Renewed National Cervical Screening Program: From Cytology to HPV Nucleic Acid Testing

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VCS Pathology

Conflict of Interest

Compass Trial has received equipment and funding contribution from Roche Molecular Systems

Renewed National Cervical Screening Program (NCSP)

- Why Renewal?
- Changes to NCSP
- Types of HPV Assays
- How does this affect the operations of the laboratory, which is primarily cytology-based?
- What are the changes from the cytologist's perspective?
- Does this have any impact on the histology laboratory?

Cervical Cancer - Human Papillomavirus (HPV)

The discovery of HPV and its pathogenesis in cervical cancer has paved the way to new technologies in the prevention of cervical cancer:

- HPV vaccines
- HPV nucleic acid testing (NAT)
- Potential cellular biomarkers for the detection of high grade (HG) disease progression.

Cervical Cancer – HPV

- HPV is necessary but not sufficient to cause cervical cancers.
- Most HPV infections are transient and will resolve within 1 to 3 years.
- Persistent infections with oncogenic HPV types may progress to a cervical HG disease or cancer.

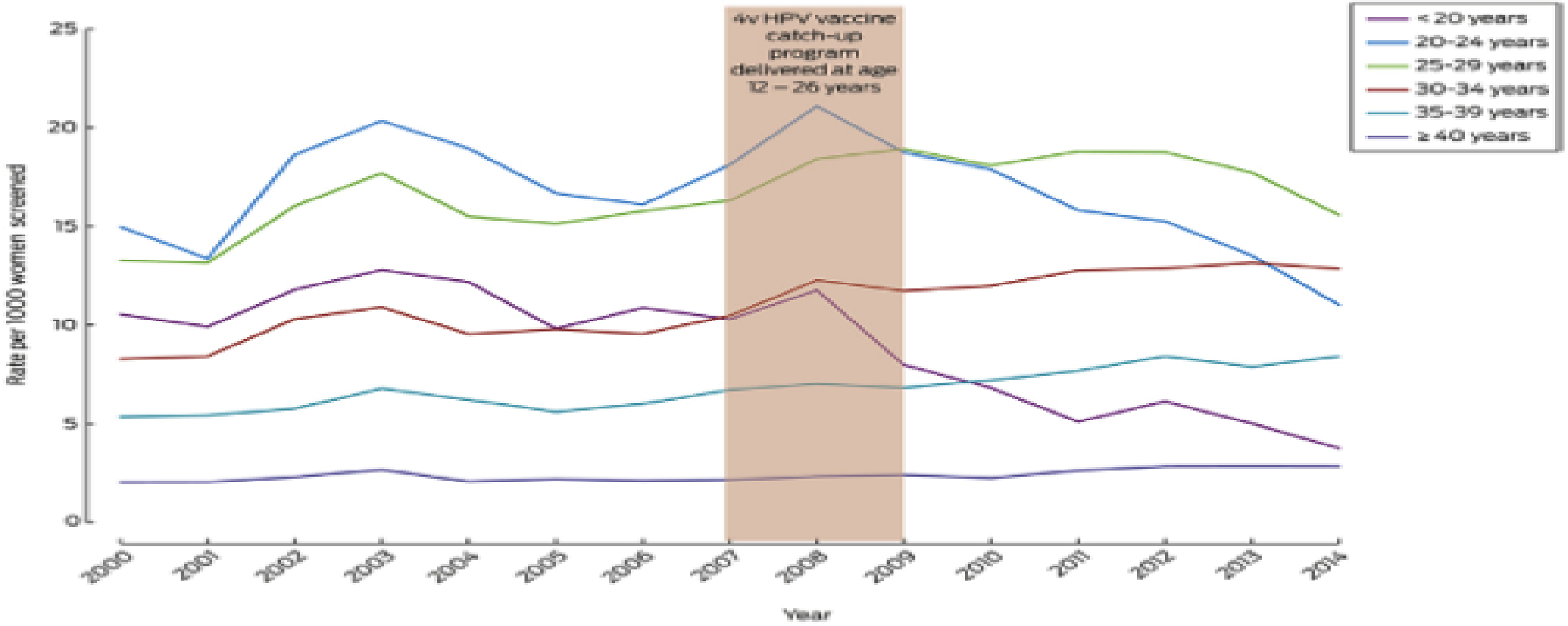
Key drivers to the Renewal

- HPV Vaccine as primary prevention

  (Cervarix – Bivalent 16/18; Gardasil – Quadrivalent 16/18/6/11)

