Reporting self-collected samples for HPV testing: what are the issues in the lab

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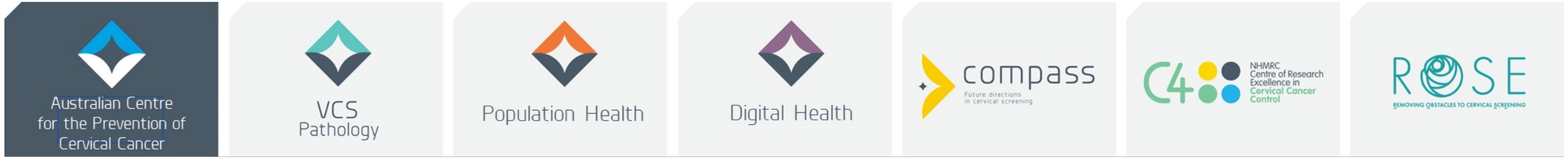
Conflict of Interest Statement

Abbott, AusDiagnostics, BD, Cepheid, Copan, Hologic, Microbiologics, MicroBix, NRL, Qiagen, Rovers, Roche, and Seegene have supplied materials for the purposes of research studies at VCS Pathology

DH has received travel funding to attend conferences and meetings from Roche, Abbott and Seegene but has had no personal gain from any diagnostics manufacturer.

ACPCC receives funding from the NHMRC as part of the Centre for Research Excellence in Cervical Cancer Control

DH is an Investigator on the Compass Trial, and the SCoPE and SCoPE2 Studies



Self-Collection for HPV-based cervical screening

- How we got here – Australian National Cervical Screening Program
- Where we started
- How we are going
- Issues and Questions

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VCS Pathology

Australian HPV Reference Laboratory

- Cervical screening focused laboratory since 1965
- Australian HPV Reference Laboratory
- Undertaken >700,000 HPV tests as part of the renewed Australian National Cervical Screening Program which began on December 1, 2017
- First laboratory in Australia to be accredited for HPV-based cervical screening on self-collected vaginal specimens
- ACPCC involved in projects in Australia, New Zealand, Papua New Guinea, Vanuatu, Fiji, Malaysia and India
- Multiple platform laboratory which has undertaken HPV testing using;

Abbott Alinity m
Cepheid Xpert
Roche cobas 4800

Abbott Realtime HPV
Hologic Aptima
Seegene STARlet/cfx

AusDiagnostics (*soon*)
Qiagen NeuMoDx

BD COR
Roche cobas 4800

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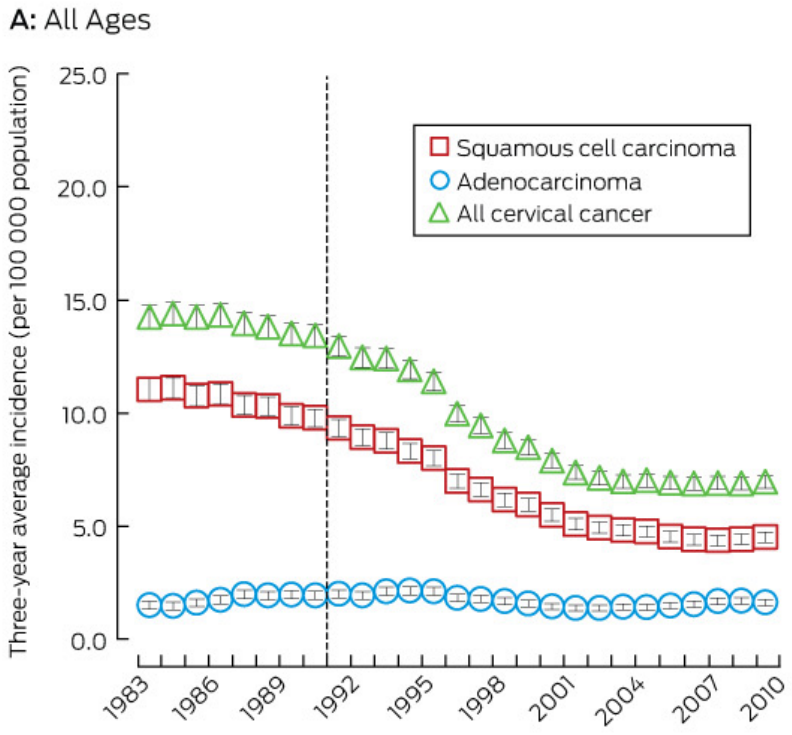
Cervical Cancer: pre and post the National Cervical Screening Program (NCSP)

- National Cervical Screening Program (NCSP) introduced in 1991
- NCSP is based around 2 yearly Pap (cytology) screening
- Drop in cervical cancers – overwhelmingly attributed to the drop in squamous cell carcinomas
- No change in rate of adenocarcinomas



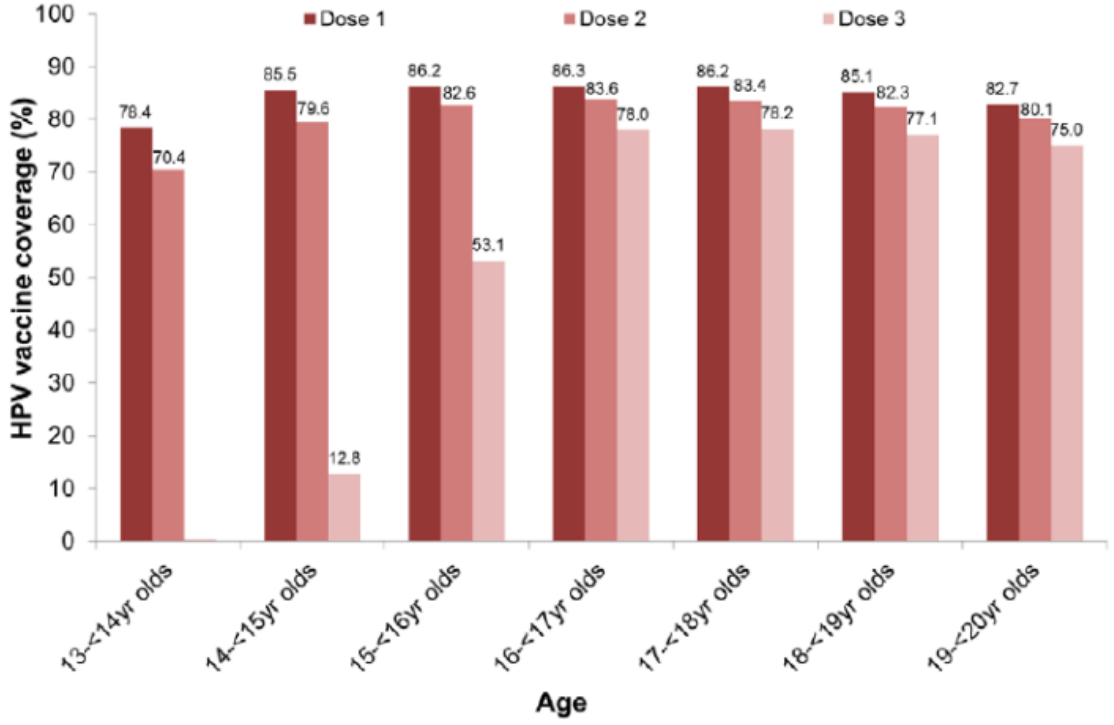
Smith and Canfell, *MJA*, 2016

HPV vaccination program in Australia

National HPV Vaccination Program

- Began in April 2007
- 3 dose Gardasil
- Catch-up program for females up to 26 years of age (born July 1, 1980 or later) until December 2009
- Gender neutral program from 2013
- 2 dose Gardasil9 from 2018

