SULLIVAN NICOLAIDES PATHOLOGY

Cervical Screening –

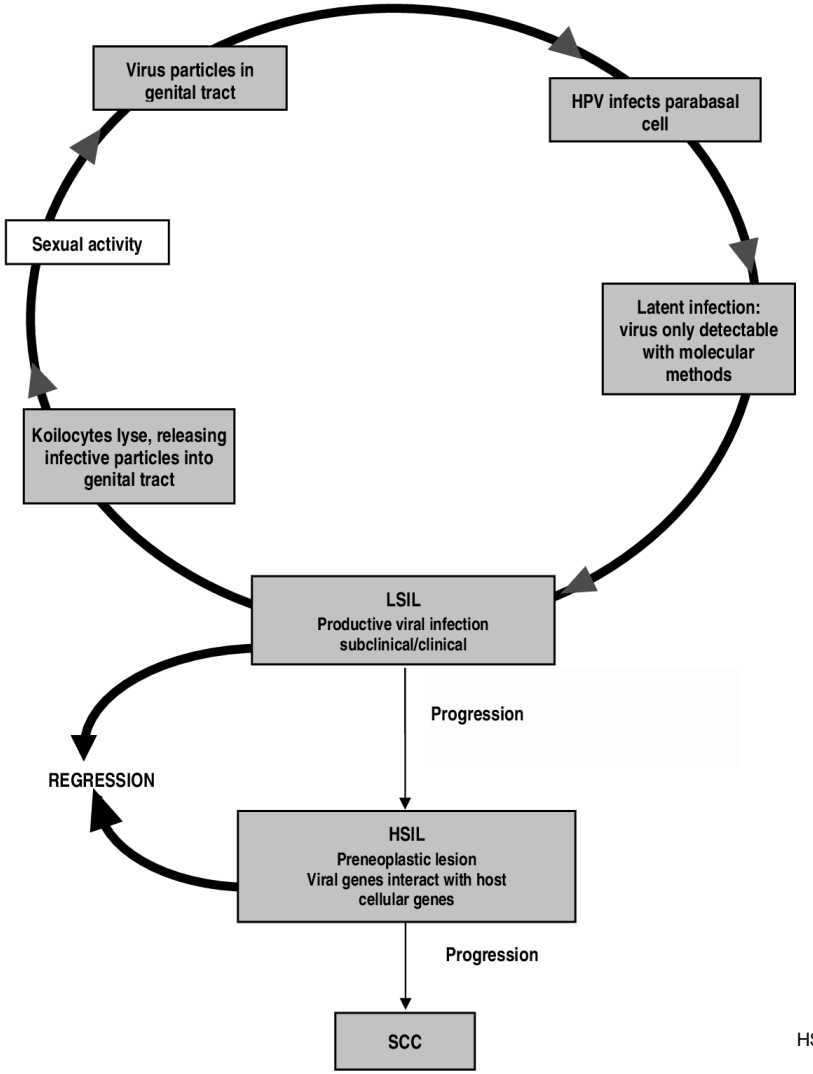
A Pathologist's Perspective

Elimination of cervical cancer

CST self-collects, latest NCSP updates, CINtec® *PLUS*, HPV-negative cancers

Dr Peta Fairweather

Natural history of HPV infection and cervical cancer precursors



LSIL = low-grade squamous intraepithelial lesion; HPV = human papillomavirus; HSIL = high-grade squamous intraepithelial lesion; SCC = squamous cell carcinoma.

Connelly, IB. and Frazer, IH. *National Health and Medical Research Council* 2005.

Global elimination of cervical cancer

World Health Organization **Cervical Cancer Elimination Initiative**

- In May 2018, the WHO Director-General announced a global call for action to eliminate cervical cancer, underscoring renewed political will to make elimination a reality and calling for all stakeholders to unite behind this common goal.
- In August 2020, the World Health Assembly adopted the Global Strategy for cervical cancer elimination.
- Campaign launch November 2020

- Australia is on track to be the first country in the world to eliminate cervical cancer – 2035.