  - ✧ 2007 – Female HPV vaccination in Australia
  - ✧ 2013 – Male HPV vaccination in Australia
  - ✧ 2018 – Gardasil 9 (Nonavalent, addition of 31/33/45/52/58)
- Reduction in HPV vaccine-type infections and disease
- Predicted to further reduce the incidence of cervical high grade disease and cancer in the screening population

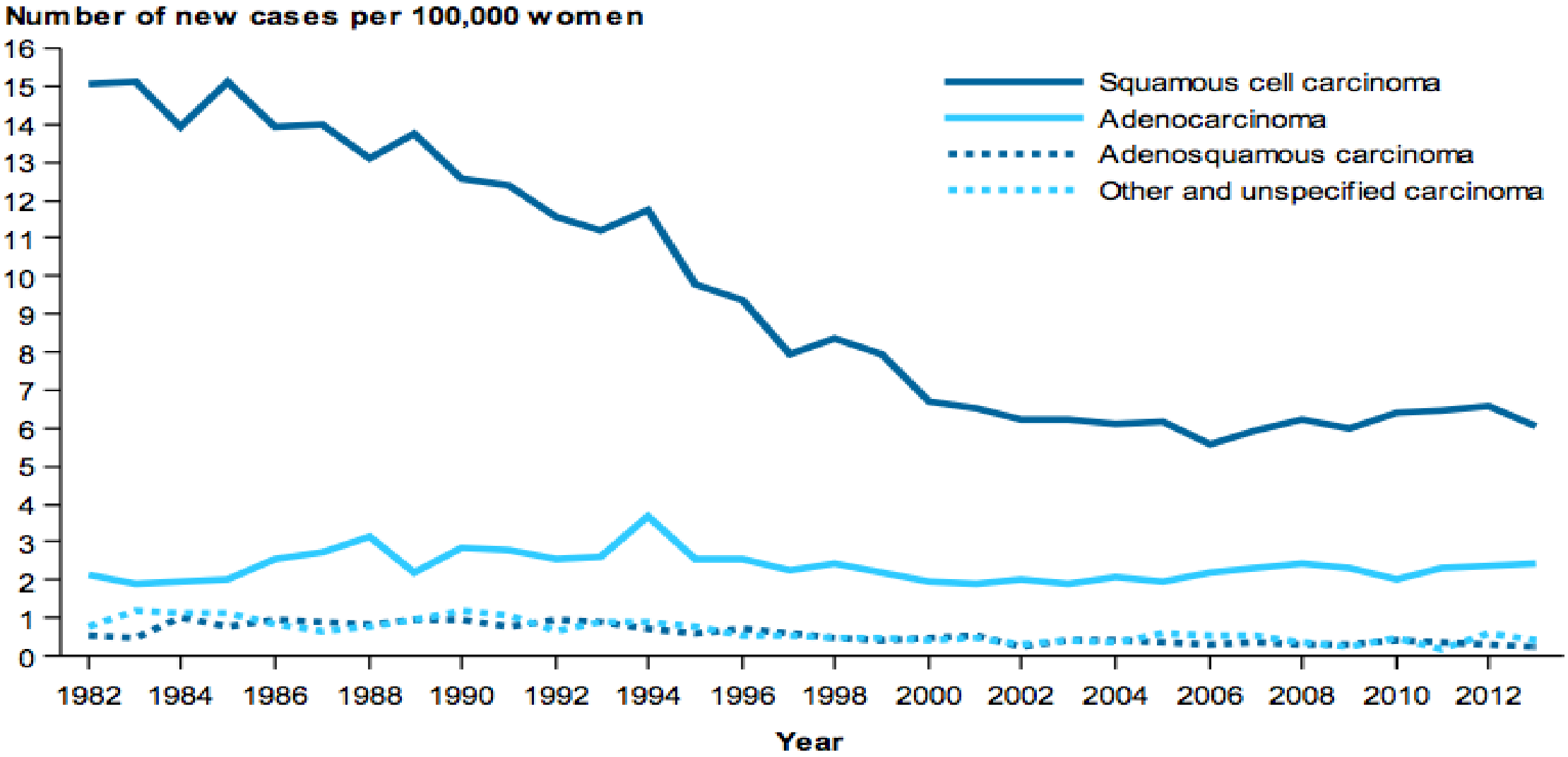
Trends in prevalence rates of high-grade histologically confirmed cervical abnormalities* diagnosed in Victorian women, by age group, 2000—2014



