, FNAB, FNAC
   • Superficial nodules and organs
   • Deep organs (by guidance of CT, US)
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Advantages of cytology in general

• Fast (preliminary) result
• Possibility of direct assessment of adequacy (DQ stain)
• Less or non-invasive procedure compared to histology
• Fewer complications
• collected easily and quickly
• easily and quickly prepared, stained and interpreted
• Inexpensive
• Little or no risk Little or no risk to the patient
• To determine next diagnostic procedures
Advantages of FNA cytology

• Some tumors may be difficult to biopsy, such as sarcomas. Other rare tumors may be dangerous to biopsy, such as pheochromocytoma. In general, a fine-needle aspiration can be done anywhere it is safe to put a needle, including liver, lung, kidney, and superficial masses.

• A "quick read" is a peek under the microscope and can tell the clinician whether enough diagnostic material was obtained. Cytological specimens must also be properly prepared so that the cells are not damaged.
• Sometimes more information about the specimen is helpful. Immunohistochemical stains and molecular testing can be performed, especially if the sample is prepared using liquid based cytology, such as HPV testing on an abnormal pap test (or vice versa) or flow cytometry on a lymphoma specimen.
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Disadvantages of cytology

• Cannot localize neoplastic lesion to an exact anatomic localization
• Cannot distinguish pre invasive from invasive cancer
• May not be able to distinguish reactive from dysplastic
• May not be able to determine tumour type
Advantages of Histopathology

• Microscopic examination usually is much less demanding (histology is generally a lot easier than cytology)

• Ability to evaluate architecture and invasion

• Ability to cut additional section cut additional section for special stains
Disadvantages of Histopathology

• Time required to create sections (fixation, processing, cutting, staining)

• Identification of certain type of cells is more difficult on histology than on cytology (small cell carcinoma vs lymphoma)

• More invasive procedure
When is histology needed

• To examine margins of a resection

• To examine stromal invasion and depth of invasion

• Gross examination to cytopathology discrepancies
Differential between follicular adenoma and carcinoma of the thyroid
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Cervical cytology
Historic aspects

• 400 BCE - Hippocrates noted that cervical cancer was incurable
• 1925 - Hinselmann invented the colposcopy
• 1928 - Papanicolaou developed the Papanicolaou technique
• 1941 - Papanicolaou and Traut: Pap smear screening began
• 1946 - Aylesbury spatula was developed to scrape the cervix, collecting the sample for the Pap smear
• 1951 - First successful in-vitro cell line, HeLa, derived from biopsy of cervical cancer of Henrietta Lacks
• 1976 - Harald zur Hausen and Gisam found HPV DNA in cervical cancer and genital warts; Hausen later won the Nobel Prize for his work
• 1988 - Bethesda System for reporting Pap results was developed
• 2006 - First HPV vaccine was approved by the FDA
HeLa cells

• Immortal cell line used in laboratories all over the world for research purposes are from cervical cancer
History of pap smear

• The Papanicolaou test (abbreviated as Pap test, known earlier as Pap smear, cervical smear, or smear test) is a method of cervical screening used to detect potentially pre-cancerous and cancerous processes in the cervix uteri. Abnormal findings are often followed up by more sensitive diagnostic procedures, and, if warranted, interventions that aim to prevent progression to cervical cancer.

• The test was invented by, and named for, the prominent Greek doctor Georgios Papanikolaou.
About 50 years after Papanicolaou developed the Pap stain and over 25 years after they started screening for cervical cancer, one of the leading causes of cervical cancer was discovered to be HPV.
Epidemiology

• Worldwide 4\textsuperscript{th} most common cancer and fourth-most common cause of death from cancer in women

• About 70% of cervical cancers occur in developing countries. In low-income countries, it is the most common cause of cancer death.