Figure 8: Cumulative coverage (%) of HPV vaccine in Australian females by dose number and, age/birth cohort* for vaccination encounters recorded up to 31 December 2019



NCIRS Report, 2021

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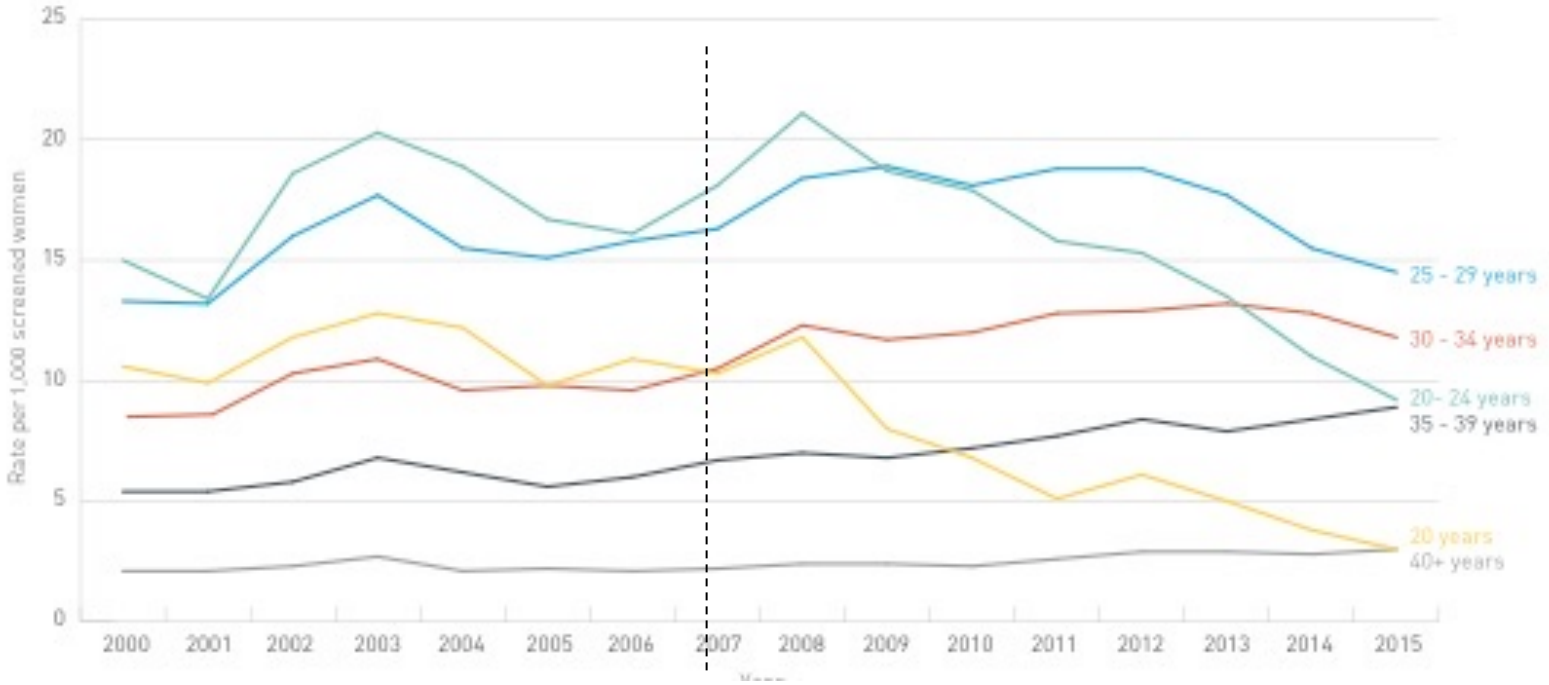
Cervical Cancer: changes in the NCSP

- Reductions in high-grade cervical abnormalities associated with HPV vaccination

FIGURE 5.2 Trends in high-grade cervical abnormalities (histologically-confirmed) by age, 2000-2015, as recorded on the VCCR.



VCSR Statistical Report, 2015

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Australian National Cervical Screening Program

NCSP Quality Framework - NPAAC Requirements for Reporting of Tests for the NCSP, 2nd Edition 2019;

- Assays must meet the ‘adapted’ Meijer Criteria
- Assays must have a cellularity and an inhibition control
- HPV positivity and invalid rates must be monitored
- Non-manufacturer QC controls are required to be run on each day that HPV testing for cervical screening is undertaken
- Only PCR-based assays can be used for self-collected specimens

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Assays available for use in the Australian NCSP

HPV Assay	NPAAC Clinical Criteria	Screening	Controls		Self-Collection	Throughput in 8 hours
			Cellularity	Inhibition	(PCR-based)	
Roche cobas 4800	✓	✓	✓	✓	✓+	288
Abbott Realtime	✓	✓	✓	✓	✓	288
Hologic Aptima	✓	✓	✓*	✓	✗	130
BD Onclarity (COR)	✓	✓	✓	✓	✓#	210
Seegene Anyplex II	✓	✓	✓	✓	✓	288
Cepheid Xpert	✓	✓	✓	✓	✓	7 - 560
Roche cobas (6800)	✓	✓	✓	✓	✓+	384
Abbott Alinity m	✓	✓	✓	✓	✓	300
Qiagen NeuMoDx (288)	✓	✓	✓	✓	✓	384

*RUO cellularity control run separately #TGA approved for self-collection +submitted to TGA for self-collection