The Global Effort to Eliminate Cervical Cancer

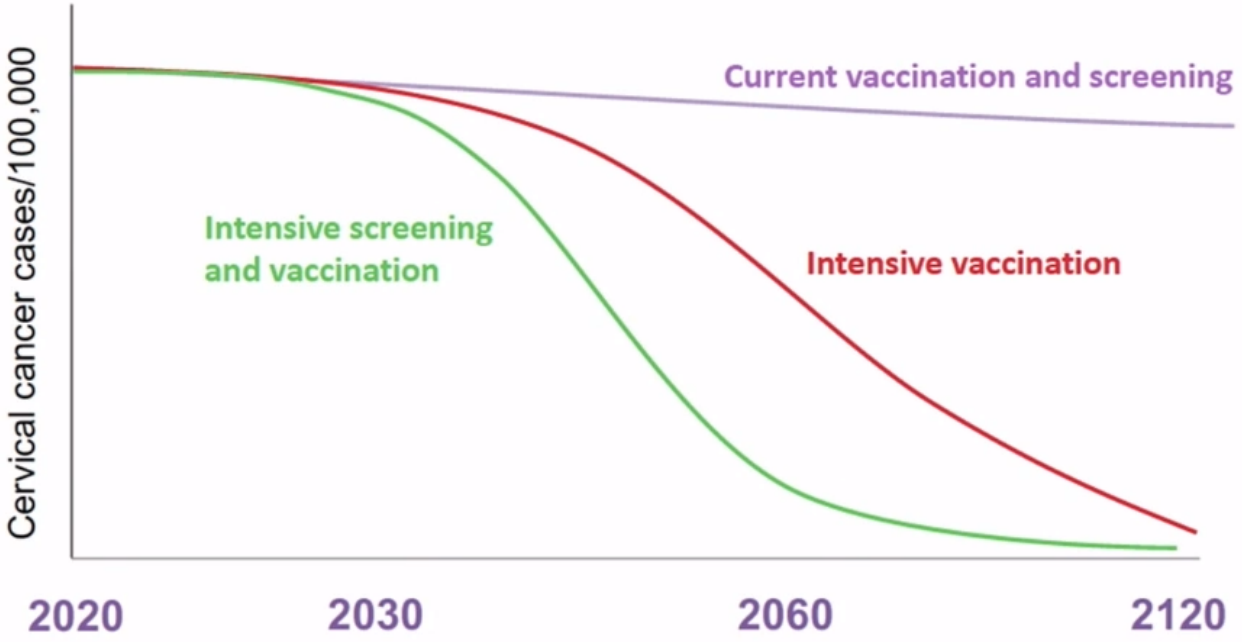
- The World Health Organization officially launched the **Global Strategy to Accelerate the Elimination of Cervical Cancer as a Public Health Problem** in November 2020:
  - Goal: <4 per 100,000 women in every country within the next 100 years

This global strategy to eliminate cervical cancer proposes:

- a vision of a world where cervical cancer is eliminated as a public health problem;
- a threshold of 4 per 100 000 women-years for elimination as a public health problem;
- the following 90-70-90 targets that must be met by 2030 for countries to be on the path towards cervical cancer elimination:

- a mathematical model that illustrates the following interim benefits of achieving the 90-70-90 targets by 2030 in low- and lower-middle-income countries:
  - median cervical cancer incidence rate will fall by 42% by 2045, and by 97% by 2120, averting more than 74 million new cases of cervical cancer;
  - median cumulative number of cervical cancer deaths averted will be 300 000 by 2030, over 14 million by 2070, and over 62 million by 2120.

CERVICAL CANCER ELIMINATION: CONCEPTUAL FRAMEWORK



Slide acknowledgement: WHO WPRO regional consultation on cervical cancer elimination

World Health Organization

Australian Centre for the Prevention of Cervical Cancer

VCS Pathology | Population Health | Digital Health

C4 | compass | ROSE

Why did screening change?

- Organised screening program since 1991.
- Cervical cancer is the 14th most common cancer affecting Australian women.
- Australia has one of the lowest rates of cervical cancer incidence and mortality in the world.

Australian women are still dying of cervical cancer

- In 2021 in Australia, there were **851 new cases** of cervical cancer.
- In 2020, there were **209 deaths** attributable to cervical cancer.

Equity – screening participation

Current participation is 62%.

~80% women diagnosed with invasive cancer are under/never screened.

- Indigenous – 2 x develop CxCa, 3.8 x likely to die from
- Geographical – NT, outer regional/remote/very remote
- Age 25–29 and >70 years
- Socioeconomic disadvantage
- Cultural and linguistic diversity
- LGBTIQ+
- Gender (versus sex) identify as male; assigned female at birth
- Disability
- History of sexual violence

Ref: C4 2021 Cervical Cancer Elimination Progress Report

VCCR. Statistical Report: Victorian Cervical Cytology Registry; 2015.