4v HPV = quadrivalent human papillomavirus. * Using Australian Institute of Health and Welfare indicator 4.2, which includes high-grade squamous abnormality, cervical intraepithelial neoplasia (CIN) grade 2, CIN grade 3 or CIN not otherwise specified; high-grade endocervical abnormality, endocervical dysplasia; and high-grade endocervical abnormality, adenocarcinoma in situ (http://www.aihw.gov.au/publication-detail/?id=60129550871). ◆

Key drivers to the Renewal

- Pap smear screening program has reached the limit of its efficacy
- NCSP has been successful in reducing the incidence of cervical cancer in Australia since its introduction in 1991
- Rate has plateaued since 2002 (9-10 new cases of cervical cancer per 100,000 women aged 20-69 years, AIHW)
- A more sensitive screening test than cytology is required



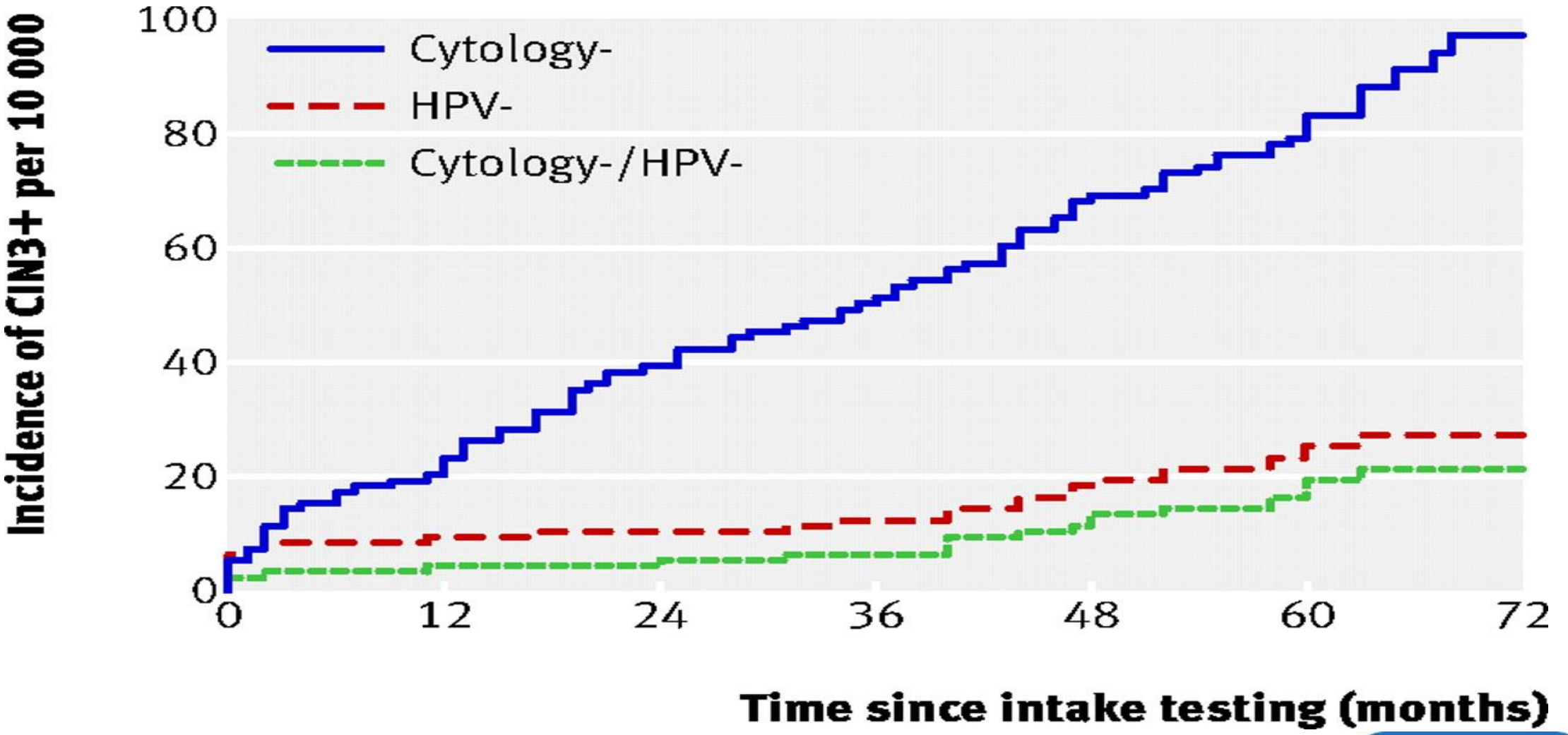
Source: AIHW Australian Cancer Database 2012. Data for this figure are available in Table A6.3.

