Using high-risk HPV testing, cervical cytology and cervical histology for cervical cancer prevention

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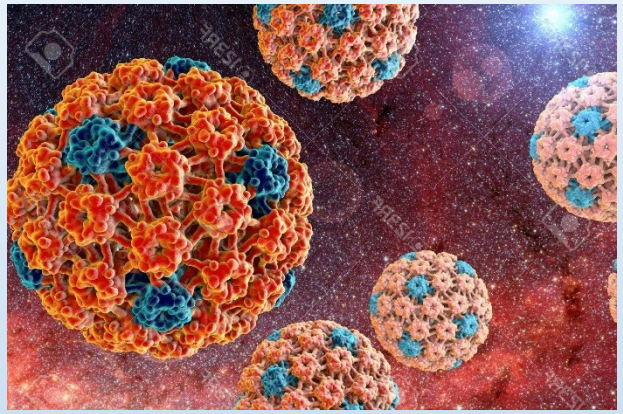
This talk aims to:

- provide an overview of the way that hrHPV testing, cervical cytology and cervical histology are used to identify cervical lesions that could lead to cervical cancer
- identify why each type of test is used at a particular time during the screening and management pathway
- show how the laboratory tests work together to achieve the best clinical outcome
- assist laboratory scientists, technicians and assistants who are new to laboratory testing or who work in one specific area, to gain a wider understanding of how their work contributes to the prevention of cervical cancer

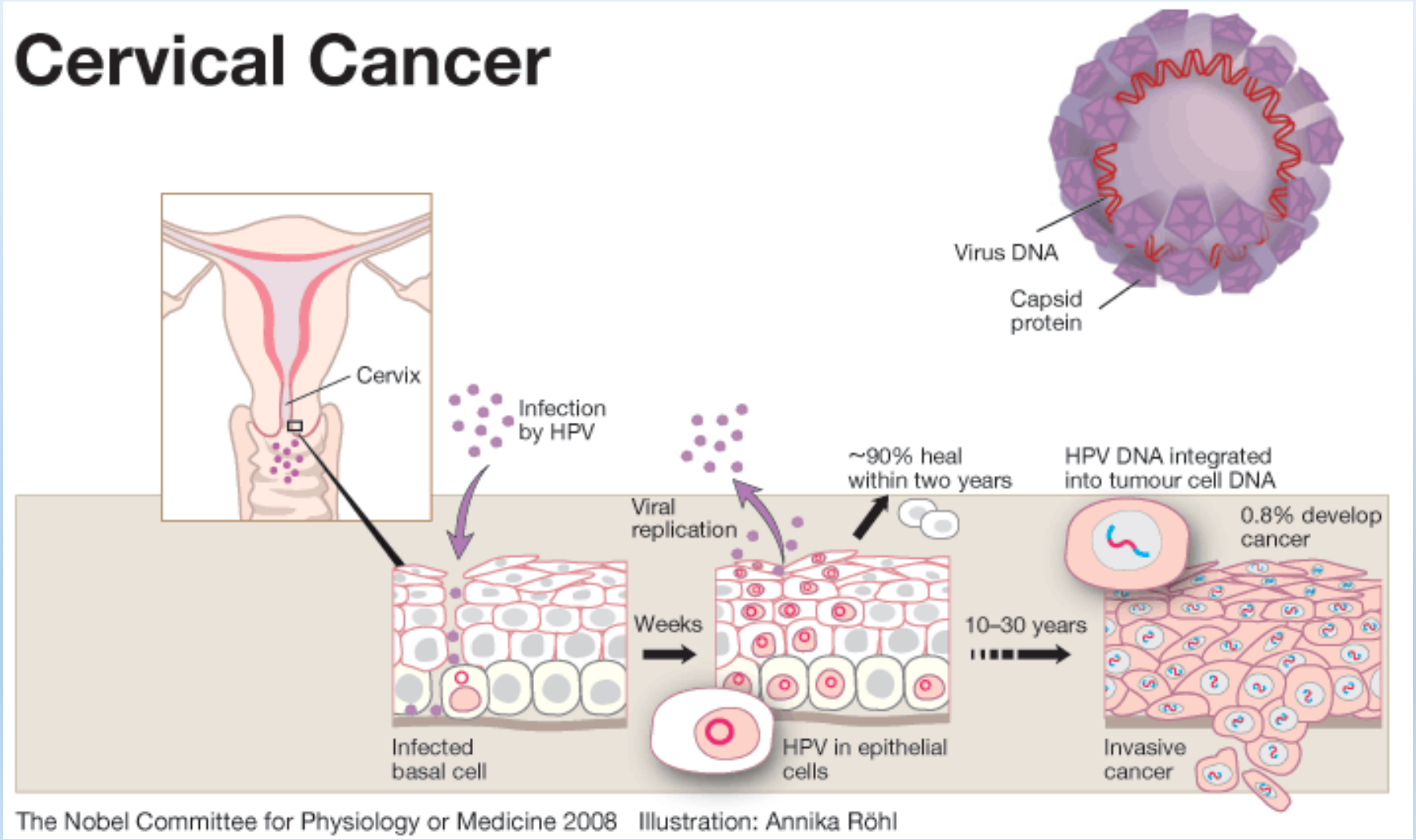
What's the problem?

- Genital tract infection with Human Papilloma Virus (HPV) is very common in sexually active men and women, particularly those who have recently commenced sexual activity or have a new sexual partner
  - 80% of sexually active adults are likely to have at least one HPV infection at some stage during their lifetime
- The vast majority of HPV infections are asymptomatic and are cleared by natural immunity. Most people wouldn't know they were infected if they weren't tested.
- In some, HPV infection causes low-grade or high-grade lesions to develop in the epithelium covering the cervix. If high-grade lesions are untreated, invasive cervical cancer will develop in a small proportion of cases.
- There is no way of identifying which individuals will develop invasive cancer if untreated so all those who are HPV positive or have abnormal cytology are at risk and need follow-up. Those with persistent HPV infection (2+ years), those with particularly "risky" HPV types (HPV-16 and HPV-18) and those with high-grade cytology, are the most at risk.

HPV Virus and infection



Human Papilloma Virus



What types of lesions can occur?