CST self-collects

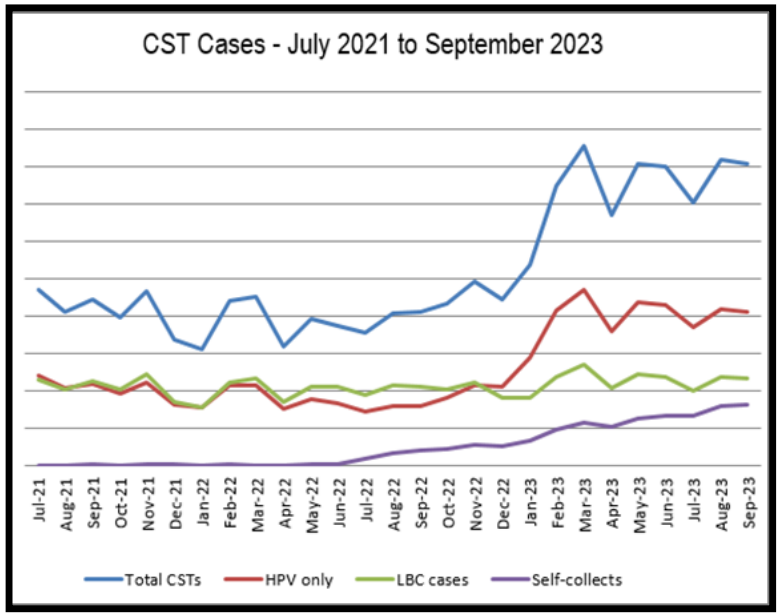
Initial hesitancy and mistrust combined with (extremely) restricted access – very low uptake (0.1%); minimal labs accredited.

2018 meta-analysis showed equivalence in accuracy between self-collection and clinician collection for PCR results; confirmed by COMPASS trial and in-house validation studies.

Ref: Inturrisi F, Aitken CA, Melchers WJG, et al. Clinical performance of high-risk HPV testing on self-samples versus clinician samples in routine primary HPV screening in the Netherlands: An observational study. *Lancet Reg Health Eur*. 2021;11:100235. Published 2021 Nov 9. doi:10.1016/j.lanepe.2021.100235

National Cervical Screening Program (NCSP)

- Self collection is now framed as a **choice** for all participants, except for the small number who are ineligible (i.e. those who you know need an LBC)
- Original modelling estimated 6% by mid 2025 (!!!!!)
- In reality there has been a surge in uptake which will hopefully be reflected in increased recruitment to screening
- At SNP self collects reached *at least* 20% of CSTs from Sept '22 onwards.



Up kick in total numbers reflection of the new 5-year cycle.
Average TAT all CSTs 7 days / HPV only 2–3 days / LBC cases 16 days

DOHA data

Self-collect HPV testing

- 27% self collects vs 16% last year
- Highest among underscreened groups
- Rural/remote areas 51%
- Northern Territory residents 47%
- 70+ 34%
- Financially disadvantaged 29%
- Never screened 33% in last 3 months of 2023 (up from 13% in previous year)
- Underscreened up from 14% to 40%

https://www.abc.net.au/news/2024-03-04/self-test-cervical-screening-hpv-test-on-the-up/103542748?utm_source=abc_news_app&utm_medium=content_shared&utm_campaign=abc_news_app&utm_content=teams

Remember ….

- If a co-test is required, then self-collection is NOT the appropriate test:
  - *symptomatic / TOC / PHx Glandular abnormality / Hyst + HSIL / DES exp in utero.*

- If HPV is detected in the self-collected sample, a reflex LBC CANNOT be performed:
  - *2% 16 or 18 Colp / Bx*
  - *6–8% non 16/18 HPV need to return to their local health provider for a clinician-collected cervical sample for LBC triage.*

NCSP Draft Update November 2023

- Six years have passed since implementation of new cervical screening program
- Guidelines are frequently undergoing review and update
- Currently in the 2nd year of ‘new’ 5 year cycle so volumes +++

A new chapter ‘Cervical screening in clinical practice’ aims to help clinicians explain the role of HPV to patients and includes discussion on the topic of latency.
Acceptable.

Screening participants with HPV non 16/18 on a self-collected specimen who do not return for a clinician collect for 9 or more months after that test, can be offered a repeat self-collect (rather than an LBC).
If the repeat HPV is negative, the infection is considered cleared and the patient can return to routine 5-yearly screening. Acceptable.

Categories of people who are considered immune deficient to the extent that different screening is needed have been clarified and expanded.
Acceptable and applauded.