Figure 4.3: Incidence of carcinoma of the cervix (squamous cell carcinoma, adenocarcinoma, adenosquamous carcinoma and other and unspecified carcinomas) in women aged 20–69, 1982 to 2013

Key drivers to the Renewal

- Primary HPV screening is a more sensitive test
- At least 20% - 30% reduction of cervical cancer and mortality
- Fewer screening tests in a lifetime

Fig 2 Kaplan-Meier plots of cumulative incidence rate for CIN3+ for women according to baseline test results in first 72 months of follow-up, excluding Denmark and Tübingen.



Joakim Dillner et al. BMJ 2008;337:bmj.a1754

Renewal NCSP

- Review the policy and operation of the NCSP commenced in 2011
- Report evaluation released and recommendations by MSAC in April 2014
- Interim Renewal Plan endorsed by AHMAC in September 2014
- Approval in 2015-16 Commonwealth Budget
- Implementation to start ~~1 May~~, delayed to 1 December 2017

Changes to the current NCSP

NCSP	Current	Renewal
Primary Screening Test	Pap (Conventional/LBC)	HPV NAT Partial Genotyping (HPV 16/18)
Reflex	N/A	LBC (manual or image-read)
Age	18 - 69 years	25 - 74 years
Screening interval	2 years	5 years (3 years if immunosuppressed)
Co-testing	Test of cure	Test of cure Symptomatic DES-exposed
Self-collection	No	Yes (Under or never-screened women)
Register	State-based	National

HPV Assays for Primary HPV nucleic acid testing (NAT)

- National Pathology Accreditation Advisory Council (NPAAC)
- *Requirements for Laboratories Reporting Tests for the National Cervical Screening Program (First Edition 2017)*
- Minimum requirements for HPV NAT and gynaecological cytology for cervical screening

Snapshot of NPAAC Requirements

4. Equipment
   - Must satisfy Meijer Criteria (sensitivity, specificity, reproducibility)
   - Must be validated for primary population based screening
   - Assay must contain a control to monitor inhibition and/or assay failure
   - Assay must contain a control for cellularity to detect inadequate or empty cervical samples
   - Self-collected specimens must be tested using a PCR test

Meijer Criteria (Meijer et al., *Int J Cancer*, 2009)

Meijer criteria as described in NPAAC Requirements, a HPV assay must show:

- Non-inferiority to a validated reference assay
- Clinical sensitivity for HSIL of ≥90% of the clinical sensitivity of the Hybrid Capture II (HC2) in women ≥25 years of age
- Clinical specificity for HSIL of ≥ 98% of HC2 in women ≥25 years of age
- Intra-laboratory reproducibility and inter-laboratory agreement with a lower confidence bound of 87%, with ≥500 samples tested and at least 30% HPV positive.