• In developed countries, the widespread use of cervical screening programs has dramatically reduced rates of cervical cancer.
Goal of cervical screening

- The test aims to detect potentially pre-cancerous changes (called cervical intraepithelial neoplasia (CIN) or cervical dysplasia; the squamous intraepithelial lesion system (SIL) is also used to describe abnormalities), which are caused by human papillomavirus, a sexually transmitted DNA virus. The test remains an effective, widely used method for early detection of pre-cancer and cervical cancer.

- While the test may also detect infections and abnormalities in the endocervix and endometrium, it is not designed to do so.
Wilson and Jungner classic screening criteria

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a “once and for all” project.
Synthesis of emerging screening criteria proposed over the past 40 years

- The screening programme should respond to a recognized need.
- The objectives of screening should be defined at the outset.
- There should be a defined target population.
- There should be scientific evidence of screening programme effectiveness.
- The programme should integrate education, testing, clinical services and programme management.
- There should be quality assurance, with mechanisms to minimize potential risks of screening.
- The programme should ensure informed choice, confidentiality and respect for autonomy.
- The programme should promote equity and access to screening for the entire target population.
- Programme evaluation should be planned from the outset.
- The overall benefits of screening should outweigh the harm
Outside of a screening program

- Clinical symptoms or signs
  - Vaginal bloodloss after menopause
  - Contact bleeding
  - Vaginal discharge

- Follow up after screening with abnormalities

- Follow up after surgical procedures for treatment of CIN/cancer
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Human papillomavirus (HPV) is a group of viruses that are extremely common worldwide.

There are more than 100 types of HPV, of which at least 13 are cancer-causing (also known as high risk type).

HPV is mainly transmitted through sexual contact and most people are infected with HPV shortly after the onset of sexual activity, 90% of infections are cleared within 2 years.

Cervical cancer is caused by sexually acquired infection with certain types of HPV.

Two HPV types (16 and 18) cause 70% of cervical cancers and precancerous cervical lesions.

There is also evidence linking HPV with cancers of the anus, vulva, vagina and penis.

Cervical cancer is the second most common cancer in women living in less developed regions with an estimated 445 000 new cases in 2012 (84% of the new cases worldwide).

In 2012, approximately 270 000 women died from cervical cancer; more than 85% of these deaths occurring in low- and middle-income countries.
Koilocytes (cells infected with HPV with specific cytopathic changes)
HPV and cervical cancer

1. During the G2 checkpoint, a functional p53 protein detects a DNA mutation.
2. Cervix cells with mutated DNA do not divide.
3. Cervix remains healthy.

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1. p53 protein is deactivated by the p53 inhibitor.
2. With p53 deactivated, cervix cells with mutated DNA successfully divide.
3. Mutated cervix cells grow uncontrollably into a tumor.

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By OpenStax College - Anatomy & Physiology, Connexions Web site.
HPV E6 oncoproteins and cancer

- The HPV E6 and E7 oncoproteins inactivate the p53 and pRB tumor suppressors.
- HPV E7 proteins interact with pRB and the related "pocket proteins" p107 and p130. Together regulate the activities of the E2F family of transcription factors that control multiple cell cycle transitions.
- E6 do not directly associate with p53 but form a complex with the cellular E6-AP protein, which is essential for p53 interaction. E6 retargets E6-AP to induce ubiquitination and rapid proteasomal degradation of p53.
- HPV E6 can activate telomerase hTERT transcription.

Schematic outline of critical steps of high-risk HPV-induced carcinogenesis. Inactivation of the pRB and p53 tumor suppressor pathways and expression of the catalytic telomerase subunit hTERT constitute a subset of the steps that have been shown to be necessary for the generation of fully transformed human epithelial cells in vitro.