Table 7.1: Conditions and therapies that may require a 3-yearly screening program

Screening Periodicity	Conditions and Medications
3-yearly screening recommended [a]	Living with human immunodeficiency virus (HIV) Solid organ transplant with immunosuppressive therapy Active haematological malignancy Haematopoietic stem cell transplant (HSCT) recipients or chimeric antigen receptor T-cell (CAR-T) therapy within 2 years of transplantation. Primary immunodeficiency including combined immunodeficiency and syndromes, major antibody deficiency (e.g. common variable immune deficiency [CVID] or agammaglobulinemia), defects of innate immunity (including phagocytic cells), defects of immune regulation, complement deficiencies and phenocopies of primary immunodeficiencies
3-yearly screening should be highly considered [b]	Long term haemodialysis or peritoneal dialysis (>6 months) Long-term treatment (>6 months) with highly immunosuppressive therapies, including: high-dose corticosteroid treatment equivalent to >20 mg/day of prednisone for ≥14 days in a month, or pulse corticosteroid therapy. selected conventional and targeted synthetic disease-modifying anti-rheumatic drugs (sDMARDS), taken for long-term treatment (> 6 months) Including: mycophenolate, methotrexate (≥10 mg/week), azathioprine (≥1mg/kg day), 6-mercaptopurine (≥0.5 mg/kg/day), alkylating agents (e.g.: cyclophosphamide, chlorambucil), and systemic calcineurin inhibitors (e.g.: cyclosporin, tacrolimus), JAK inhibitors (e.g.: tofacitinib, baricitinib, ruxolitinib, upadacitinib) Excluding: hydroxychloroquine or sulfasalazine when used as monotherapy or low-dose corticosteroid biologic therapies that deplete T cells (e.g.: muromonab, teplizumab) Excluding: other biologics that have other targets, for example anti-CD20 (e.g. rituximab) or anti-TNF (e.g. infliximab, adalimumab) multiple immunosuppressants where the cumulative effect is considered to be severely immunosuppressive
[a] Based on documented significantly higher risk of cervical precancer and cancer [b] Based on an increased risk expected through pathological mechanisms and trends on observational studies Note: Most standard chemotherapy regimens for solid tumours are not considered to be highly immunosuppressive, as well as other antineoplastic treatments, such as hormone therapy, immunotherapy and targeted therapy. People with history of cancer and those being treated with chemotherapy short-term (< 6 months) for solid tumours, should **not** be considered immune-deficient in this context. Particular cases, especially with prolonged treatments or multiple prior lines of cytotoxic therapy, may be discussed with the oncologist.	

Post-treatment management for people who have been successfully treated for HSIL and AIS (‘Test of Cure’) have changed:

- People treated for HSIL are recommended to have annual HPV tests (rather than co-tests) until they are negative on two consecutive occasions, at which point they can return to routine screening. Acceptable.
- Follow-up testing after completely excised AIS (annual co-tests) can be extended to 3-yearly testing if all tests have been negative for 5 years, and can cease 25 years after completely excised AIS if all tests have been negative. Acceptable but confusing for cytology staff regarding management guidelines. NCSR should take over issuing management guidelines. What about incompletely excised AIS?

Where testing is indicated after a total hysterectomy, this has been simplified to annual testing (co-testing or HPV testing depending on cervical pathology and history) until the patient tests negative on two consecutive occasions. These scenarios remain multiple and complex, making them difficult to apply for cytology staff. Consideration should be given to transitioning away from laboratory-based issuing of management guidelines to the NCSR, which would offer consistency as the register has most comprehensive overview of medical history.

Section 8 Investigation and management at colposcopy: no visible lesion or Type 3 TZ.

- In some circumstances, recommendations now state that endocervical curettage could be considered, by those who are confident and trained in the technique and have appropriate equipment.

- Option to defer re-referral for those with HPV (16/18) detected, negative LBC and normal colposcopy, if 12-month follow-up results are again HPV (16/18) detected and negative LBC (the HPV test could be repeated in another 12 months rather than immediate referral to colposcopy). CINtech® *PLUS****

- Clarification of recommendation against diagnostic excision of the TZ in the absence of high-grade cytology or histology.

Rec	Results reported				Recommended clinical action
	Screening test	LBC	Colposcopy	Cytopathology review*	
8.2	Oncogenic HPV (any type)	pHSIL/HSIL	No abnormality detected	***Indicated***	*Pending Cytopathology review* Consider discussion at MDT
8.3					Colposcopic examination of the vagina to exclude VAIN before considering excision
8.4				HSIL *(confirmed)*	**Diagnostic excision of TZ**
8.5				pHSIL *(confirmed)*	**Consider diagnostic excision of TZ**
8.7				Negative/ pLSIL/ LSIL *(downgraded)*	As per reviewed cytology (see REC X)
8.15	Oncogenic HPV (any type)	pHSIL/HSIL	Type 3 TZ	***Indicated***	*Pending Cytopathology review* Consider discussion at MDT Consider ECC (if proficient)
8.14				pHSIL/ HSIL *(confirmed)*	**Diagnostic excision of TZ**
8.7				Negative/ pLSIL/ LSIL *(downgraded)*	As per reviewed cytology (see REC X)
8.1	Oncogenic HPV (any type)	Negative/ pLSIL/ LSIL	No abnormality detected	Not indicated	**Repeat HPV test at 12 months**
8.10	Oncogenic HPV (16/18)	Negative	Type 3 TZ	***Indicated***	Consider discussion at MDT Consider repeat cytology (collected using both endocervical brush and broom)
8.11		Negative/ pLSIL/ LSIL			Consider ECC (if proficient) if previously never screened patient aged 26+
8.9	Oncogenic HPV (not 16/18)	Negative	Type 3 TZ	Not indicated	Consider discussion at MDT **Repeat HPV test at 12 months**
8.10	Oncogenic HPV (any type)	pLSIL/ LSIL	Type 3 TZ	***Indicated***	*Pending Cytopathology review*
8.9	Oncogenic HPV (any type)	Negative/ pLSIL/ LSIL	Type 3 TZ	Negative/ pLSIL/ LSIL	Consider discussion at MDT