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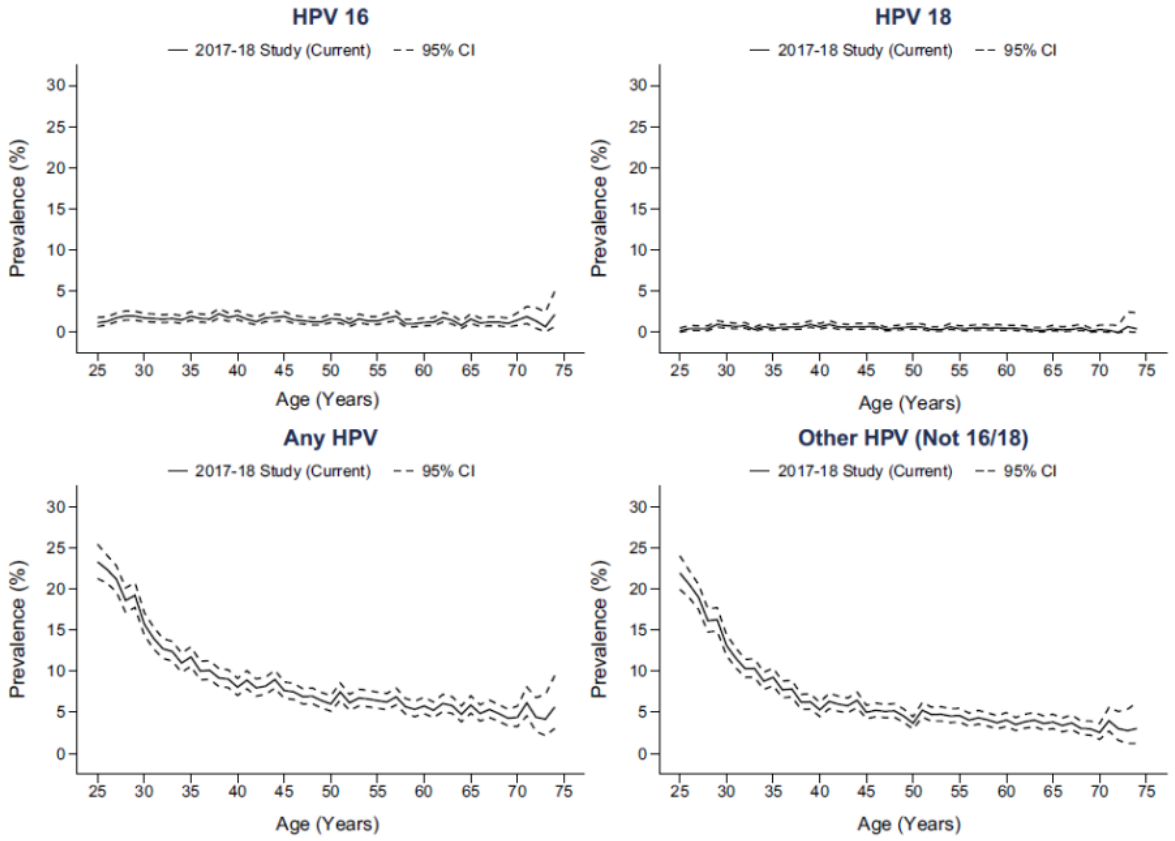
HPV positivity rates in the NCSP

Data from the NCSP (December 2017 – June 2018) shows

- HPV positivity is age related
- HPV16/18 do not show a similar pattern, likely as a result of a highly successful vaccination campaign
- Participants aged 37 or under would have been eligible for HPV vaccination during the time period of this study



Brotherton et al, *Vaccine*, 2019

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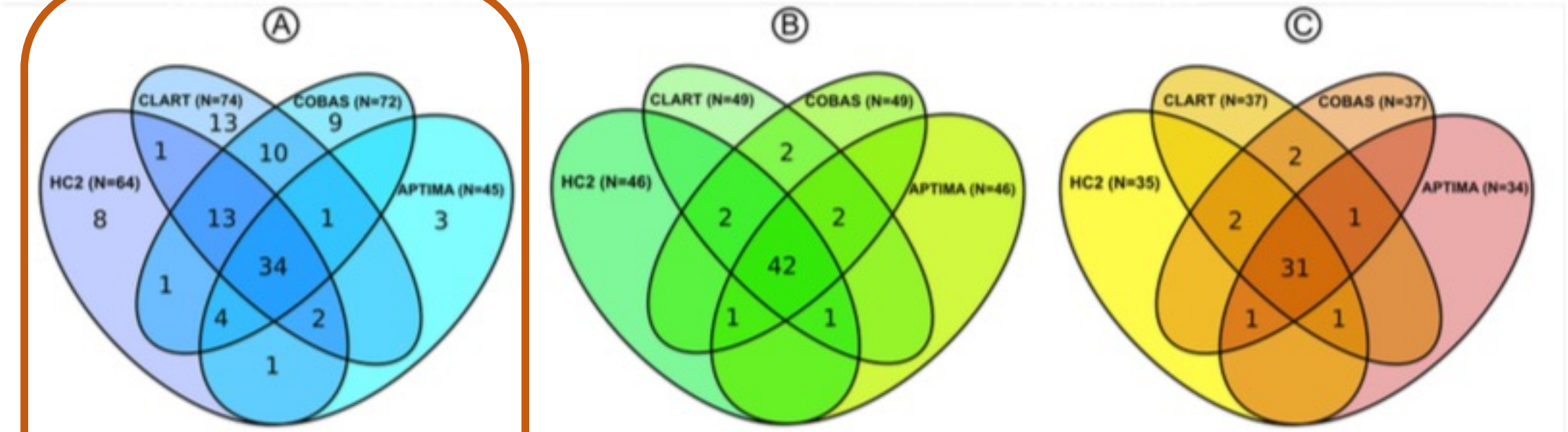
HPV is a *risk stratification* rather than diagnostic test

There is some variability in HPV positivity in normal histology



<CIN1 (n = 100)
34% positive for
all four assays

≥CIN2 (n =50)
84% positive for
all four assays

≥CIN3 (n = 38)
82% positive for
all four assays

Rebolj et al., *J Clin Microbiol*, 2016

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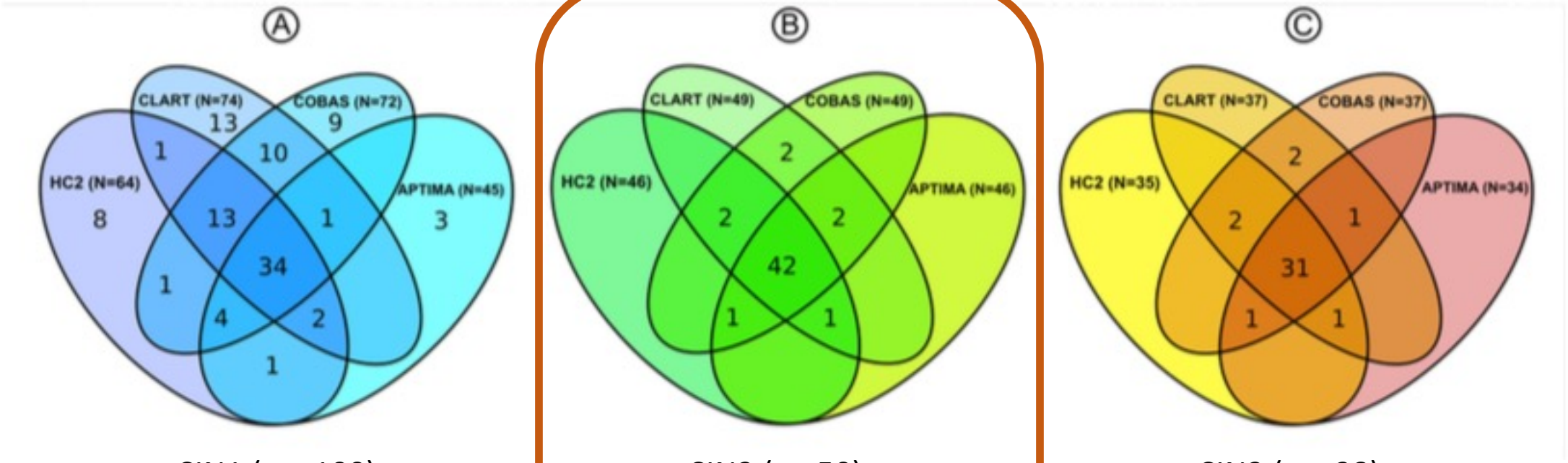
HPV is a *risk stratification* rather than diagnostic test