Meijer Criteria Publications – in chronological order

- Roche cobas® 4800 – Heideman et al., *J Clin Microbiol*, 2011
- Abbott RealTi*me* – Hesselink et al., *J Clin Microbiol*, 2013
- Hologic Aptima – Heideman et al., *J Clin Microbiol*, 2013
- BD Onclarity™ – Ejegod et al., *J Med Microbiol Diagn*, 2014
- Seegene Anyplex™ II – Jung et al., *Arch Pathol Lab Med*, 2016
- Cepheid GeneXpert® – Cuschieri et al, *J Clin Microbiol*, 2016
- Roche cobas® 6800 – Saville et al., *manuscript in preparation*

Roche cobas® 4800 HPV test

- DNA, real time PCR
- **Target:** L1 region
- **Partial Genotyping:** HPV16; HPV18; Other High Risk (HR) HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)
- 288 samples processed in 8 hours
- Internal cell control (β-globin)

Roche cobas® 6800 HPV test

- DNA, real time PCR
- **Target:** L1 region
- **Partial Genotyping:** HPV16; HPV18; Other High Risk (HR) HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)
- 384 samples processed in 8 hours
- Internal cell control (β-globin)

VCS Pathology

Abbott RealTi*me* High Risk HPV test

- DNA, real time PCR
- **Target:** L1 region
- **Partial Genotyping:** HPV16; HPV18; Other High Risk (HR) HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)
- 288 samples processed in 8 hours
- Internal cell control (β-globin)

Gen-Probe/Hologic APTIMA HPV Assay

- mRNA, TMA
- **Target:** E6/E7 region
- **Partial Genotyping:** HPV16; HPV18/45; nonHPV16/18/45 high risk HPV
- 270 samples processed in 8 hours (may be reduced with addition of cellularity control)
- Internal cell control (currently being tested)

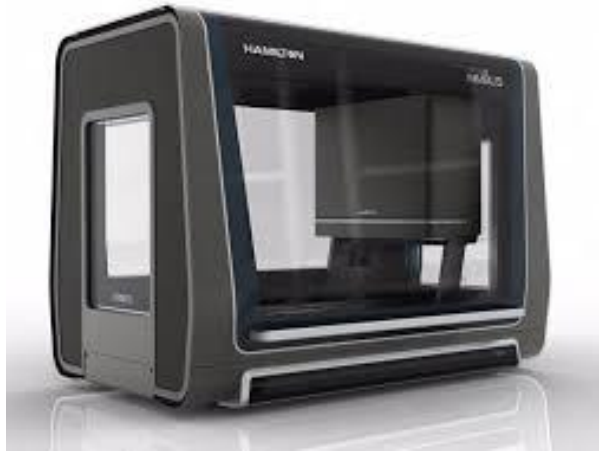
BD Onclarity™ HPV Assay

- DNA, real time PCR
- **Target:** E6/E7 region
- **Partial Genotyping:** HPV16; HPV18; HPV31; HPV45; HPV51; HPV52; HPV33, 58; HPV39, 35, 68; HPV56, 59, 66
- 90 samples processed in 8 hours
- Internal cell control (β-globin)

VCS Pathology

Seegene Anyplex™ II HPV HR test

- DNA, real time PCR
- **Target:** L1 region
- **Full HR Genotyping:** HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (optional 26, 53, 69, 73, and 82)
- 288 samples processed in 8 hours
- Internal cell control (β-globin)