The cervix is covered by two different types of epithelium: squamous and glandular

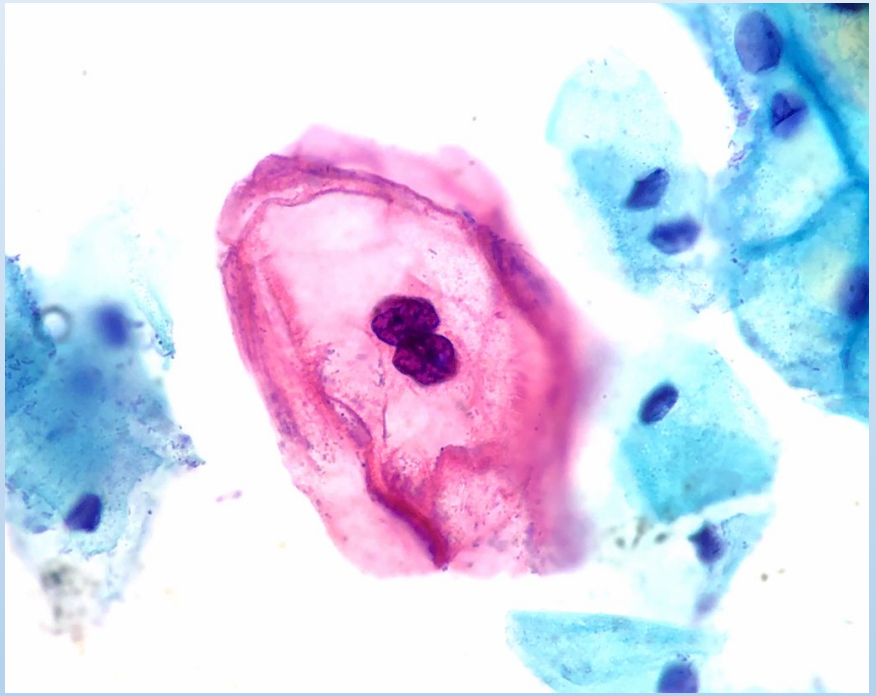
Squamous cells cover the outer surface of the cervix and are the more common type of cell infected by HPV

- Low-grade squamous lesions often result from HPV infection but are not pre-neoplastic and usually clear naturally
- High-grade squamous lesions are pre-neoplastic with a higher risk of progression to cancer, particularly if they persist for months to years. Some high-grade lesions still resolve naturally, particularly in young women

- Glandular cells line the endocervical canal in the interior of the cervix and lesions caused by HPV are less common
  - Adenocarcinoma in situ is the high-grade pre-neoplastic lesion arising from glandular cells
- About 70% of cervical cancers are squamous cell carcinomas, with 30% endocervical adenocarcinomas
- Cervical cancer has become much less common since New Zealand introduced an organised cervical screening programme in 1990

HPV infection and low-grade lesions

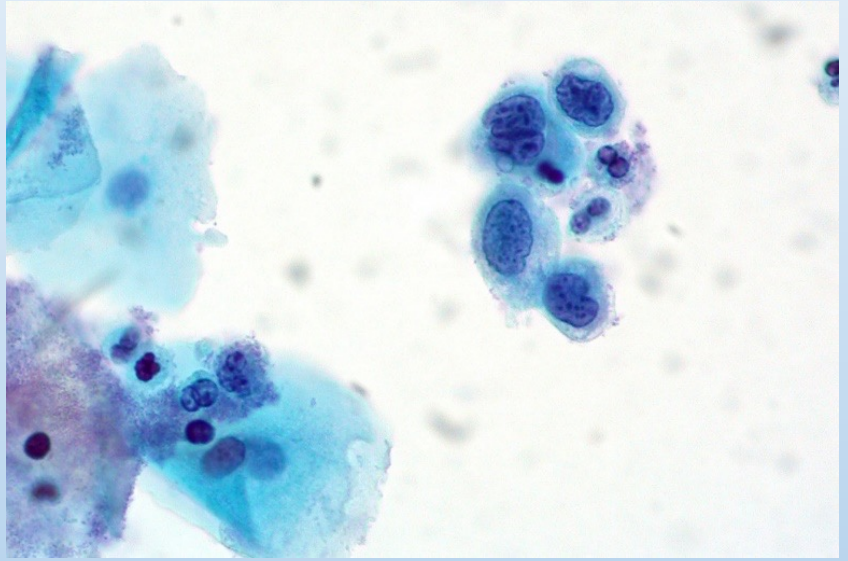
- When HPV infects the cervical epithelium, it usually causes a productive viral infection
- The virus enters the cell nucleus and uses the host cell mechanisms to replicate itself producing many more viral particles, which are released into the vagina when the mature epithelial cell is shed from the surface
- Low grade changes are seen in HPV infected cells when the upper layers of the epithelium are sampled for cytology
- The typical HPV-infected cell in a productive viral infection is called a koilocyte. This is not a pre-neoplastic cell but does mean that an HPV infection is present.
- The viral DNA is within the host cell nucleus but remains separate from the host DNA