There is much less variability in HPV positivity with histologically confirmed disease



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Quality Assurance in the laboratory

Sources of variability for which controls are used monitor quality

- Different instruments give different results
  - External QC controls run on each machine being used
- Different reagents (or QC controls) can give different results
  - Good record keeping so that all reagents used for producing each result can be traced
- All reagents and processes are possible sources of contamination
  - Run ‘empty’ controls to monitor for HPV or cellular contamination
- Monitor HPV positivity to detect changes to be able to investigate whether they are assay related

HPV QC in the laboratory

Commercial QC products

- Acrometrix
  - RUO; HPV16, HPV18, HPV68, or Negative; material in PreservCyt-type solution
- Microbiologics
  - IVD; HPV16/HPV18/HPV45/HPV31/HPV68, or Negative; lyophilized pellet
- NRL
  - IVD; HPV16/HPV18/HPV68, or Negative; material in PreservCyt-type solution
- MicroBix
  - IVD: HPV16, HPV18, HPV45 or Negative; material in PreservCyt-type solution
  - *Coming Soon* RUO: FLOQSwab HPV16, HPV18, HPV45, or Negative; dry swab
- SeraCare
  - RUO; HPV16, HPV18, HPV51, or Negative; material in PreservCyt-type solution

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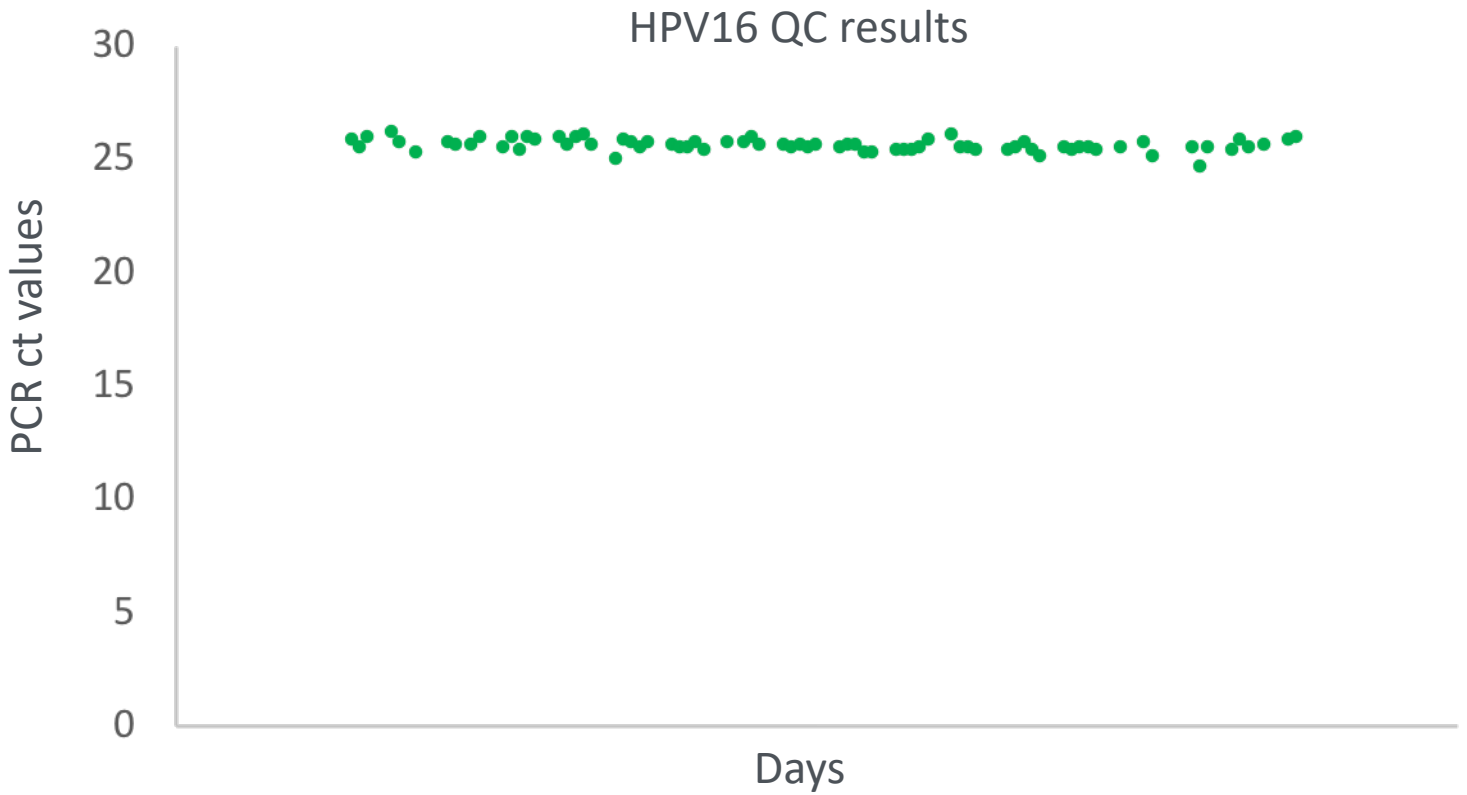
Quality for HPV NAAT in the NCSP

