# Primary HPV Cervical Screening – A Laboratory Perspective

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THE NATIONAL CERVICAL SCREENING PROGRAMME

# Learning Outcomes of the Presentation

To highlight and discuss the following:

- What is HPV, its carcinogenic role in cervical cancer and its detection as an application in Cervical Screening
- The importance of an evidence based approach
- The HPV test, including practical considerations and challenges about its implementation as a screening test
- The importance of Quality Assurance including validation and verification processes
- Limitations of the test including potential for false negatives
- Sample taker responsibilities and optimising laboratory screening services

# Facts about Laboratory Cervical Screening

- Detect pre-cancerous conditions in a timely manner to prevent progression to invasive disease
- Reduce morbidity and ultimately mortality
- A HPV Test is a test of risk of cervical disease
  - Has a high sensitivity and negative predictive value
  - Poor specificity
- Therefore a cervical screening programme also requires a test of disease
  - High specificity
  - High positive predictive value
  - Cytology
  - Molecular Biomarkers
  - Computer Assisted Screening
  - Digital Artificial Intelligence Screening
- Current measure of outcome of a cervical screening programme is cervical histopathology

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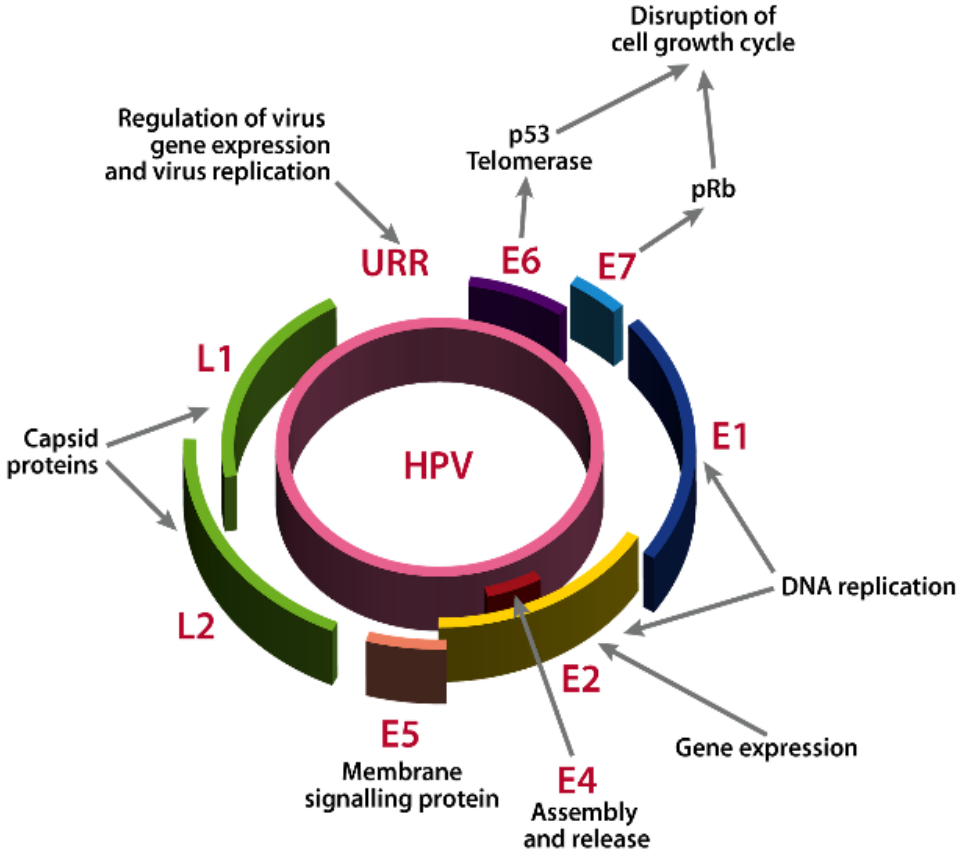
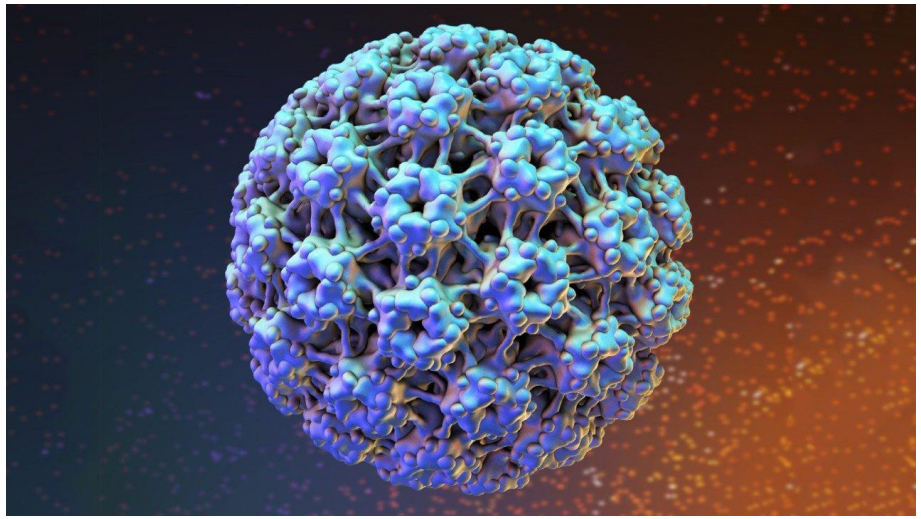
# Following the Evidence ……