Koilocyte indicating HPV infection in a low-grade lesion

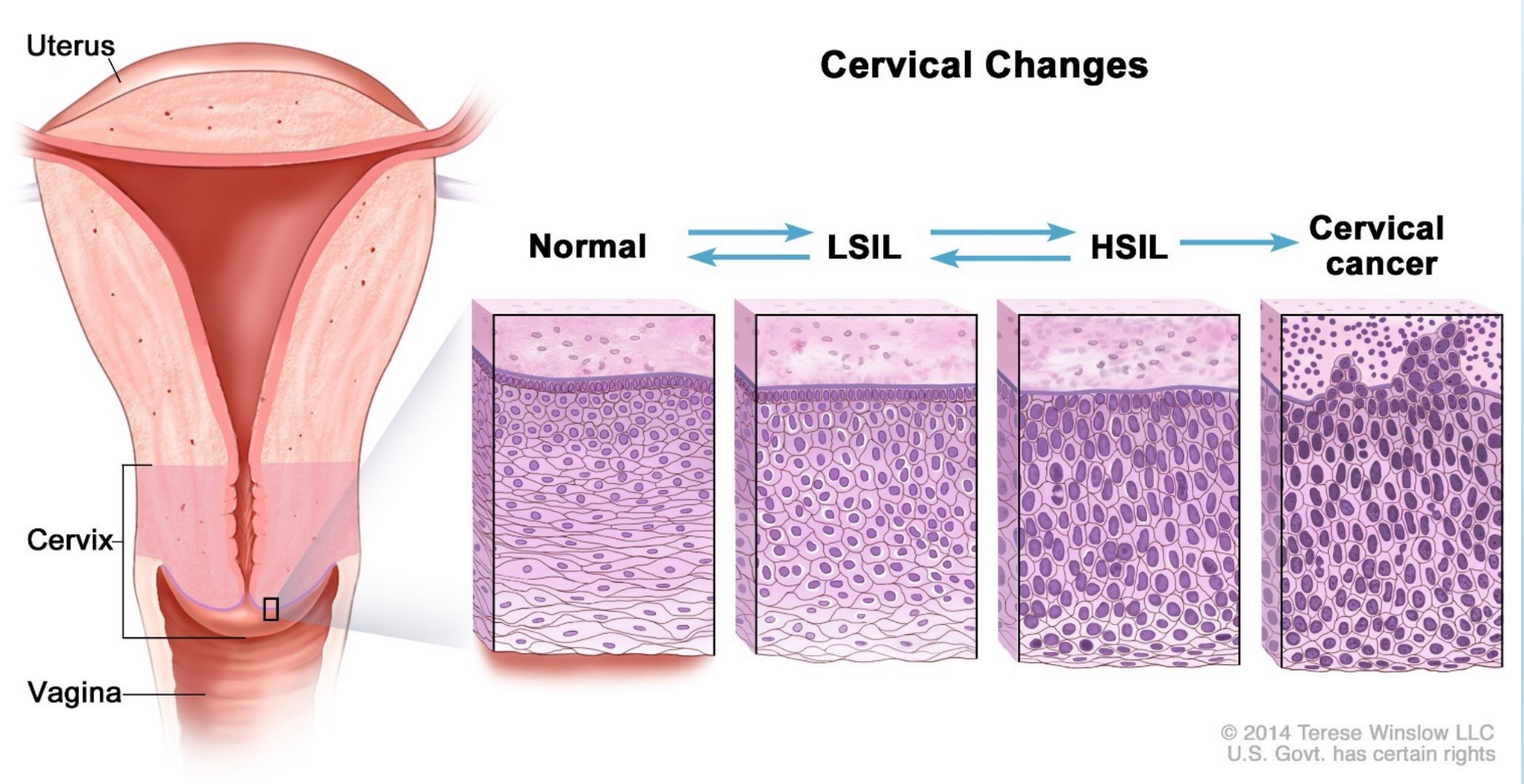
HPV infection and high-grade lesions

- High-grade squamous intraepithelial lesions (HSIL) have abnormal cervical cells that could become cancer in the future if not treated
- HSIL is pre-neoplastic change in squamous epithelium
  AIS is pre-neoplastic change in glandular epithelium
- HSIL and AIS are not invasive cancer: HSIL and AIS are treated to prevent cancer from developing
- Progression to cancer usually takes many years so even if lesions are initially missed by cytology screening, with repeat screening there is time to detect and treat the pre-neoplastic change before invasion occurs



HSIL cells have large nuclei compared to the size of the cell and the nuclei are often irregular in shape

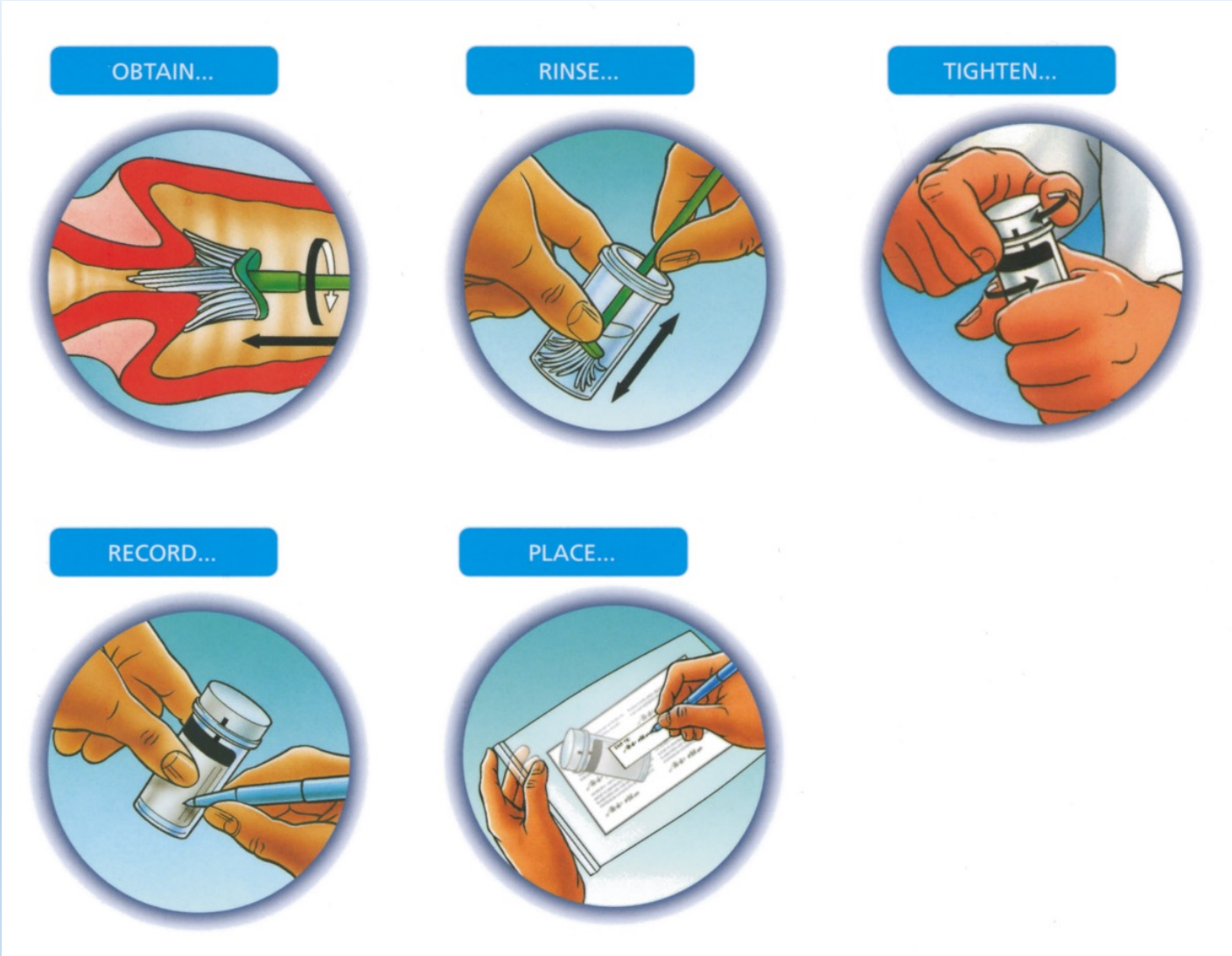
HPV Infection and high-grade lesions



What is the role of cervical cytology?

- Pre-neoplastic lesions are asymptomatic: a cytology screening test is currently used to identify those with lesions that can lead to cancer
- Cervical cytology involves looking at individual cells collected into fluid and transferred on to a glass slide so they can be examined under a microscope
- The sample of cells is taken from the cervical epithelium usually by a GP or nurse sample taker, using a brush-like plastic device. The sample is then placed in a small vial containing fluid fixative and sent to the laboratory for processing
- Cervical cytology isn't 100% accurate because sometimes abnormal cells don't make it through sampling and processing on to the glass side seen by the cytologist. Abnormal cells can also be missed or misinterpreted at screening.
  - For this reason, the test needs to be repeated regularly. In New Zealand, cervical cytology screening is recommended every three years, if the results are normal.
- High-grade pre-neoplastic lesions are often present for at least 10 years before cancer develops so there is time for repeat testing to be effective, even if a lesion is missed on one or more of the initial tests.
- Of course if someone has high-grade cells detected, no one knows how long they have already been there for, so referral for follow-up and treatment should occur quickly.