Non-manufacturer QC material must be run on every day that HPV NAT is undertaken



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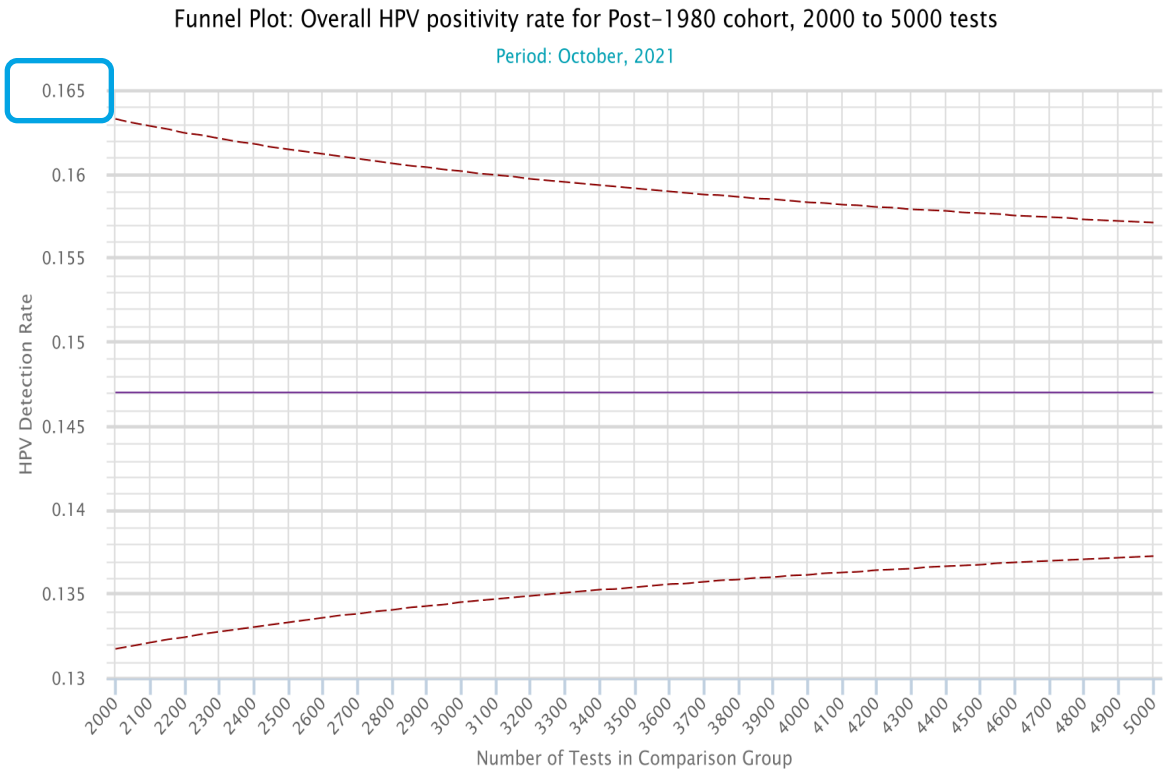
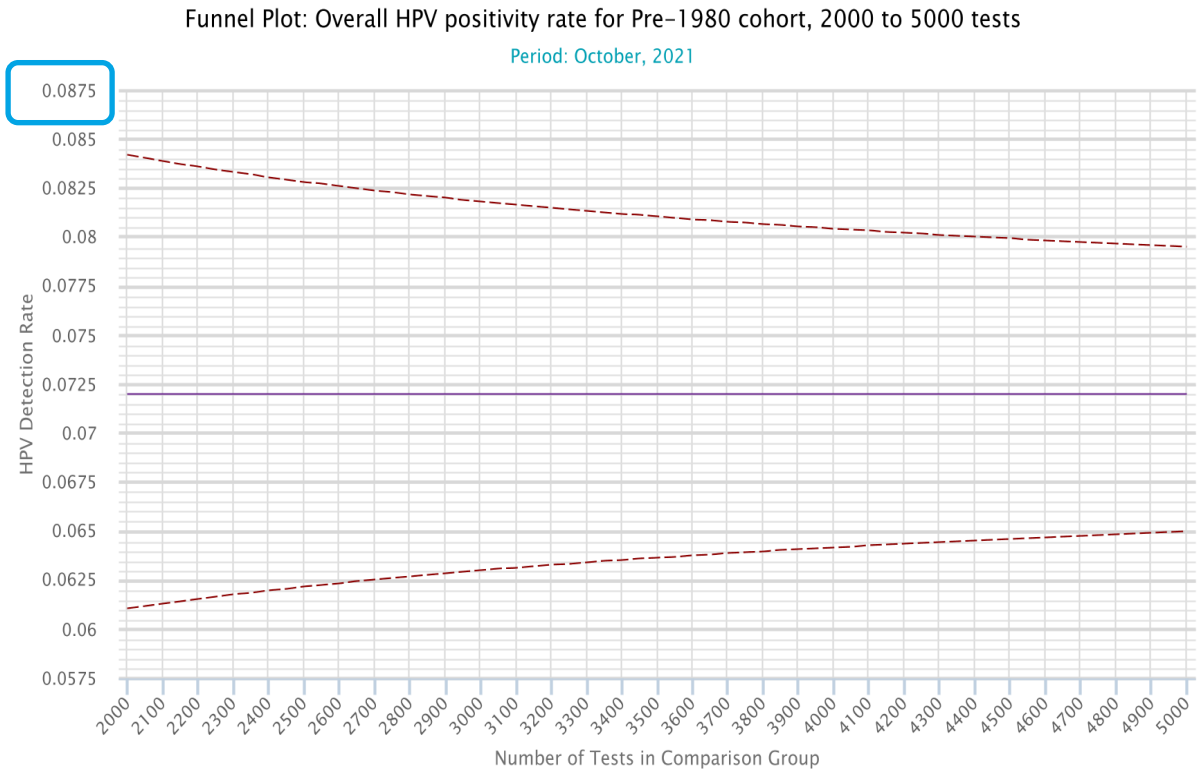
Quality for HPV NAAT in the NCSP

- Non-manufacturer QC material must be run on every day that HPV NAAT is undertaken
- HPV positivity must be monitored
- Retrospective analysis of previous screening episodes for histologically confirmed HSIL
- Must be enrolled in an external Quality Assurance Program

HPV positivity in the Australian NCSP





Australian NCSR , 2021

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Self-collection for HPV-based screening in the Australian NCSP

From December 1, 2017

In the Australian National Cervical Screening Program self-collection is an alternative pathway that can only be accessed by those that meet specific criteria;

- Must be over 30 years of age
- Must be more than two years overdue for screening
- Must have refused a practitioner collection (specula examination)
- Must be collected within a clinical setting

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Self-collection for HPV-based screening in the Australian NCSP

From December 1, 2017

In the Australian National Cervical Screening Program self-collection is an alternative pathway that can only be accessed by those that meet specific criteria;

- Since 2018 >8800 under- or never-screened women have accessed self collection
- VCS Pathology was the first accredited laboratory in Australia
  - In-house validation required as no PCR assays have manufacturer validated self-collection*
- Copan FLOQSwab 552C used for collection and transport