- 1842 – Domenico Antonio Rigoni-Stern – Statistical facts about cancers – IV Conference of Italian Scientists
- George Herbert Green, “The Unfortunate Experiment” and the Cartwright report
- 1976 – Harald Zur Hausen - HPV related to cervical cancer
- HPV testing initially introduced as a risk stratification tool
- HPV triage of low grade cytology
- Test of Cure following treatment
- Primary HPV test – numerous clinical trials - ARTISTIC, ATHENA
- Countries  now implementing primary HPV screening include:
- Australia, Denmark, England, Holland, Ireland, Scotland, Wales

# Human Papilloma Virus - HPV

Over 100 sub-types, but only a relatively small number have been implicated in cervical disease

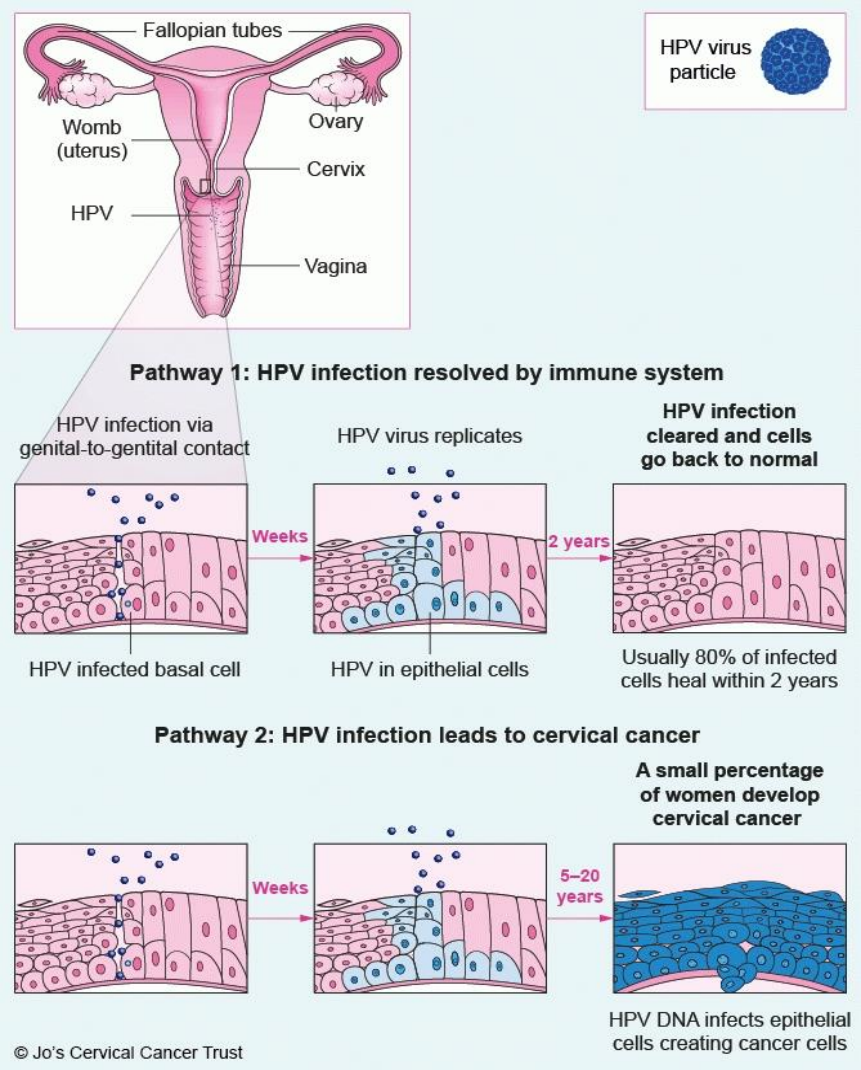


**HPV mediated carcinogenesis**

- HPV infection of epithelium
- Immune system deficiencies
- Expression of E6, E7 oncogenes
- Cellular alteration and transformation
- Dyskaryosis and malignancy

# HPV Related Cervical Carcinogenesis




- 80% of infections are cleared within 2 years
- Lead time to developing cancer is 5-20 years
- Association with persistent infection

# The challenges of HPV testing in cervical primary screening

**HPV Not Detected Result**

- Negative predictive value 99.9%
- Long-term protective effect is well established
- Safely increase the screening interval

**HPV Detected Result**

- Poor specificity of a positive HPV test
- Risk of over referral to colposcopy
- Increased cost and patient anxiety

**Specificity NPV** **VS** **Sensitivity PPV**

# The HPV Test – Clinical Validation

- Currently over 200 available HPV assays
- CervicalCheck has a policy of rigorous validation of HPV tests
- Those that satisfy programme requirements are added to the Approved Assay List
- HPV detection threshold – approved tests have been clinically validated so that a positive or negative result can be trusted. There is no need to be cautious of results that are “close to the cut-off”
- This is why a negative result is has such a high negative predictive potential
- The test is very reliable, **IF** the CervicalCheck standards and processes are followed

# Operational considerations of introducing primary HPV testing in the laboratory

# Acknowledgments and the future!

- The dedication and professionalism of our laboratory providers
  - Quest Diagnostics
  - Coombe Women and Infants University Hospital

- National Cervical Screening Laboratory development
  - An exciting project and very positive for cervical screening in Ireland
  - Challenging – staffing in Ireland at critically low levels
  - Training infrastructure for Cytopathologists needed
  - Advanced clinical medical scientist practitioners role needed urgently

# The HPV Test – General Information

- Platform currently in use – Roche Cobas 4800
- Tests for 14 high risk sub-types
- Partial genotyping results are not provided for screening samples
- Test for infection – therefore a test of **RISK** for cervical disease