Taking the sample using a cervixbrush

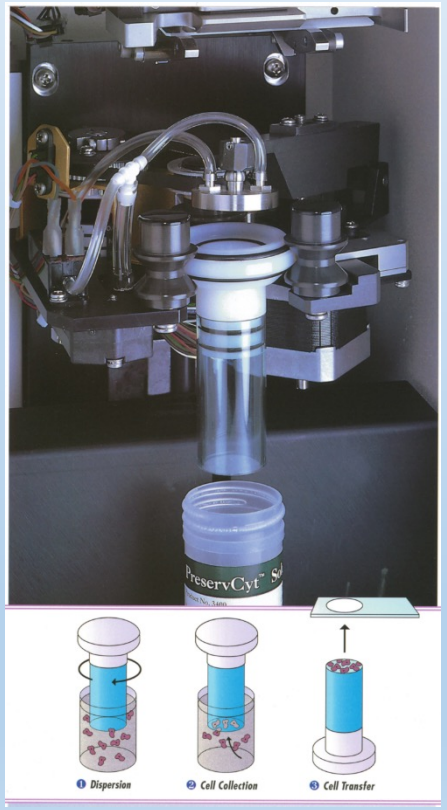


Processing cytology samples

- The collection of samples into a fluid-filled vial is called liquid-based cytology (LBC), and has been used for all cervical cytology since 2009 in New Zealand.
  - Previously, the cells were smeared directed on a glass slide (called a cervical smear) which was then inserted into a liquid fixative, or sprayed with fixative.

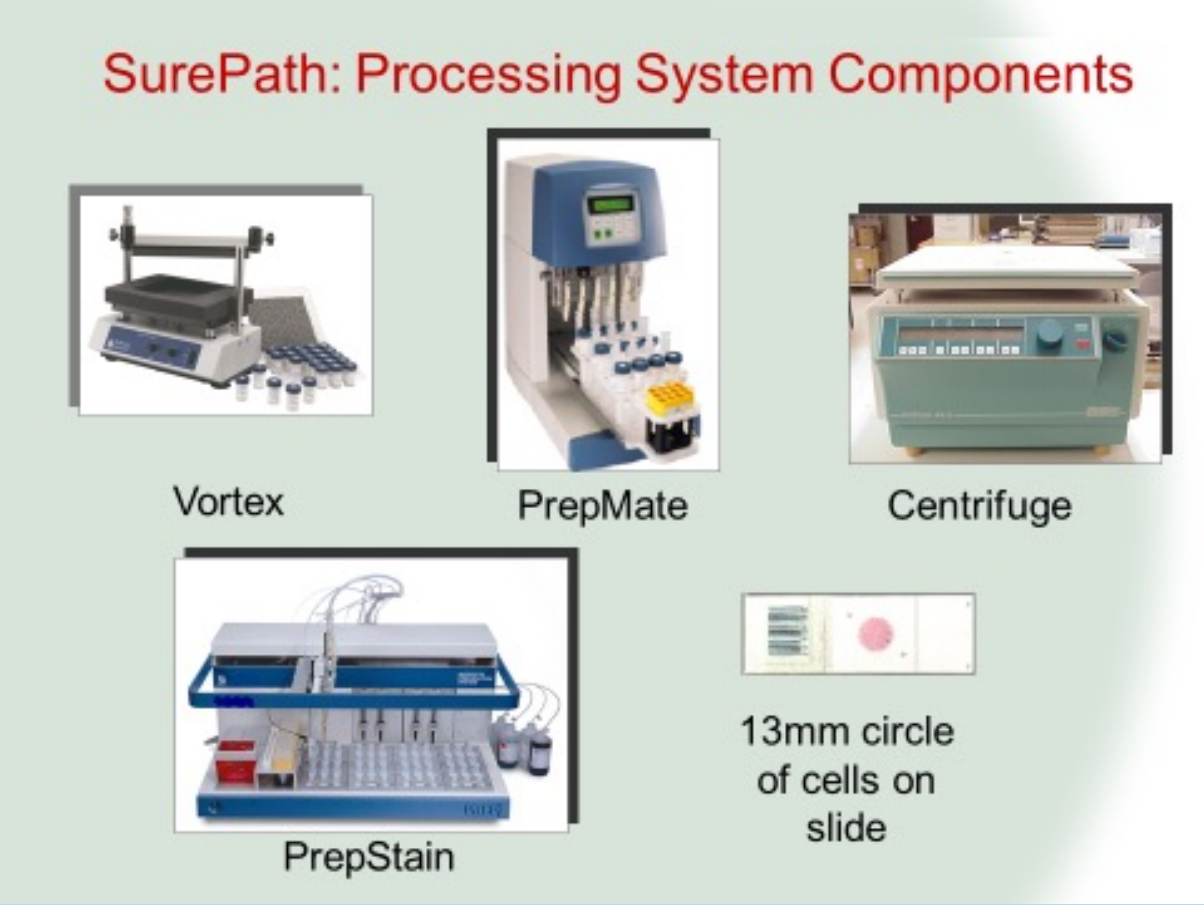
- Two types of LBC are in use in New Zealand: ThinPrep and SurePath
- Each has a different way of processing the sample but both techniques result in a circle of well-preserved evenly spread epithelial cells on a glass slide while removing much of the blood and inflammatory cells that used to make interpretation difficult in conventional cervical smears
- A coverslip is applied over the cells before microscopic examination

ThinPrep processing



- The vial is placed in a ThinPrep processor which mixes the contents and pulls the fluid up through a filter
- Once there are sufficient cells on the filter, it inverts to deposit the cells onto a glass slide
- Each filter is used only once
- The result is a 20mm circle of cells on the slide

SurePath processing



- After thoroughly mixing the contents of the vial by vortex, the PrepMate removes the blood, mucus and inflammatory cells producing an enriched cell pellet
- The sample is then centrifuged twice to concentrate the cell pellet further
- The PrepStain machine allows the cells to settle onto a glass slide before staining them
- The result is a 13mm circle of cells on the slide

Microscopic examination

- A satisfactory LBC cytology slide contains 5000-200,000+ cells. Sometimes even less than 10 of these cells are abnormal.
- Cytoscreeners (cytoscientists and cytotechnicians) are trained to screen the slides to detect **any** abnormal cells that are present – it takes years to become skilled at this difficult task.
- In recent years, automated imagers have been developed to pre-screen the slides, and identify which microscopic fields of view on each slide, are the most likely to contain abnormal cells. The cytoscreener then only needs to look at the selected microscopic fields – if these are normal, the rest of the slide doesn't need to be examined.
- If an abnormality is identified by the first cytoscreener, a second cytoscreener will look at it too, because the interpretation of the visual appearance of cells is fairly subjective so a second opinion is very useful. If the screeners agree that an abnormality is present, the slide is sent to a cytopathologist for reporting.