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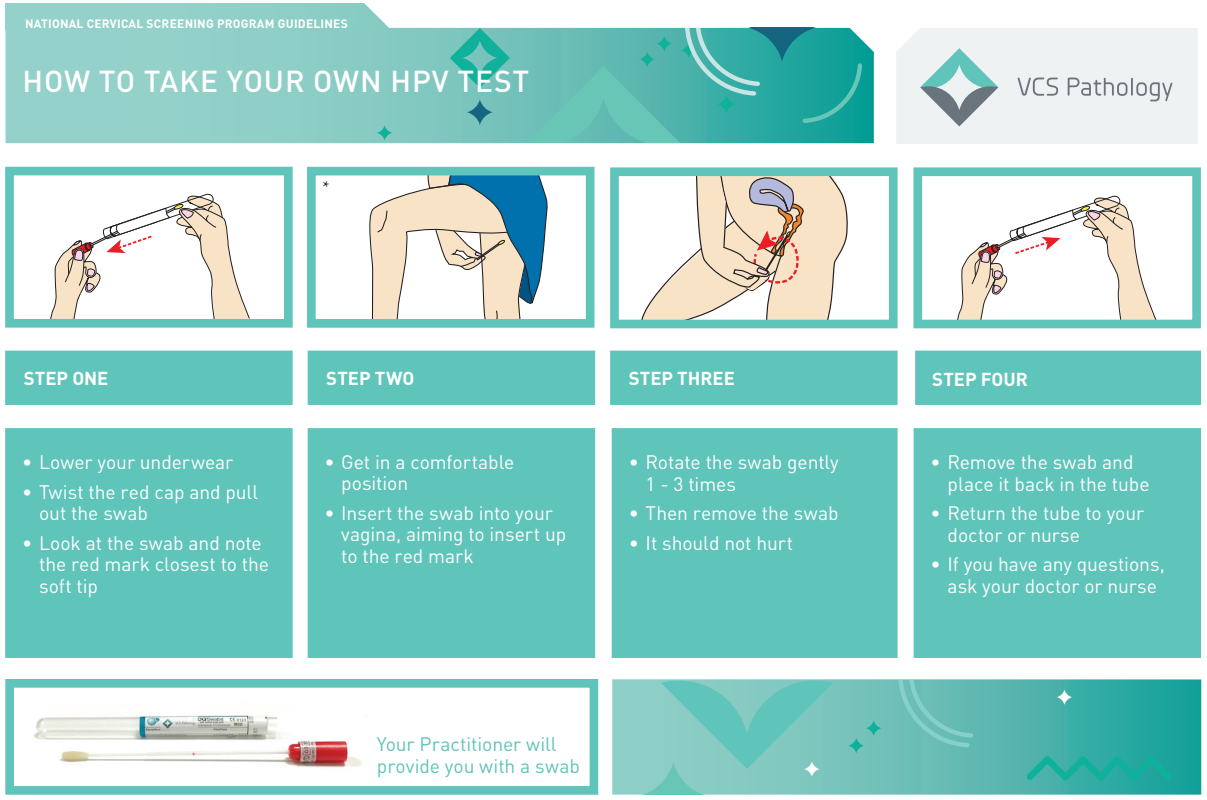

https://www.vcs.org.au/pathology/hpv-self-collection/

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Self-collection Devices

There are many self-collection devices available,

some include

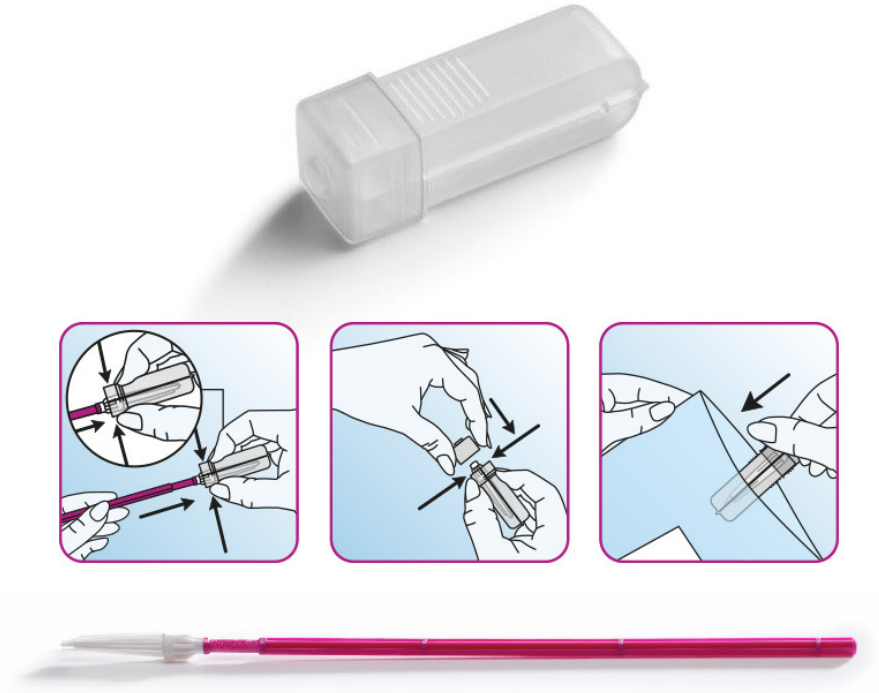
- Copan FLOQSwab
  - 552C/552C.80 – 552C.80 is validated for self-collection (including claim for Roche assays)
  - 5E087N – validated for the BD Onclarity Assay
- Rovers

Self-collection Devices

There are many self-collection devices available,

some include

- Copan FLOQSwab
- Rovers
  - Evalyn Brush – used in the Netherlands and Denmark
  - Viba-brush – simpler, cheaper version of Evalyn



Clinical validation of self-collection

- PCR-based assays
  - Not less sensitive than clinician collected samples (95% CI 0.87 – 1.04 depending on collection device)
  - Marginally less specific , depending on the collection device

Table 2 | Variation in relative sensitivity and specificity of high-risk human papillomavirus (hrHPV) assays on self samples versus clinician samples, by self sampling device and storage medium

Covariate	No of studies	Relative sensitivity (95% CI)	Relative specificity (95% CI)
Self sampling device			
hrHPV assay based on signal amplification			
Brush	13	0.84 (0.78 to 0.90)*	0.93 (0.91 to 0.96)*
Swab	7	0.85 (0.78 to 0.91)*	0.93 (0.90 to 0.95)*
Lavage†	2	0.84 (0.69 to 1.04)	0.74 (0.55 to 0.98)*
Tampon	1	0.86 (0.78 to 0.96)*	1.02 (1.00 to 1.03)
hrHPV assay based on polymerase chain reaction			
Brush	12	0.98 (0.95 to 1.02)	0.95 (0.91 to 0.99)*
Swab	4	0.98 (0.93 to 1.03)	0.93 (0.89 to 0.98)*
Lavage†	4	0.95 (0.87 to 1.04)	1.09 (0.91 to 1.30)
Tampon	0	NA	NA
Storage medium			
hrHPV assay based on signal amplification			
Cell preserving†	3	0.84 (0.78 to 0.90)*	0.93 (0.91 to 0.96)*
Virological†	15	0.86 (0.81 to 0.91)*	0.95 (0.92 to 0.98)*
Dry samples	0	NA	NA
Other	1	0.90 (0.71 to 1.13)	0.92 (0.71 to 1.21)
hrHPV assay based on polymerase chain reaction			
Cell preserving	6	1.00 (0.96 to 1.04)	0.92 (0.88 to 0.97)*
Virological†	3	0.97 (0.91 to 1.04)	0.94 (0.89 to 0.99)*
Dry samples†	7	0.96 (0.90 to 1.02)	1.01 (0.94 to 1.10)
Other	1	0.95 (0.80 to 1.13)	1.05 (0.69 to 1.58)

Relative values were computed by using a bivariate normal model, separating studies using a hrHPV assay based on signal amplification or a hrHPV assay based on polymerase chain reaction. Pooling was performed using a bivariate normal model.
NA=not available.
*Relative accuracy statistically significantly different from unity.
†When the bivariate model containing covariates did not fit or when the number of studies <4, a separate pooling of the relative sensitivity and relative specificity using a model for ratios of proportions was run.