- Negative HPV test means very, very small chance of abnormality
- Cytology is the test used as reflex to a HPV positive result. It is a test of **DISEASE**, specifically employed to qualify the **RISK**
- All high risk HPV positive samples have a reflex cytology test performed to check for the presence of cervical disease
- Automated and therefore has a faster TAT than a cytology primary test

# Partial Genotyping

- Widely published that most (up to 70%) of HG cervical lesions attributable to HPV subtypes 16,18
- HPV testing demonstrates that HPV vaccination is performing, with declines in HPV 16 and 18 prevalence recorded
- This evidence[1,2] has informed CervicalCheck primary HPV partial genotyping policy – reporting only presence or absence of high risk subtypes
- All positives referred for colposcopic assessment on second HPV positive
- Facilitates benchmarking with primary HPV based programmes e.g. UK
- Use of partial genotyping in colposcopy setting currently under review


1. Type-specific HPV in sexually active young females in England, to end 2018. Public Health England. PHE publications gateway number GW-1027; HPR 14(2). Available at: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/858872/hpr0220_HPV_2018.pdf
2. Age-specific HPV prevalence among 116,052 women in Australia's renewed cervical screening program: A new tool for monitoring vaccine impact. Julia ML. Brotherton, David Hawkes, Farhana Sultana, Michael J. Malloy, Dorothy A. Machalek, Megan A. Smith, Suzanne M. Garland, Marion Saville. https://doi.org/10.1016/j.vaccine.2018.11.075

# HPV Vaccinated Participants

- HPV vaccinated persons are increasingly tested as part of a cervical screening programme as they reach eligible screening age
- HPV vaccination results in fewer persistent infections, with subsequently fewer positive results
- Older HPV assays were not clinically validated in a vaccinated population and so further validation studies are required:

    - Performance evaluation of the assay concerned in other primary HPV based screening programmes where HPV vaccinated eligible participants are screened (Australia, U.K.) is important

# Vaginal Vault Samples

- Traditionally, eligible post hysterectomy samples followed up with cytology
- Currently, no HPV tests are FDA approved for HPV testing on vaginal samples
- Non-FDA in-house analytical validation carried out – good concordance with cervical sample testing
- Clinical validation carried out on samples with known cytology outcomes
- Initial correlation results are promising
- Now looking to perform histology outcome correlation

# The “HPV – indeterminate” result

This is a relatively rare event, however, there are a number of causes, including :

- Assay quality control failure
- Technical issues with the sample or equipment
- Sample contamination

In many instances, the test can be repeated on the sample and a result obtained. However, that is not always the case – hence the indeterminate result.