We find the summary of recommendations difficult to navigate; it will be difficult for the screener to find the information needed. We would like this table translated into flow diagrams. Another reason for NCSR to issue management guidelines. And a Medicare rebate for reviews.

New platform. The Cancer Council Australia NCSP Guidelines are now hosted on a new clinical practice guideline platform – MAGIC App. Acceptable.

THEN!!!!

- Separate to draft changes proposed in November 2023; email sent February 23 2024 regarding changes to follow-up and recall, effective Monday 4 March 2024

- Summarised as:

  1. If patient has a total hysterectomy with no previous screening history, this will be alerted in the register. Patient can exit program following two separate negative HPV tests.

  2. Intermediate risk tests must have a HPV test (non 16/18) and are no longer triggered by LBC result of pLSIL or LSIL alone. Eg., if cotest done with HPV negative and LSIL/pLSIL, the recommendation will be follow-up in 5 years. Does not apply to TOC, DES, AIS follow-up.

  3. NCSR will no longer recall participants based on a negative or low grade LBC result, without an accompanying positive HPV result.

  4. NCSR will follow-up on unsatisfactory cytology results under the following circumstances:
  - Cotest for symptoms and signs; provider will receive reminder at 3 months, then patient will get 5 year reminder
  - Cotest for DES
  - Ongoing TOC
  - Follow-up of AIS

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- HPV subtypes are changing and moving towards less aggressive types with non-16/18 HPV subtypes increasing.
- Vaccinated cohorts are now entering the screening system.
- Colposcopy versus surveillance poses a challenge to follow up, for which HPV 16/18 genotyping together with *dual staining* may provide a potential efficient strategy for stratification.