Arbyn et al, *BMJ*, 2018

Clinical validation at VCS Pathology: SCoPE

- PCR-based assays
  - Not statistically less sensitive than practitioner- collected sample (when PC was the reference standard)
  - Marginally less specific but this is intrinsic to the study design (PC as standard)

HPV assay type	Oncogenic HPV type	Sensitivity		Specificity	
		%	(95% CI)	%	(95% CI)
cobas 4800	HPV 16	93.8	(79.2-99.2)	96.5	(93.5-98.4)
	HPV 18	100	(47.8-100)	99.0	(97.0-99.8)
	Other	94.6	(89.6-97.6)	73.1	(65.1-80.1)
	Any HPV	94.4	(89.7-97.4)	68.7	(60.0-76.5)
cobas	HPV 16	87.2	(72.6-95.7)	97.1	(94.2-98.8)
	HPV 18	100	(66.4-100)	97.8	(95.2-99.2)
	Other	94.6	(89.6-97.6)	76.9	(69.2-83.6)
	Any HPV	95.2	(90.7-97.9)	71.4	(62.7-79.1)
Onclarity	HPV 16	83.3	(62.6-95.3)	97.8	(95.2-99.2)
	HPV 18	100	(39.8-100)	99.3	(97.5-99.9)
	Other	86.1	(78.9-91.5)	79.0	(72.1-85.0)
	Any HPV	86.5	(79.8-91.7)	76.1	(68.6-82.6)
GeneXpert	HPV 16	82.1	(63.1-93.9)	97.7	(95.1-99.2)
	HPV 18/45	82.4	(56.6-96.2)	97.5	(94.8-99.0)
	Other	93.3	(87.3-97.1)	79.0	(72.1-84.8)
	Any HPV	93.0	(87.5-96.6)	73.6	(65.8-80.5)
Anyplex II	HPV 16	84.9	(68.1-94.9)	98.5	(96.1-99.6)
	HPV 18	100	(59.0-100)	99.3	(97.5-99.9)
	Other	91.2	(85.7-95.1)	81.0	(73.4-87.2)
	Any HPV	92.5	(87.5-95.9)	78.9	(70.6-85.7)
Abbott	HPV 16	88.5	(69.9-97.6)	98.9	(96.8-99.8)
	HPV 18	80.0	(28.4-99.5)	99.3	(97.5-99.9)
	Other	86.0	(79.1-91.4)	82.5	(75.7-88.1)
	Any HPV	88.7	(82.5-93.3)	80.1	(72.7-86.3)

Saville et al, *JCV*, 2020

Clinical validation at VCS Pathology: SCoPE

- In this study the self-collected sample (‘vaginal specimen’) was taken prior to the practitioner collected sample (‘cervical specimen’)
- It appears that the first sample to be collected is more sensitive for oncogenic HPV detection which leads to lower concordance

Virus and vaginal specimen type	No. of cervical specimens		
	Positive	Negative	Total
HPV-16			
Positive	33	4	37
Negative	2	966	968
Total	35	970	1,005
HPV-18/45			
Positive	13	12	25
Negative	3	977	980
Total	16	989	1,005
Other hrHPVs			
Positive	82	48	130
Negative	8	867	875
Total	90	915	1,005
All hrHPVs			
Positive	112	54	166
Negative	12	827	839
Total	124	881	1,005

Toliman et al, *J Clin Microbiol*, 2016

Clinical validation at VCS Pathology: SCoPE2

Study Design

- Recruitment at the Royal Women's Hospital Dysplasia Clinic, Melbourne
- Three specimens collected
  - Self-collection: Copan 552C.80 Swab (Dry)
  - Self-collection: Rovers Via-brush (Dry)
  - Practitioner-collection: usual device (e.g. Rovers Cervex-brush) in ThinPrep vial
  - Self-collected specimens stored for 7 days at room temperature before resuspension in 5 ml Copan MSwab media (#6E067N)
- Any histological outcomes for up to six month after recruitment linked to each case
- 400 participants to be recruited – this will be the sensitivity arm of a VALHUDES validation

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Self-collection for HPV-based screening in the Australian NCSP

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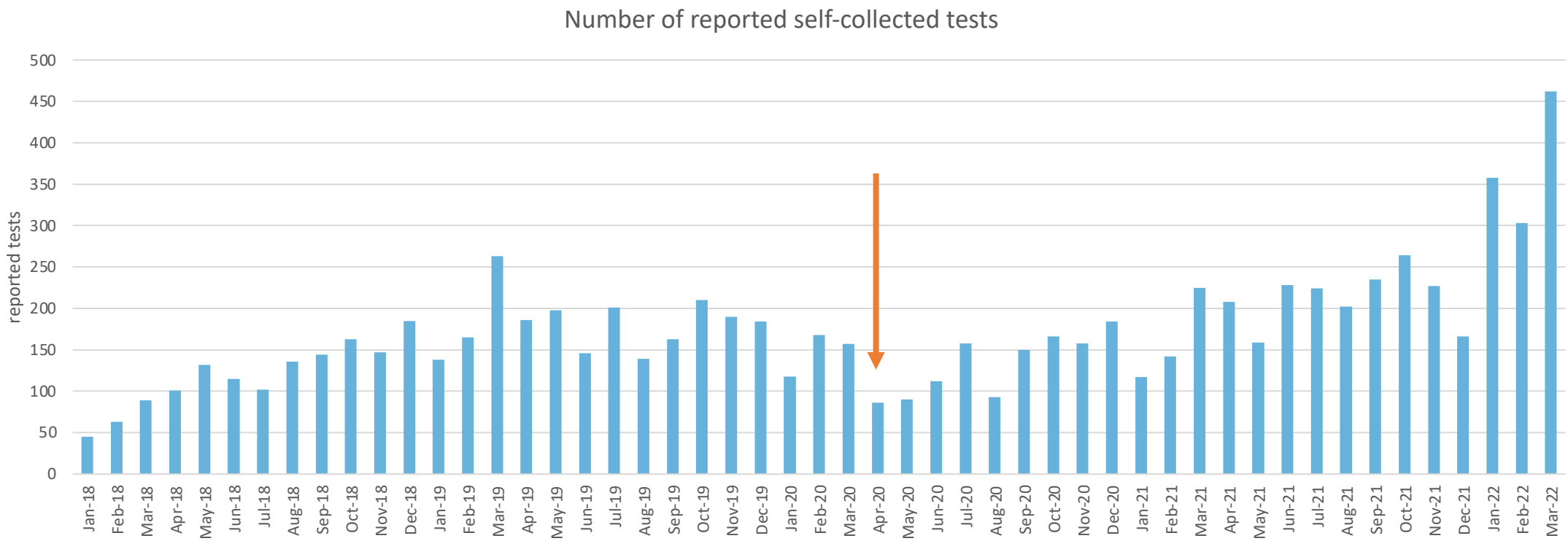