Cepheid GeneXpert® HPV test

- DNA, real time PCR
- **Target:** E6/E7 region
- **Partial Genotyping:** HPV16; HPV18/45; HPV31/33/35/52/58; HPV51/59; HPV39/56/66/68
- 128 - 640 samples processed in 8 hours
- Internal cell control

HPV Diagnostic Tests – Sample Controls

- Roche cobas® 4800 – cellularity and inhibitory controls
- Roche cobas® 6800 – cellularity and inhibitory controls
- Abbott *R*ealTi*me* – cellularity and inhibitory controls
- Hologic Aptima – synthetic inhibition (process) control – also currently adding cellularity control
- BD Onclarity™ – cellularity and inhibitory controls
- Seegene Anyplex™ II – cellularity and inhibitory controls
- Cepheid GeneXpert® – cellularity and inhibitory controls

HPV assays for use with Renewed NCSP:

NPAAC Requirements

	NPAAC Requirements				
HPV Assay	Meijer Criteria	Screening	Controls		Self-Collection
			Cellularity	Inhibition	PCR-based
Roche cobas 4800/6800	✓	✓	✓	✓	✓
Abbott Realtime HPV	✓	✓	✓	✓	✓
Hologic Aptima	✓	✓	✓	✓	X
BD Onclarity	✓	✓	✓	✓	✓
Seegene Anyplex II	✓	✓	✓	✓	✓
Cepheid GeneXpert	✓	✓	✓	✓	✓

Changes to a cytology-based laboratory

- Specimen processing from slides to LB vials, ThinPrep (TP) or SurePath (SP)
  - TP PreservCyt – methanol based, storage of samples (up to 6 weeks from 4-37^{0}C)
  - SP Preservative Fluid – ethanol based with formalin, storage of samples (up to 4 weeks at ambient temperature, 6 months at 4^{0}C)
- The samples can be used for other molecular testing such as Chlamydia, Gonorrhoea, Trichomonas vaginalis, Candida, etc.
- However, samples will need to be first used for cervical screening testing (HPV and LBC) before any other molecular testing.

Issues to consider

- Pre-analytical (Collection/Transport/Education)
- Analytical
  - Specimen Triaging for testing (IT)
  - Sharing of specimens between the Molecular and Cytology Laboratories
- Post-analytical
  - Storage of samples – 1 month post processing (if cytology is performed)
  - Disposal of samples
  - Combined reporting (IT)
  - Quality Assurance (IT)– HPV positivity from batches of greater than 2000 tests