Conventional pap smear slide preparation

A cervical sample containing precancerous cells (red)

Non-representative sample may not reflect patient’s actual condition

Missing cells, obscuring elements limit accurate diagnosis

Over 80% of collected sample discarded

Smear spray-fixed and sent to lab
Conventional cervical cytology

- Cheap
- Possibility of limited quality
  - Thick smears
  - Degeneration
  - Blood
  - Fixation and staining artefacts
- No additional stains or techniques possible
Liquid based cytology
A cervical sample containing precancerous cells (red)

Increased opportunity to detect early signs of abnormality

Representative thin layer of cells is clear of obscuring elements

Virtually 100% of collected sample rinsed into ThinPrep vial

Sample immediately preserved and sent to lab

Filtration process disperses, randomizes cells
The ThinPrep Sample Preparation Process

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<table>
<thead>
<tr>
<th>Conventional Pap Smear</th>
<th>ThinPrep Pap Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Conventional Pap Smear Image" /></td>
<td><img src="image" alt="ThinPrep Pap Test Image" /></td>
</tr>
<tr>
<td>• Majority of cells not captured</td>
<td>• Virtually all of sample is collected</td>
</tr>
<tr>
<td>• Non-representative transfer</td>
<td>• Randomized, representative transfer</td>
</tr>
<tr>
<td>• Clumping and overlapping</td>
<td>• Even distribution</td>
</tr>
<tr>
<td>• Obscuring material</td>
<td>• Minimizes obscuring material</td>
</tr>
</tbody>
</table>
Limaye, 2003: 233% increase in HSIL detection

A 2003 comparison of the ThinPrep Pap Test vs. the conventional pap smear showed a 233% increase in HSIL detection.

Diaz-Rosario 1999: 102% increase in HSIL detection

A 1999 comparison of the ThinPrep Pap Test vs. the conventional pap smear found a 102% increase in HSIL detection.
Disadvantages of LBC

• Expensive
• Larger containers with fluid
• Storage issues
• Transport issues
Advantages:

• More representative slides
• Better evaluation and detection of abnormalities
• Possibility of making additional stains/extra specimens for quality purposes
• HPV and cytology possible from one smear
• Less pap 0 smears
KOPAC-B and Bethesda systems

• Differences and interpreting the results
NORMAL VS ABNORMAL SMEAR

Cervical Cell Pathology in Squamous Tissue

Grades and cell types:
NORMAL  INFLAMMATORY  CANCER  PRECANCER

Normal  CIN I  CIN II  CIN III

Superficiales normales  SIL-L colocios  SIL-H, CIN II  SIL-H, CIN III
<table>
<thead>
<tr>
<th>Kompositie</th>
<th>Ontstekings-Verschijnselen</th>
<th>Plaveiselepitheel</th>
<th>Andere afwijkingen endometrium</th>
<th>Cilindercelepitheel endocervix</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>onvoldoende</td>
<td>n.v.t.</td>
<td>n.v.t.</td>
<td>n.v.t.</td>
</tr>
<tr>
<td>1</td>
<td>endocervix (ec)</td>
<td>Virusinfectie</td>
<td>geen afwijkingen</td>
<td>geen afwijkingen</td>
</tr>
<tr>
<td>2</td>
<td>squameuze metaplasie (sm)</td>
<td>Trichomonas vaginalis</td>
<td>abnormale plaveiselcellen</td>
<td>epitheelatrofie</td>
</tr>
<tr>
<td>3</td>
<td>endometrium (em)</td>
<td>Bacteriële infectie</td>
<td>atypische squameuze metaplasie</td>
<td>geen endocervical cilinderlepitheel</td>
</tr>
<tr>
<td>4</td>
<td>ec&lt;sup&gt;a&lt;/sup&gt; + sm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Candida albicans</td>
<td>geringe dysplasie</td>
<td>geringe atypie endocervicale cilindercellen</td>
</tr>
<tr>
<td>5</td>
<td>ec + em&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Haemophilus vaginalis</td>
<td>matige dysplasie</td>
<td>matige atypie endometrium</td>
</tr>
<tr>
<td>6</td>
<td>sm + em</td>
<td>Geen ontsteking</td>
<td>ernstige dysplasie</td>
<td>endocx. ernstige atypie</td>
</tr>
<tr>
<td>7</td>
<td>ec + sm + em</td>
<td>Actinomyces</td>
<td>carcinoma in situ</td>
<td>adenocarc. endometrium</td>
</tr>
<tr>
<td>8</td>
<td>uitsluitend plaveiselcellen</td>
<td>Chlamydia</td>
<td>micro-invasief carcinoom</td>
<td>metastase van elders</td>
</tr>
<tr>
<td>9</td>
<td>slechte fixatie</td>
<td>Aspecifieke</td>
<td>invasief plaveiselcellencarcinoom</td>
<td>n.v.t.</td>
</tr>
</tbody>
</table>