A cytoscreener viewing cytology slides

ThinPrep Imager
used to image
ThinPrep slides

FocalPoint Profiler
used to image
SurePath slides

Cytology results

A range of interpretations are given in cervical cytology reports and these determine the next step in the screening pathway

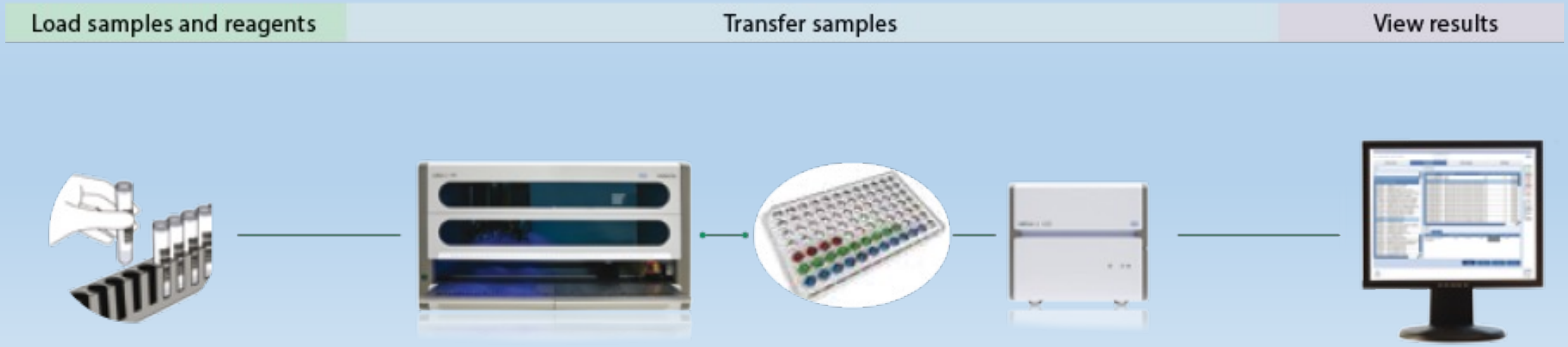
- Low-grade lesions (ASC-US, LSIL): the cytology sample is repeated in 12 months for those under 30 years of age to see whether the lesion resolves naturally.
  - Referral for investigation occurs if the repeat test is also abnormal, even if still low-grade
- In those 30+ years of age an HPV test is performed on the first low-grade sample
  - Referral for investigation occurs if the HPV test is positive. If the HPV test is negative, the cytology is repeated in 12 months with investigation if the repeat sample is still abnormal
  - While LSIL isn't pre-neoplastic, some people with LSIL cytology have high-grade lesions detected at colposcopy so follow-up after LSIL cytology is very important
- High-grade lesions (HSIL, AIS) are referred directly to colposcopy for further investigation
- Sometimes a definite result can't be given but there are still suspicious features. When this occurs the follow-up is the same as LSIL if the sample is suspicious of a low grade lesion, or referral to colposcopy if suspicious of a high-grade lesion

What is hrHPV testing?

- The HPV test used in the cervical screening pathway tests for 14 different types of the HPV virus simultaneously. All 14 are high-risk types of HPV (hrHPV) in that they are all associated with the development of invasive cervical cancer, although some types are riskier than others
  - If any one of the 14 high-risk types are present, the test result is positive and reported as *hrHPV Detected*. Because HPV-16 and HPV-18 are the two most risky types, these are specifically identified in the report while the remaining 12 types are all reported together as *hrHPV Other*
- about 40 HPV types can infect the genital tract – the rest are low-risk HPV types that only very rarely cause cancer. HPV-6 and HPV-11 are well known low-risk HPV types because together they cause 90% of cases of genital warts.
  - The low-risk types are not detected in the hrHPV test used for cervical screening

HPV testing

- HPV testing involves extracting nucleic acids (DNA) from the cells, and then running real-time PCR (polymerase chain reaction), which amplifies the amount of HPV DNA (if present) so it can be detected
- Automated multi-channel pipetting instruments are used to extract, purify, and prepare nucleic acid for subsequent PCR testing, for multiple samples simultaneously
- Real-time PCR measures DNA amplification as it occurs, cycle-by-cycle, allowing quantitative measurements to be made as the PCR proceeds, because samples with a high HPV viral load will show up as positive faster than those with a low viral load
- The testing system can be connected to a computer which controls the Real-time PCR machine and stores real-time PCR data collected from the reaction plate



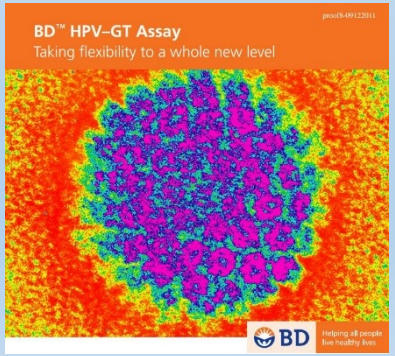
HPV Assays in use in New Zealand (2022)

Abbott Alinity

Roche Cobas®4800 HPV test

BD Onclarity HPV Assay

All are nucleic acid amplification assays and all detect the presence of HPV DNA



How is HPV testing used in the screening pathway?

Cytology-based screening:

Currently New Zealand uses cytology as the primary screening test

HPV Testing is used in three ways as a second test:

1. To determine which 30+years women with low-grade cytology should be referred for colposcopy
2. As part of a Test of Cure after treatment of a high-grade lesion, to see if the treatment is complete
3. Specialist colposcopists can order HPV tests to assist with case management

HPV-based screening:

In July 2023, New Zealand will change to HPV testing as the primary screening test

- Cytology will only be done on those who are hrHPV positive
- more disease will be detected because HPV testing is a more sensitive primary screening test than cytology
- cervical cancer rates will drop further as a result

What is the role of histology?

- Histology is the examination of whole pieces of tissue (rather than individual cells) in order to diagnose the presence and type of a cervical abnormality
- a small tissue biopsy is taken from the cervix to confirm the diagnosis, followed later by excision of the affected area to remove the abnormality
- The samples can only be taken by a specialist colposcopist at the time of a colposcopy
- Accurate histological grading of pre-invasive cervical lesions is important for clinical management of patients, because low-grade and high-grade lesions are treated differently
- Another important role of histology for large excision specimens, is to check that the abnormal area has been completely excised and does not extend to the edge of the piece of tissue removed

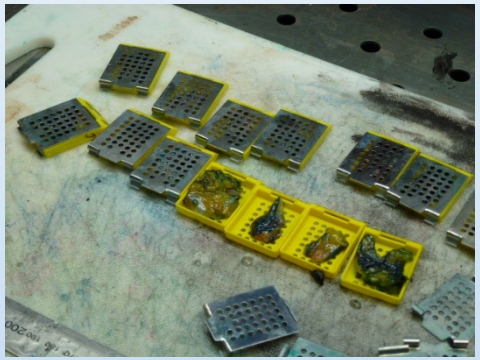
What happens to samples in histology?