# Sample Contamination

- All HPV assays can be affected by contamination which inhibits the test reaction, potentially resulting in a false negative result
- From the programme perspective, this mainly concerns blood and certain lubricants
- The tolerance for contamination with whole blood varies with assay, generally the newer assays have higher tolerance
- Important that the laboratory has a standardised, reproducible process that minimises uncertainty around the test

# Role of the Doctor/Nurse

- Fundamental to the success of the screening programme – interfacing directly with the woman
- Poor sampling can lead to failure in detecting pre-malignant abnormalities - before the sample has even reached the lab!
- Sampling is somewhat less critical with primary HPV testing, but is still a major consideration for reflex cytology
- Fact - Abnormal results are relatively commonplace but cancer is relatively rare
  It is vital, therefore, that the smear taker visualises the cervix when sampling - the lab can only give a result on the material presented in the vial
- **HELP THE LAB TO HELP YOU!**

# Specimen Collection – Request Form

- Ensure that the current version of the form is used
- For electronic requests, ensure that the GP IT system provider has the current request order version
- Check that the patient details are correct – WHILE THE PATIENT IS THERE!
- Rejection by the laboratory because of incompletely completed request forms may result in the sample becoming date expired by the time a corrected form is received
- Ensure that all relevant clinical history is captured
- CONSENT – ensure that this is captured to latest requirements

# Specimen collection – Date Expired Samples

- GLP requires that all lab investigations producing test results are controlled where possible, to ensure the most accurate results
- Laboratories therefore estimate and minimise uncertainty wherever and whenever possible
- The sample collection fluid is effective in preserving cells for 42 days, after which its properties begin to decline
- HPV is stable up to 6 months from taking the sample
- Because of the potential requirement for reflex cytology, a 42 day expiration limit is operated
- Samples in date expired vials will be rejected, ensure availability of in-date vials
- Expiry dates include the 42 day period, and so stock control in your areas is absolutely vital
- Do not delay vials to save on postage, could make the difference in none, some or even all samples being rejected and the woman having to attend for a repeat test

# Specimen collection – taking the sample

- Bin spare labels and forms – minimise the chance of transcription/ID errors
- The sample life for a HPV test and subsequent cytology test (if required) is 42 days – this has implications for the sample taker
- Make sure that the vial is not date expired, nor likely to in the near future that might impact on the validity of the test
- Sample transport – ensure that the samples get to the laboratory in a timely manner, the 42 day clock is ticking as soon as the broom enters the sample collection fluid
- Batching samples together to save postage can have serious impact on the test validity

# Common Reasons for Test Rejection by the Lab

- Patients outside programme eligible screening age
- Request form incomplete/incorrect version of the form
- Sample not labelled
- Consent to test not adequately recorded/no signature
- Sample vial date expired or within 42 days of expiry
- Sample leaked
- Broom left in vial