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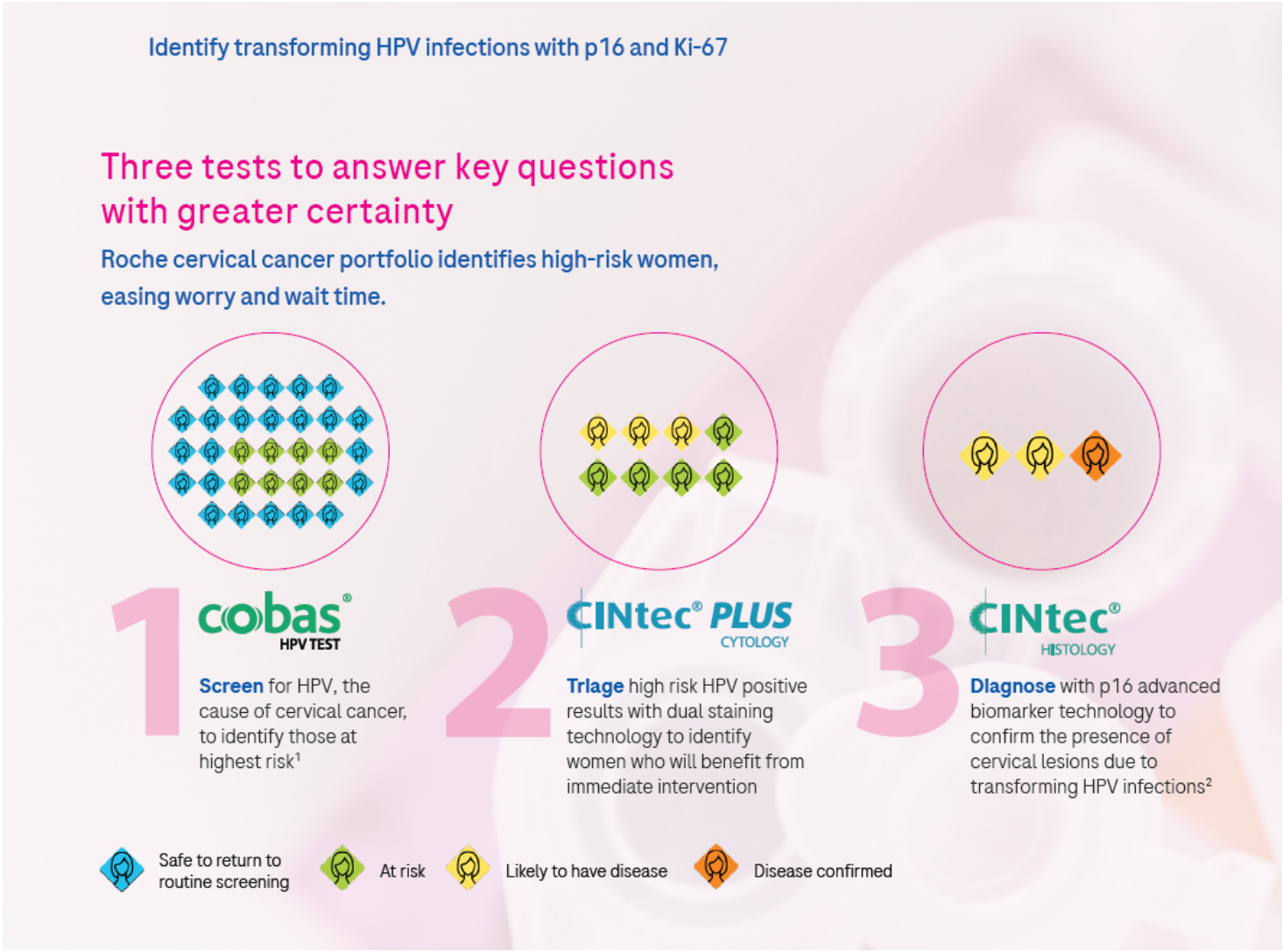
- Use of biomarkers as a tool to identify women at increased risk for harbouring cervical cancer precursors.
- These biomarkers offer improved specificity over HR-HPV testing as their altered expression represents evidence of true cellular neoplastic transformation.
- p16INK4a protein (p16) is a biomarker of transforming HPV infections and precancerous cervical lesions.
- Ki67 indicates cell cycle activity.
- P16 and Ki67 are mutually exclusive in normal cells, therefore their presence within the same cell is a hallmark of cell cycle deregulation.
- Identifies women that may benefit most from immediate colposcopy.



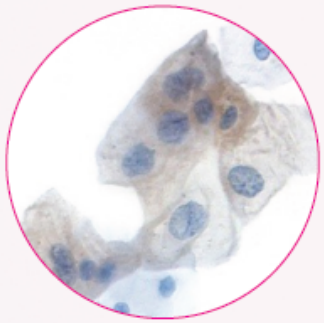
Source: Cervical disease risk identified with grater confidence. Roche 2023.

CINtec® *PLUS* Cytology increases clarity

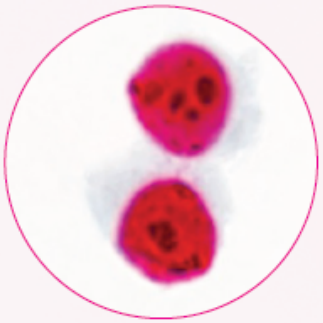
Identify transforming HPV infections with p16 and Ki-67

CINtec® *PLUS* dual stain technology based on immunocytochemistry staining, provides objective and enhanced visual clarity. This is in comparison to Pap cytology, where disease identification is based on morphological characteristics which can be difficult to assess objectively.[3]

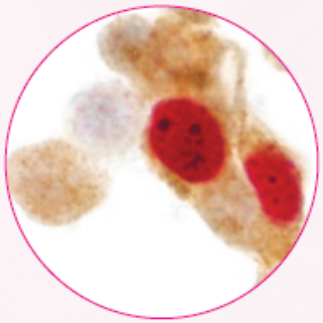
CINtec® *PLUS* Cytology



Negative
Expression of p16 ***(brown)*** signals halting of cell division

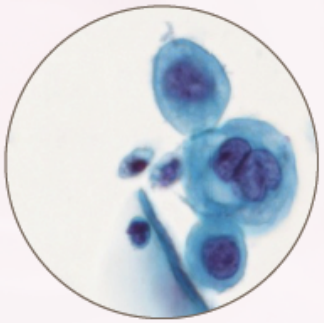


Negative
Expression of Ki-67 ***(red)*** signals progression of cell division



Positive
Co-expression of p16 & Ki-67 ***(brown and red)*** indicates cell cycle deruglation

Pap cytology



Subjective
Reliant on interpretation of morphology only

Source: Cervical disease risk identified with grater confidence. Roche 2023.