Histology slide preparation involves 5 steps: fixation, processing, embedding, sectioning and staining.

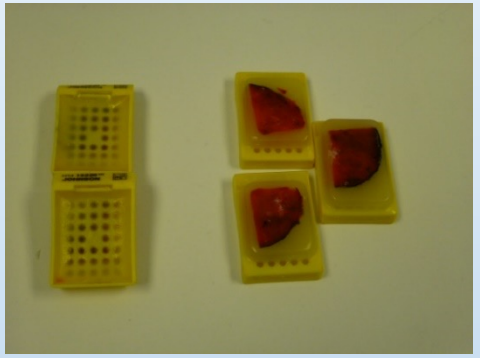
- To prevent decomposition, the tissue is placed in liquid formalin (fixed) as soon as the biopsies are taken
- At the laboratory, a histoscientist or pathologist describes and measures what has been received (part of the final report) and selects the tissue for processing
    - Small biopsies are transferred directly for processing. Selected pieces of tissue of interest are cut from larger tissue specimens
- The selected tissue is placed into plastic cassettes and then processed in a machine to remove all the water and replace it with paraffin wax before embedding in a larger wax block so that thin sections can be cut, slid onto a glass slide and stained.
- Sectioning is a highly skilled job. Sufficiently thin slices must be prepared in order to enable clear and accurate microscopic observation of the tissue.
    - The sections that are cut by the histoscientist determine what the histopathologist will see down the microscope.

Histology processing

Scientist cutting and describing the samples



Tissue going into cassettes before processing



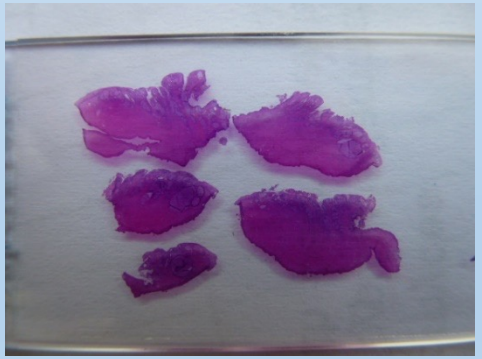
Embedded tissue in wax blocks

Scientist cutting sections from wax blocks

H&E Autostainer

QC checking blocks against slides

Slides ready for pathologist



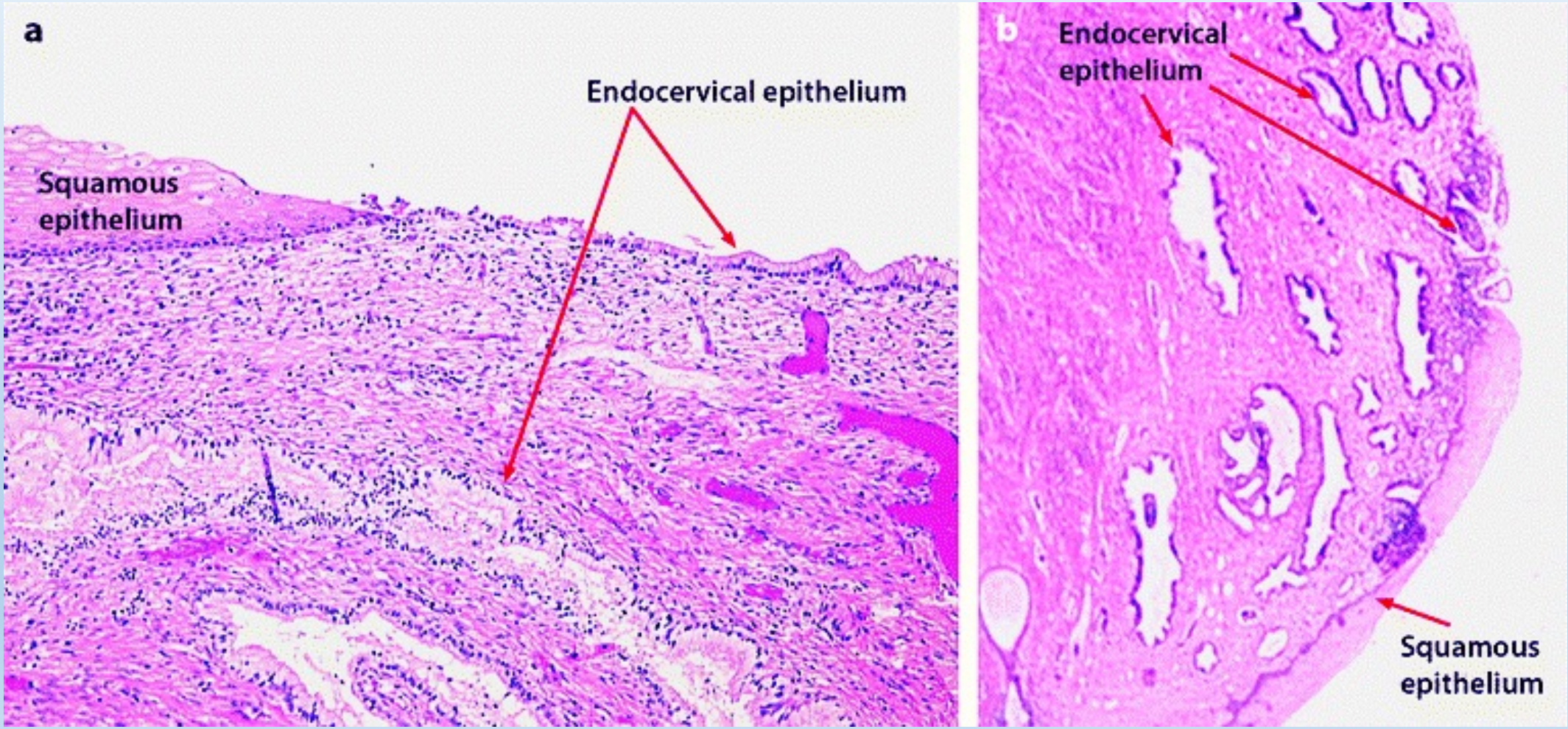
Slices of cervix (treatment sample)