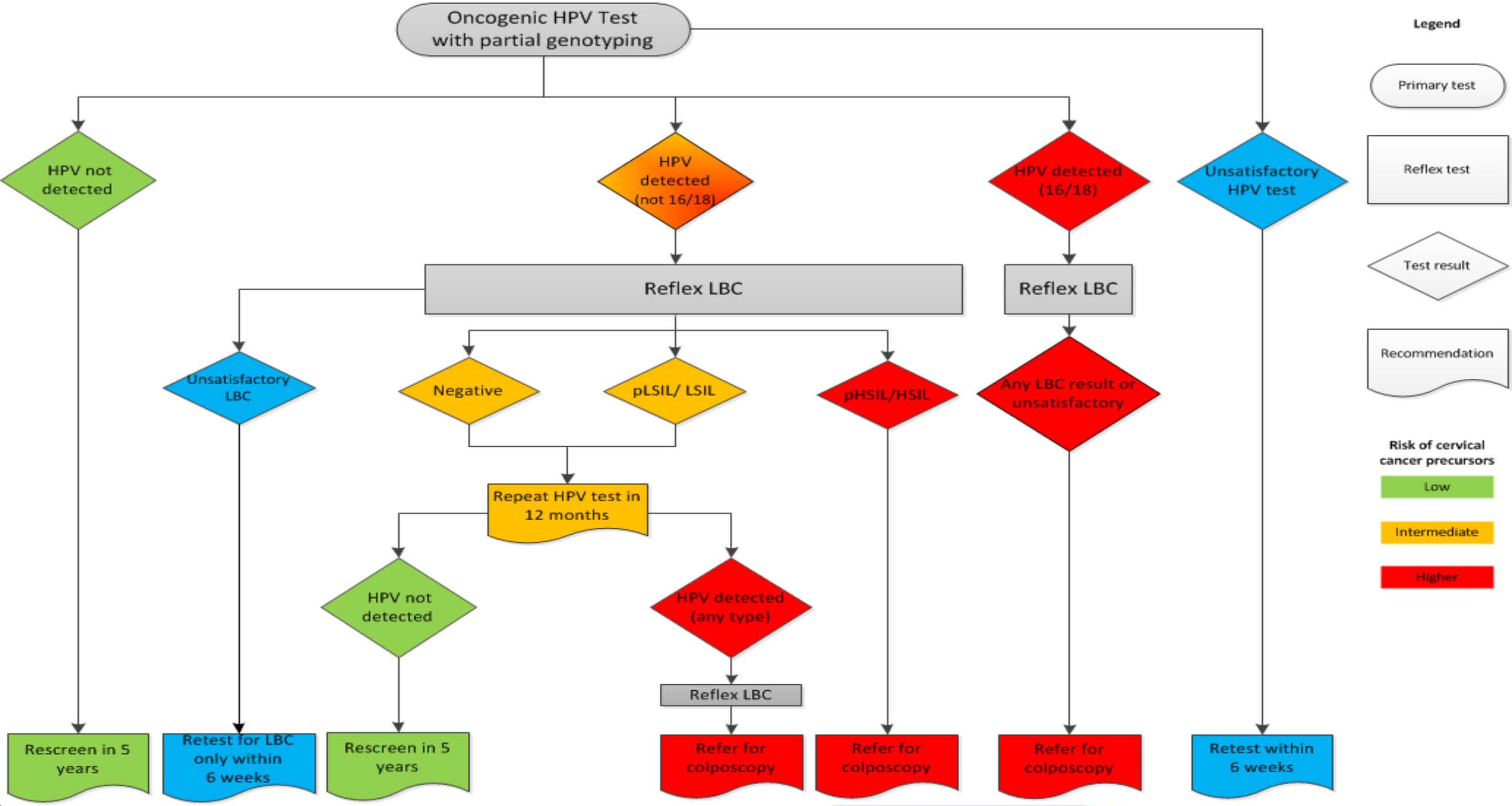
Cytologist’s perspective

- Reduction in cytology workload
- Training for LBC (TP or SP)
- Internal Quality Assurance
- Recruitment and retention of skilled staff

Cytologist's perspective

- NPAAC requirements
  - CTASC
  - 20 abnormal viewed per month
- Except for co-testing, all cases reflexed to cytology are HPV positive and are therefore, diagnostic
- Not all positive HPV tests will be abnormal on cytology
- Approximately 40% - 60% are abnormal (LSIL and above)
- Composite reporting combining both HPV and cytology test results with recommendations and risk categories

Cervical screening pathway



Histology Laboratory

- A move to a more sensitive test will initially increase the colposcopy rate by moving forward cases that are detected earlier and at a higher risk of HG disease progression
- Modelled evaluation by Canfell and colleagues predicted an increase in colposcopy rate in the unvaccinated cohort but not in the vaccinated cohort
- Initial increase in cervical biopsies for the histology laboratory but will taper off as program matures

Summary

- Renewed NCSP commencing 1 December
- Changes to NCSP
- Seven HPV assays are likely to be acceptable (regulatory/workflow)
- Significant changes to the cytology-based laboratory
- Initial increase workload expected in histology
- Predicted outcome of a 20-30% reduction in cervical cancer and mortality

Acknowledgements

Principal Investigators of Compass Trial

- Assoc. Prof. Marion Saville (Executive Director, VCS Ltd)
- Prof. Karen Canfell (Cancer Council NSW)

VCS Ltd

- Dr David Hawkes (Dir. Molecular Biology)
- Diana Stockman (Supervisor Cytology and Histology)

For Compass trial go to http://www.compasstrial.org.au/