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Self-collection for HPV-based screening in the Australian NCSP

From July 1, 2022

In the Australian National Cervical Screening Program self-collection will be universally available to anyone with a cervix undergoing cervical screening;

- Must be over 24 years and 9 months of age
- Must be due for screening (e.g.,> 4 years 9 months since negative screening result)
- Must not require co-test
- Must be collected within a clinical setting

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Self-collection for HPV-based screening in the Australian NCSP

From July 1, 2022

In the Australian National Cervical Screening Program self-collection will be universally available to anyone with a cervix undergoing cervical screening;

- Estimate ~16,000 screening tests per week
- Begin of second HPV-based screening cycle from December 1, 2022
- Estimate ~45,000 screening tests per week
- Current self-collection protocol is manual elution – that's a big problem with higher volumes

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Pre-analytic for self-collection?

Current pre-analytics for HPV-based screening;

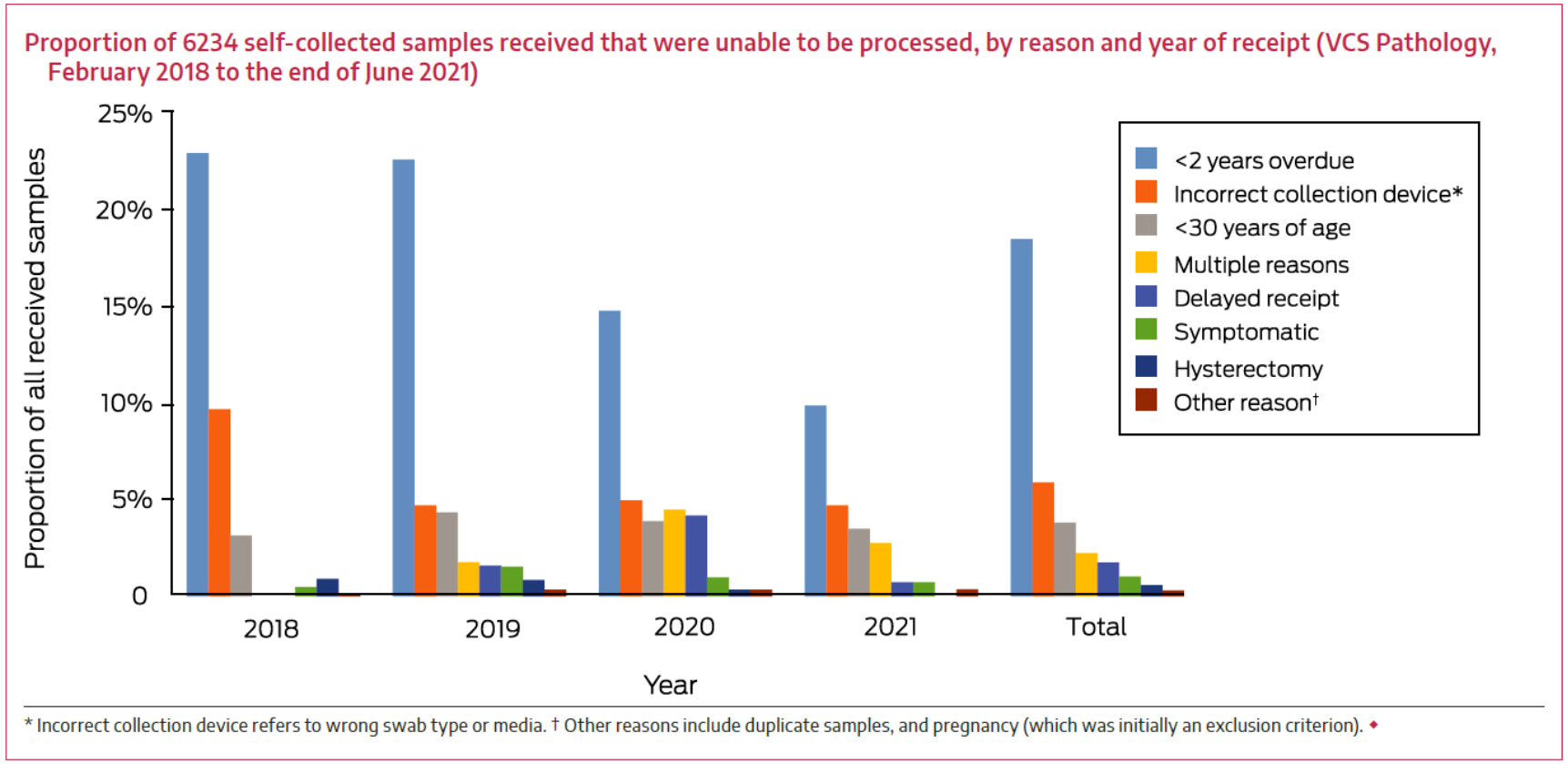
- Abbott
  - Abbott MP – Thinprep/SurePath; 228/8 hours
- BD
  - PX module of BD COR – ThinPrep/SurePath, self-collection swab in diluent tube; ~300/8 hours
- Roche
  - p480 – ThinPrep/SurePath(with heat step); ~500 h ThinPrep (less for SurePath)/8 hours
  - Prime – Large high volume pre-analytic (not yet available)
- Copan Universe
  - FLOQSwab in Mswab media; ~700/ 8 hours
  - ThinPrep/SurePath capacity to be added ~Q3, 2022

Self-collection for HPV-based screening in the Australian NCSP

Why we reject self-collected specimens for testing;

- Not under- or never-screened
- Incorrect collection device
- Under 30 years of age
- Received beyond known specimen stability
- Not a screening test, e.g., symptomatic requiring co-test

Self-collection for HPV-based screening in the Australian NCSP



Brotherton, Hawkes and Saville, *MJA*, 2022

Australian Centre for the Prevention of Cervical Cancer

VCS Pathology

Population Health

Digital Health

compass Future directions in cervical screening

C4 NHMRC Centre of Research Excellence in Cervical Cancer Control

ROSE Removing Obstacles to Cervical Screening

HPV-based screening: LIMS

Issues to consider for LIMS

- Pathway for self-collection is different; no reflex cytology but matching LBC episode for a combined result and risk status/recommendation
- Instrument interfaces, potentially including stand alone pre-analytic instruments
- Capture as much genotype data as possible in the LIMS
- Try and build in the ability to modify the pathways

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Acknowledgements

ACPCC

Professor Marion Saville
Professor Julia Brotherton
Grace Tan
Dr Mark Hiu Tat Chan
Dr Saugata Choudhury

Molecular Microbiology Laboratory

Marco Ho Ting Keung
Joanne Romano
Ivy Nguyen
Khurram Karim
Daisy Gurung
Stephanie Fabiano
Alaiza Gacutan
and all of our lab technicians