Histopathologist reporting

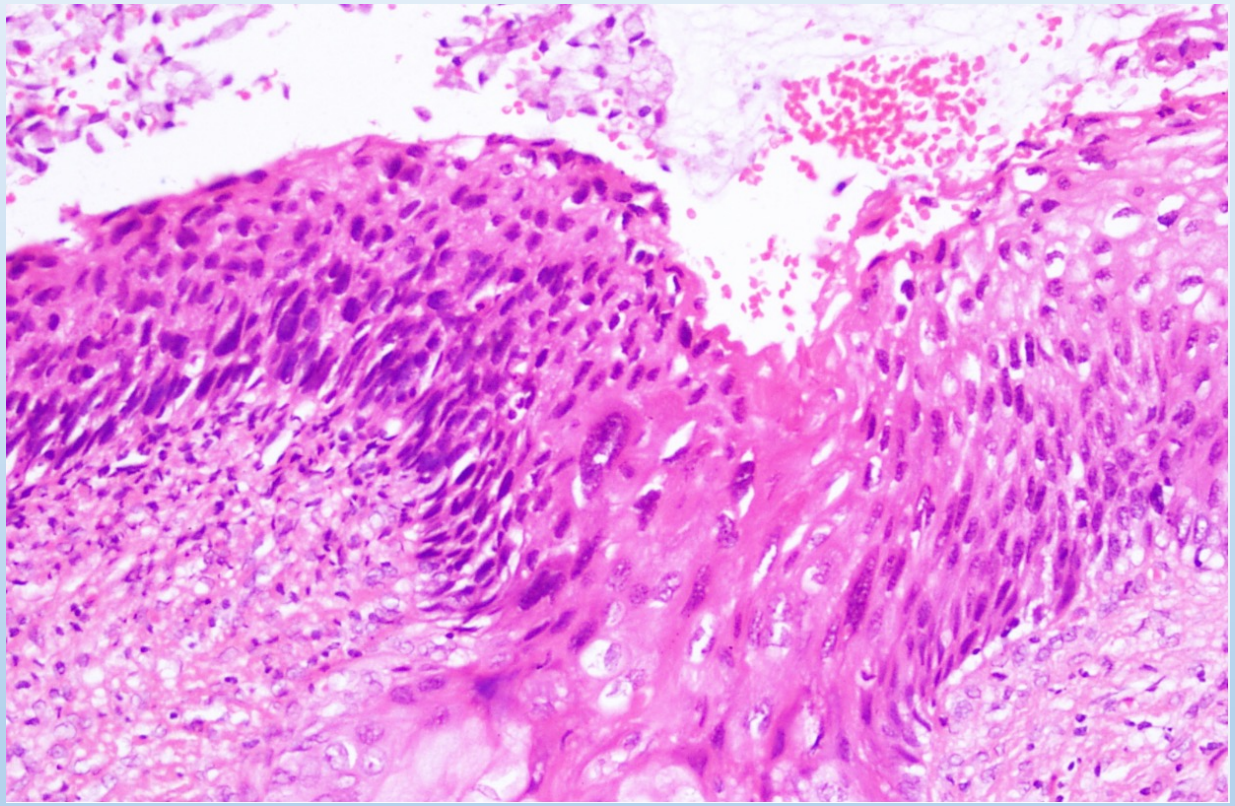
All histology is reported by a histopathologist

- The glass slides are given to the histopathologist for reporting once the processing, slide preparation and staining is complete. The tissue is covered by a thin glass coverslip.
- Histopathologists are trained to know what normal cervical tissue looks like, and to recognise all of the different changes that can be seen in different diseases
  - Small cervical biopsies are used to confirm the diagnosis
  - Excision of larger pieces of cervix, usually a LLETZ biopsy or a cone biopsy, are used to remove the whole affected area for treatment
  - Special stains are often used as well as the basic H&E (Haematoxylin and Eosin) stain to assist with interpretation
- The colposcopist who takes the specimens and treats the person needs to work closely with the histopathologist to make sure that everyone has a clear understanding of the abnormality being diagnosed and treated
- Multidisciplinary meetings (MDMs) are held regularly throughout New Zealand, where difficult cases are discussed by the colposcopist, the histopathologist and the cytopathologist, to ensure the best outcome for the patient

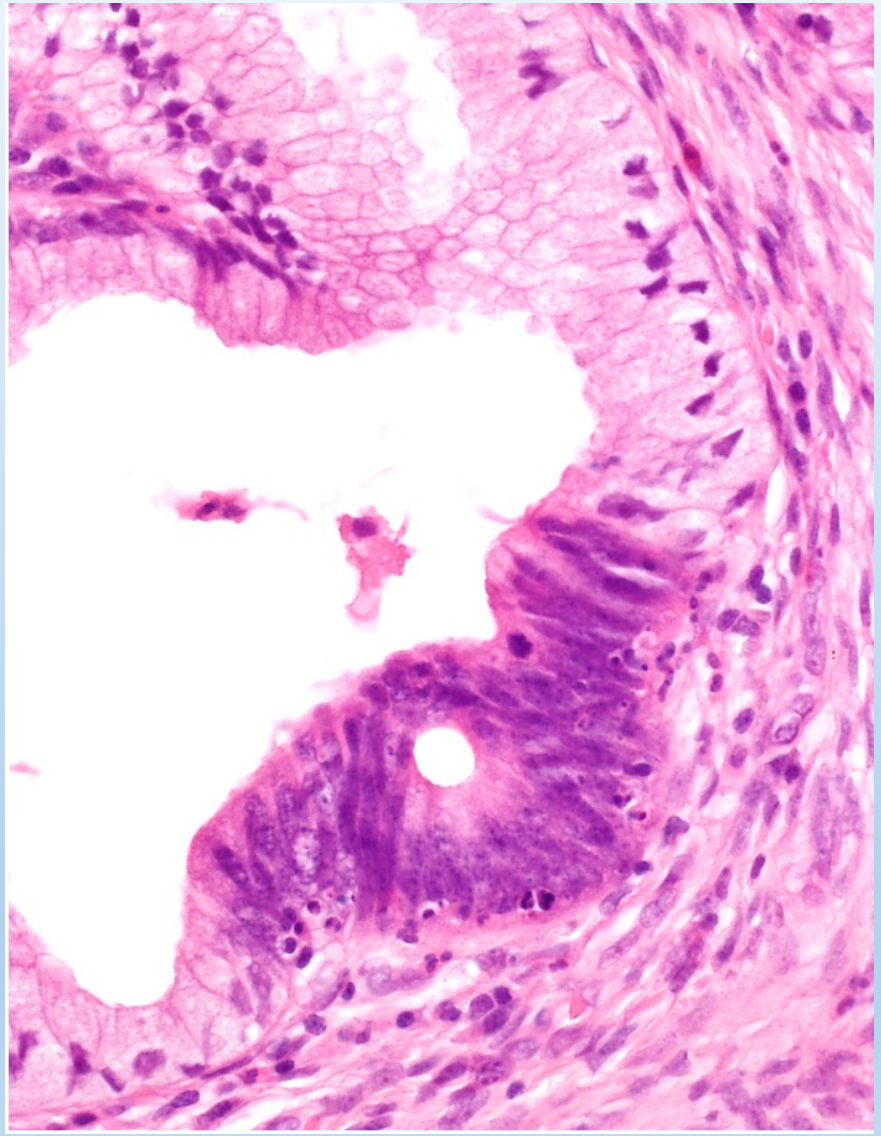
Normal histology of the cervix



What do high-grade cervical lesions look like in histology?