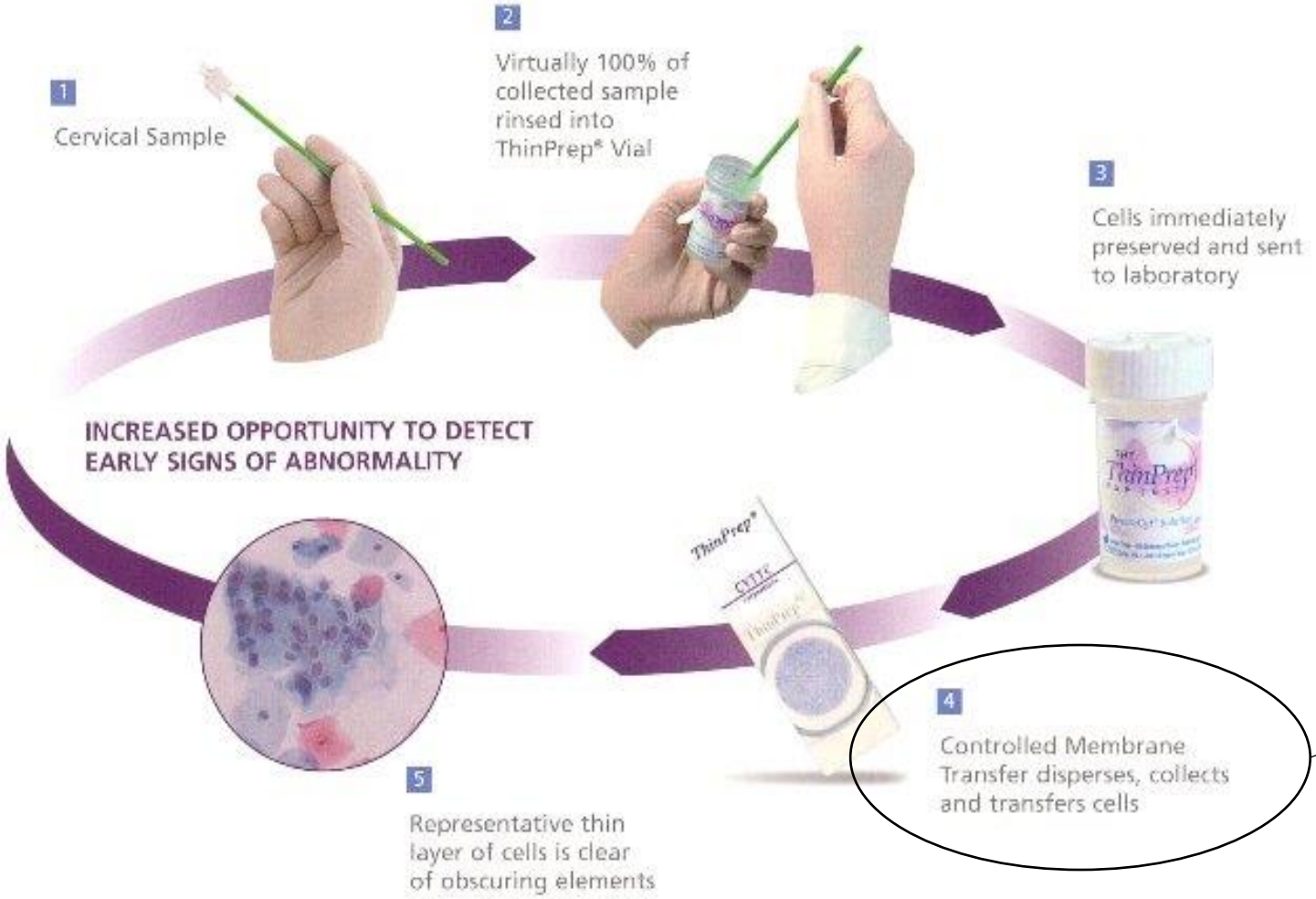
# Tests Queried: October 1st to November 15th, 2020

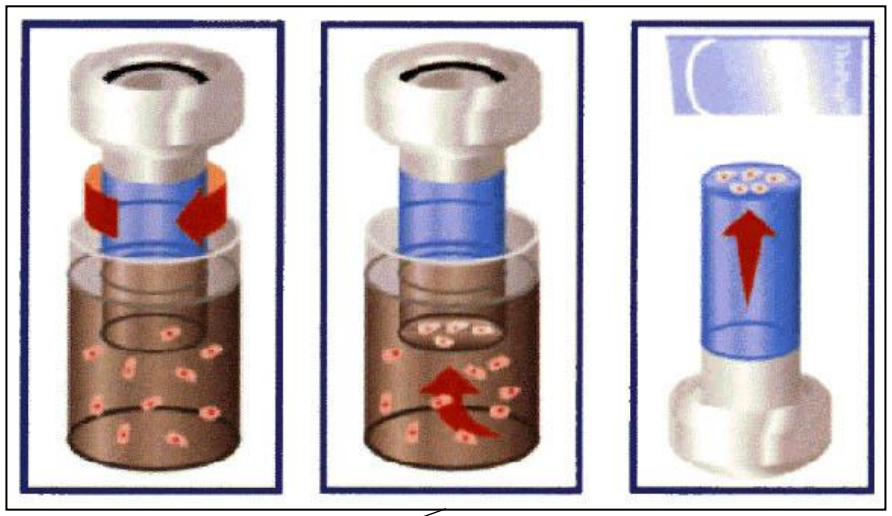
| Expired Vials | 237 | 10.9% |
|---|---|---|
| Address Mismatch | 8 | 0.4% |
| DOB mismatch | 37 | 1.7% |
| DOS mismatch | 69 | 3.2% |
| MCRN missing/incorrect | 1551 | 71.1% |
| Blank Vial | 84 | 3.8% |
| Name Mismatch | 22 | 1.0% |
| Others | 10 | 0.5% |
| Incorrect Forms | 163 | 7.5% |
| TOTAL | 2181 | |