Preliminary COMPASS result findings

- Dual staining (DS) can improve risk stratification in conjunction with HPV genotyping.
- Potentially more effective than LBC as a triage (that is, following +HPV result). Interestingly more so in young vaccinated cohort than older age group.
- DS brings forward the diagnosis of CIN2+/CIN3+ disease.
- Negative DS is a very strong marker of safety in non16/18 positive patients.***

Cases

Understanding unusual cases

- Cases
  1. MM (Dr DP)
  - April 2018 HPV not detected
  - June 2023 self-collect specimen HPV non 16/18 detected
  - August 2023 clinician collect specimen HPV not detected
  2. GG (Dr SL)
  - March 2018 HPV non16/18 detected (641021905) – then ‘no partners’
  - April 2019 HPV18 (647426781)
  3. KZ (Dr EM)
  - 2016 HPV16 + non16/18 HPV (633304896)
  - 2017 Not Detected (639146296) – then ‘no partners’
  - 2018 non 16/18 HPV (7/11/18 retest 10/11/18) (644262085)
    - 13/11/18 non 16/18 HPV (648683699)
  4. JB (Dr LO)
  - 5 March 2019 non 16/18 HPV (646646053)
  - 8 March 2019 Not Detected (646646358)
  5. SCC with HPV Not Detected (?)

Topics of interest

Arising from case reports

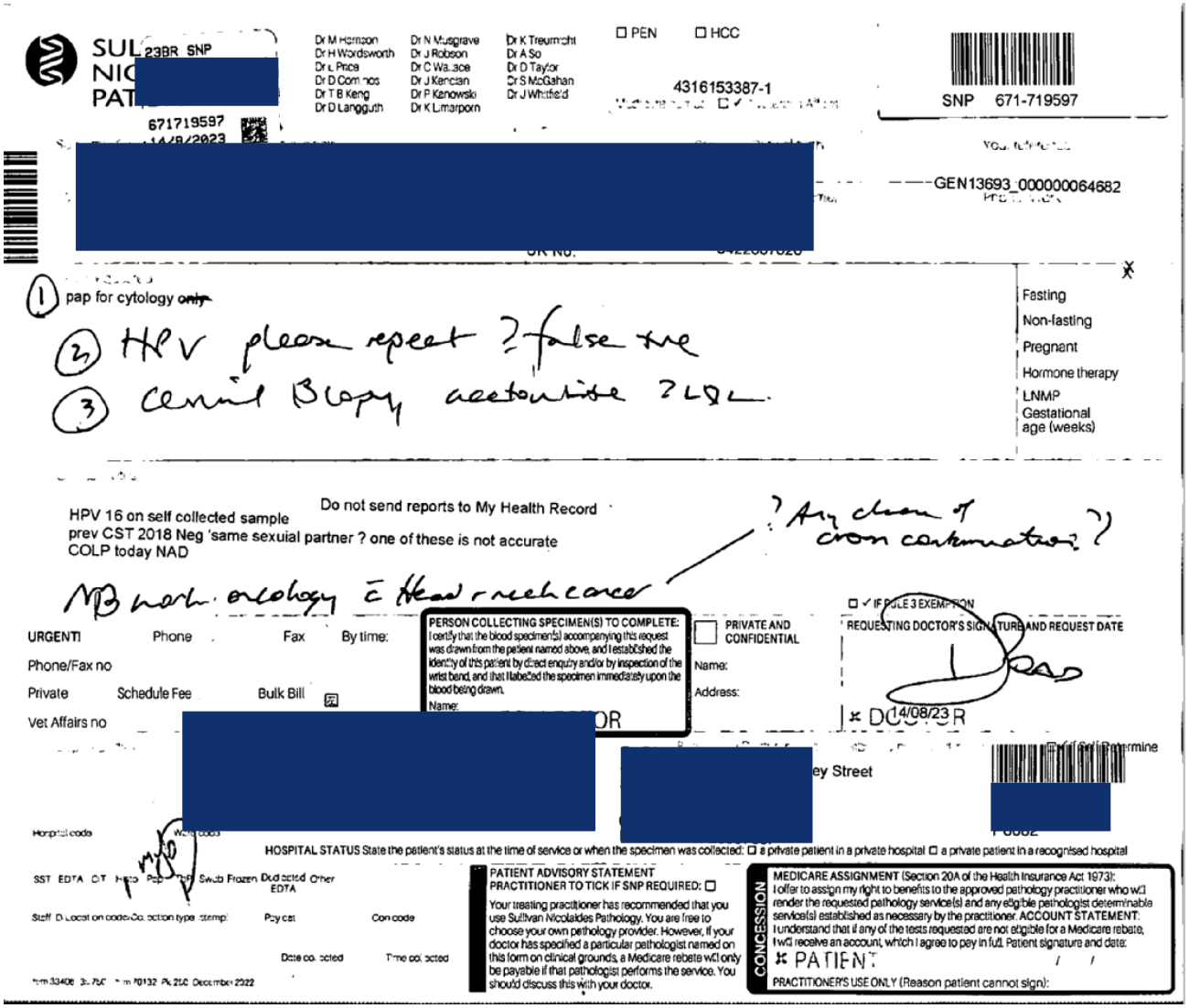
- Basically about fluctuating HPV results, test sensitivity and false negative rates and causes.
- It would be good to hear what CT, IC(globin) and Cp mean / are