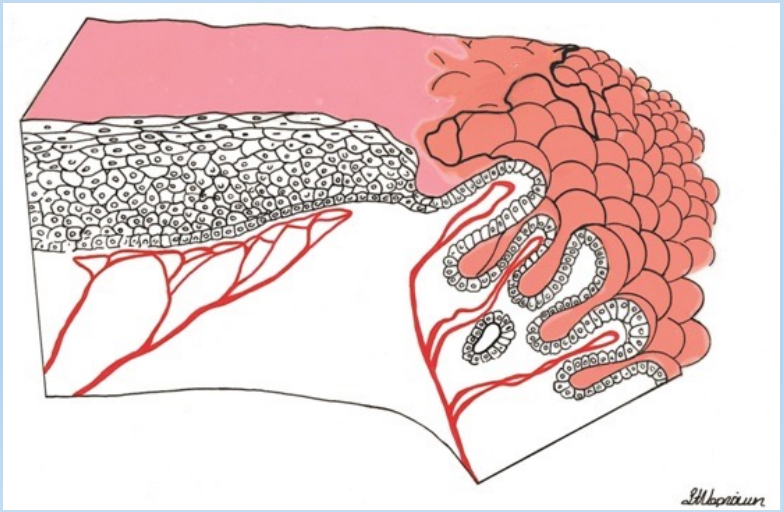
HSIL extending into an endocervical crypt



Adenocarcinoma in situ (AIS)

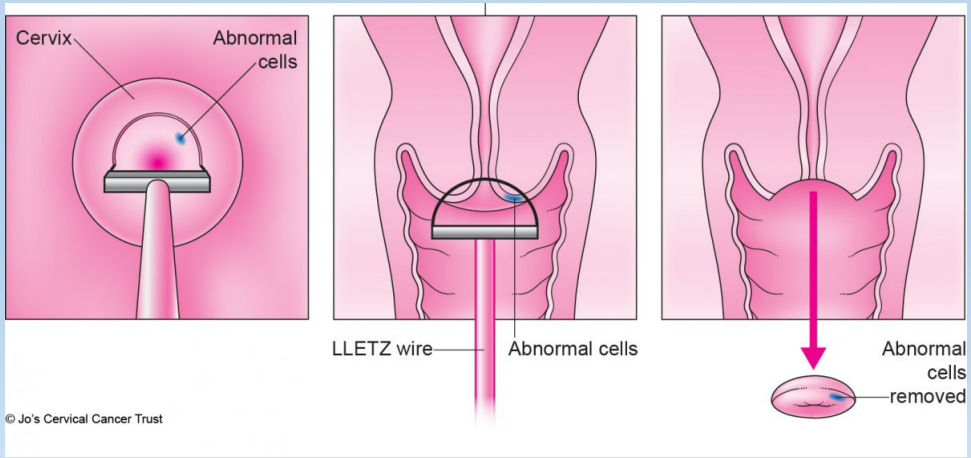
What is colposcopy?

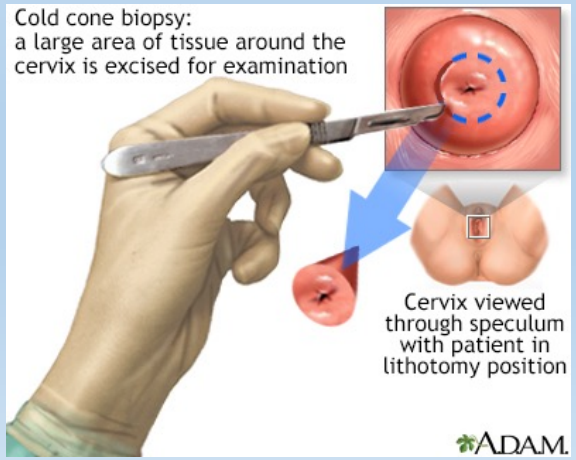
- The cervix can be magnified using a special microscope called a colposcope, to look for changes in appearance that indicate cervical lesions
- The colposcopist is a gynaecologist with special training to recognise and treat cervical lesions
- If an abnormality is suspected, biopsies are taken for histology to confirm the abnormality



What happens when a lesion is treated at colposcopy?

- If the histology is low-grade, the person is usually discharged back to primary care for follow-up to make sure it resolves – or is referred back to colposcopy if abnormalities persist
- If the histology is high-grade, the colposcopist will excise the affected area using a LLETZ (Large Loop Excision of the Transformation Zone) or a Cone biopsy
- The histopathologist reports the type and extent of any cervical lesion present and also confirms that the lesion has been completely excised





A typical screening journey for a 35 year old woman who has a HSIL detected and treated…

- Attends her local general practice for a regular cervical cytology screening test. She doesn't have any symptoms and her screening cytology tests in the past have always been normal.
- Cytology identifies LSIL so hrHPV testing is performed on the same sample and hrHPV is detected so she is referred for colposcopy
- At colposcopy, the appearance of the cervix is suspicious of HSIL. Cervical biopsies are taken for histology which are reported a few days later confirming HSIL. There is no invasion present
- She goes back to colposcopy for treatment, where a LLETZ excision is performed to remove the affected area. Histology confirms completely excised HSIL with no invasion.
- After treatment she has a Test of Cure: cytology and HPV cotesting at both 6 months and 18 months after treatment. If all results are normal cytology/negative for HPV, she is considered cured and returns to the regular cervical screening interval, currently 3 years under the cytology-based screening programme.
- Her risk of further lesions is no greater than that of the rest of the screening population

Why does cervical screening work so well to prevent cancer?

- The cervix is relatively accessible
- There is a cheap simple screening test (cytology) that can be used across the whole population to detect pre-invasive lesions
- Cytology has reasonable sensitivity and good specificity for detecting cervical lesions
- Even though the sensitivity of cytology is only reasonable, there is a long pre-invasive stage usually of many years, so repeat testing detects most cases before invasion develops.
- Because the cervix can be visualised at colposcopy, pre-invasive lesions can be confirmed using histology and then treated at colposcopy before invasive cancer develops
- Treating precursor lesions has a significant impact on the incidence of invasive disease and mortality from cervical cancer

But we can do even better!

- The explosion of knowledge over the past 4 decades about the role that HPV plays in causing cervical cancer plus the technological advances in HPV testing will improve cervical screening
- By improving the sensitivity of the primary screening test, we can detect more lesions and detect them earlier compared with screening with cytology
- Using HPV testing for primary screening has been shown internationally to significantly reduce cervical cancer rates.
  - about 10% of those tested will be hrHPV positive whereas about 7% have abnormal cytology
  - cytology will continue as an important second test for those who are HPV positive, in order to sort out who needs early referral to colposcopy, rather then repeat HPV testing because cytology is better than HPV testing at identifying who actually already has a cervical lesion (greater specificity)

Further:

- hrHPV testing (but not cytology) can be accurately performed using a simple vaginal swab sample, which is a much more acceptable way of being screened for many people compared with requiring a speculum examination for an LBC sample, as is needed for cytology

Primary screening using hrHPV testing instead of cytology, will be introduced in New Zealand in July 2023.

Kia ora

Thanks for your interest and attention