# Impact of Rejected/Queried Tests for Patient and Lab

- In 6 weeks, over 2000 samples were rejected or queried
- In total the lab would expect to receive about 36000 samples, so about 6% of samples are either queried or rejected
- Follow up of these samples has a major impact on available resources
- A significant number of women undergo a repeat sample
- Programme reputation potentially damaged with every incident of this nature
- The vast majority of these issues are AVOIDABLE!

# The ThinPrep Liquid Based Cytology test



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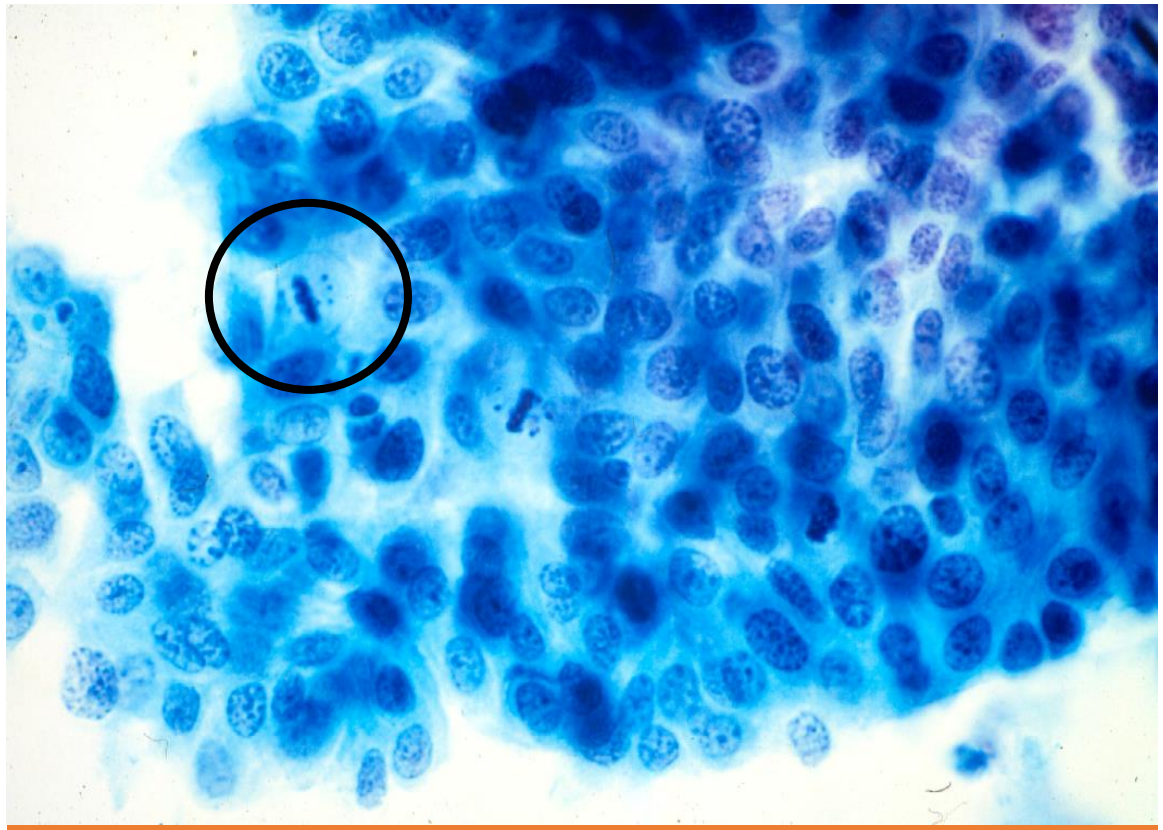
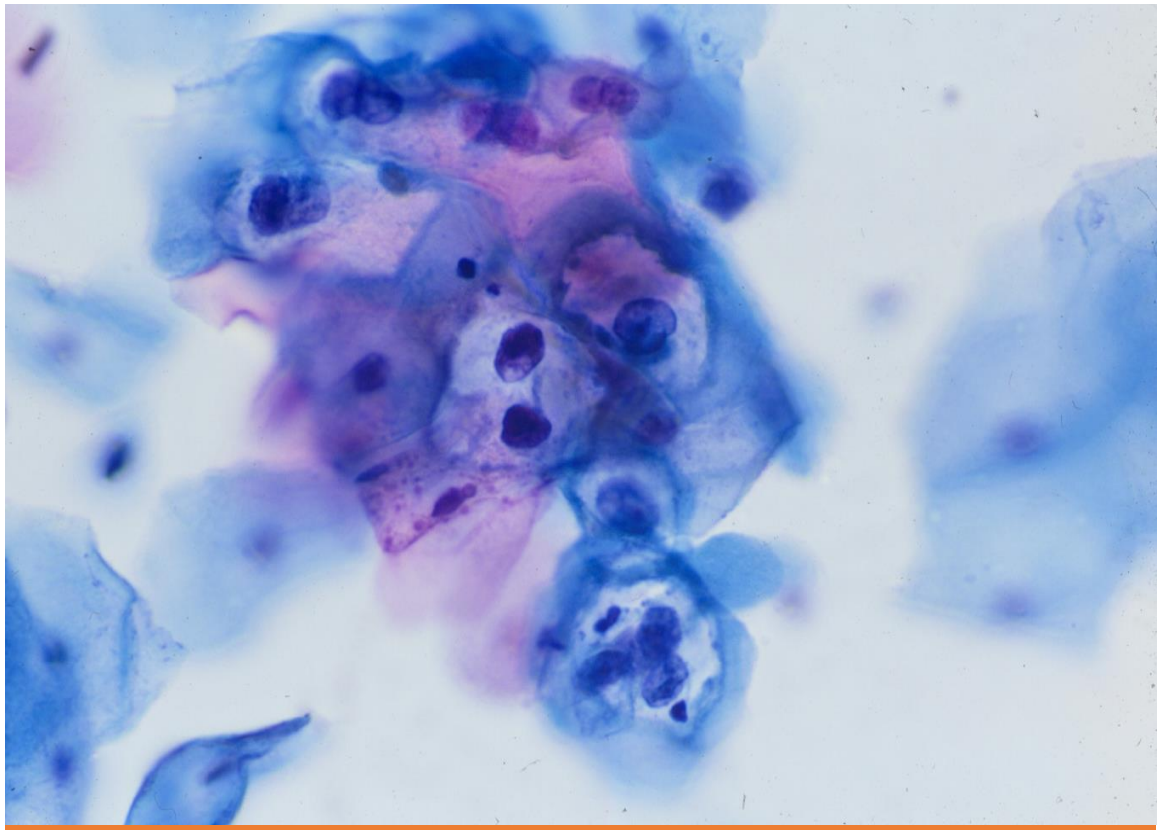


HSIL – microbiopsy



LSIL – Koilocytes

# Cytology and TZ Sampling

- Data will no longer be collected on Transformation Zone sampling as this will only be available on HPV positive results – up to 13% of the total workload
- Sample taker proficiency will be quality assured in other ways – approved training, sample taker register, updates and inadequacy rates

# The Clinically Indicated Cervical Screening Sample

- Should not be taken - a clinical referral is more appropriate
- HPV primary screening test is **not** a diagnostic test (designed to detect HPV as causative to cervical pre cancer – can be negative where the HPV DNA is integrated into the host genome)
- Sample takers must act appropriately in managing symptomatic patients

# The Private Test

A HPV or cytology test can be negative or positive for a number of reasons – not necessarily associated with cervical disease. Most labs are accredited in one form or another, however:

- CervicalCheck quality assures the labs it contracts with, which are then approved
- Labs are re-assessed if circumstances change and routinely within 2 - 3 years
- CervicalCheck cannot accept results from non-approved labs and when the programme is aware of the existence of such a result, further steps will be taken to assure the validity of that result
- CervicalCheck is currently reviewing the process for managing results from non-approved sources to ensure they are appropriately incorporated into the governance and assurance processes of the programme

# Thanks for Listening!

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