<sup>a</sup> ec: endocervix; <sup>b</sup> sm: squameuze metaplasie; <sup>c</sup> em: endometrium
<table>
<thead>
<tr>
<th>Pap-classificatie</th>
<th>KOPAC-B-codering</th>
<th>Pap-classificatie</th>
<th>KOPAC-B-codering</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap 0</td>
<td>KO (B3)</td>
<td>Pap 3B</td>
<td>P6 A5 C6</td>
</tr>
<tr>
<td>Pap 1</td>
<td>P1 A1-2 C1</td>
<td>Pap 4</td>
<td>P7 A6 C7</td>
</tr>
<tr>
<td>Pap 2</td>
<td>P 2-3 C3</td>
<td>Pap 5</td>
<td>P8-9 A7-8 C9</td>
</tr>
<tr>
<td>Pap 3A</td>
<td>P4-5 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Comparison of the Categories Specimen Adequacy and Diagnosis in Bethesda 1991 and 2001

<table>
<thead>
<tr>
<th>Category</th>
<th>Modified a Bethesda 1991</th>
<th>Bethesda 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen adequacy</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Satisfactory but limited by… (SBLB)</td>
<td>Satisfactory with quality indicator</td>
</tr>
<tr>
<td></td>
<td>Unsatisfactory</td>
<td>Unsatisfactory</td>
</tr>
<tr>
<td>No epithelial cell</td>
<td>Within normal limits (WNL)</td>
<td>Negative for intraepithelial lesion or malignancy</td>
</tr>
<tr>
<td>abnormality</td>
<td>Reactive/reparative cellular changes (R/R)</td>
<td>(NIL)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative for intraepithelial lesions or malignancy with reactive /</td>
</tr>
<tr>
<td></td>
<td></td>
<td>reparative cellular changes</td>
</tr>
<tr>
<td>Epithelial cell</td>
<td>ASCUS – favor reactive</td>
<td>ASC-US</td>
</tr>
<tr>
<td>abnormalities</td>
<td>ASCUS – not otherwise specified (NOS)</td>
<td></td>
</tr>
<tr>
<td>Squamous cells</td>
<td>ASCUS – favor SIL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atypical metaplastic cells&lt;sup&gt;6&lt;/sup&gt;</td>
<td>ASC, cannot exclude HSIL (ASC-H)</td>
</tr>
<tr>
<td></td>
<td>LSIL</td>
<td>LSIL</td>
</tr>
<tr>
<td></td>
<td>HSIL</td>
<td>HSIL</td>
</tr>
<tr>
<td>Glandular cells</td>
<td>AGUS–favor reactive, AEC–favor reactive</td>
<td>AGC, AEC</td>
</tr>
<tr>
<td></td>
<td>AGUS, NOS, AGUS-EC NOS</td>
<td>AGC-favor neoplastic, AEC-favor neoplastic</td>
</tr>
<tr>
<td></td>
<td>AGUS-favor neoplastic, AGUS-EC-favor neoplastic AGUS-EM (AMC)</td>
<td>AMC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenocarcinoma in situ</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>Benign endometrial cells over age 40 years</td>
</tr>
</tbody>
</table>
Any questions?