> We dragged out the archive files to check the results and look at the Cp values. Results and controls are all good for the runs.
>
> 646646053 on 5/3 has IC (Globin) of 22.94 and the Other HR was Cp 30.14
>
> 646646358 on 8/3 has IC (Globin) of 24.72

- We would like to hear more about the "imperfect homogeneity" and volumetric considerations that you mentioned.

> So quite possible the combination of less cells and lower Other HR HPV content 3 days later may have combined to result in a Not Detected. Ideally it would have been good to have been able to retest the not detected sample but we have none here from March. I think I have mentioned before that it does become a numbers game due to the sampling aspect and the imperfect homogeneity – i.e. 20mL Preservcyte, the test only takes 400uL and even with shaking intra or extracellular viral particles may not end up in the 400uL that gets DNA extracted/tested.

- Causes of unsatisfactory tests would also be good to learn more about



SUL 23BR SNP [redacted]
NIC
PAT
671719597
14/8/2023

Dr M Harrison Dr H Wordsworth Dr L Price Dr D Commos Dr T B Keng Dr D Langguth
Dr N Musgrave Dr J Robson Dr C Wallace Dr J Kenclan Dr P Kenowski Dr K Limarpoxn
Dr K Treurnicht Dr A So Dr D Taylor Dr S McGahan Dr J Whitfield

☐ PEN ☐ HCC

4316153387-1

SNP 671-719597

[redacted]

GEN13693_000000064682

UR No.

(1) pap for cytology ~~only~~

(2) HPV please repeat ? false +ve

(3) cervical Biopsy acetowhite ? LSIL

Fasting
Non-fasting
Pregnant
Hormone therapy
LNMP
Gestational age (weeks)

HPV 16 on self collected sample
prev CST 2018 Neg 'same sexual partner ? one of these is not accurate
COLP today NAD

Do not send reports to My Health Record

? Any chance of cross contamination ??

MB work: oncology & Head + neck cancer

URGENT Phone Fax By time:

Phone/Fax no

Private Schedule Fee Bulk Bill ☑

Vet Affairs no

PERSON COLLECTING SPECIMEN(S) TO COMPLETE:
I certify that the blood specimen(s) accompanying this request was drawn from the patient named above, and I established the identity of this patient by direct enquiry and/or by inspection of the wrist band, and that I labelled the specimen immediately upon the blood being drawn.
Name:
[redacted]OR

☐ PRIVATE AND CONFIDENTIAL
Name:
Address:

☐ ✓ IF RULE 3 EXEMPTION

REQUESTING DOCTOR'S SIGNATURE AND REQUEST DATE

✕ DOCTOR 14/08/23

[redacted]

[redacted]ey Street

Hospital code Ward code

HOSPITAL STATUS State the patient's status at the time of service or when the specimen was collected: ☐ a private patient in a private hospital ☐ a private patient in a recognised hospital

SST EDTA CT Hep Pap Swab Frozen Dedicated EDTA Other

Staff ID Location code Collection type (temp) Pay cat Con code

Date collected Time collected

Form 33406 3rd Edition Form 70132 PK 250 December 2022

PATIENT ADVISORY STATEMENT
PRACTITIONER TO TICK IF SNP REQUIRED: ☐

Your treating practitioner has recommended that you use Sullivan Nicolaides Pathology. You are free to choose your own pathology provider. However, if your doctor has specified a particular pathologist named on this form on clinical grounds, a Medicare rebate will only be payable if that pathologist performs the service. You should discuss this with your doctor.

CONCESSION

MEDICARE ASSIGNMENT (Section 20A of the Health Insurance Act 1973):
I offer to assign my right to benefits to the approved pathology practitioner who will render the requested pathology service(s) and any eligible pathologist determinable service(s) established as necessary by the practitioner. ACCOUNT STATEMENT:
I understand that if any of the tests requested are not eligible for a Medicare rebate, I will receive an account, which I agree to pay in full. Patient signature and date:

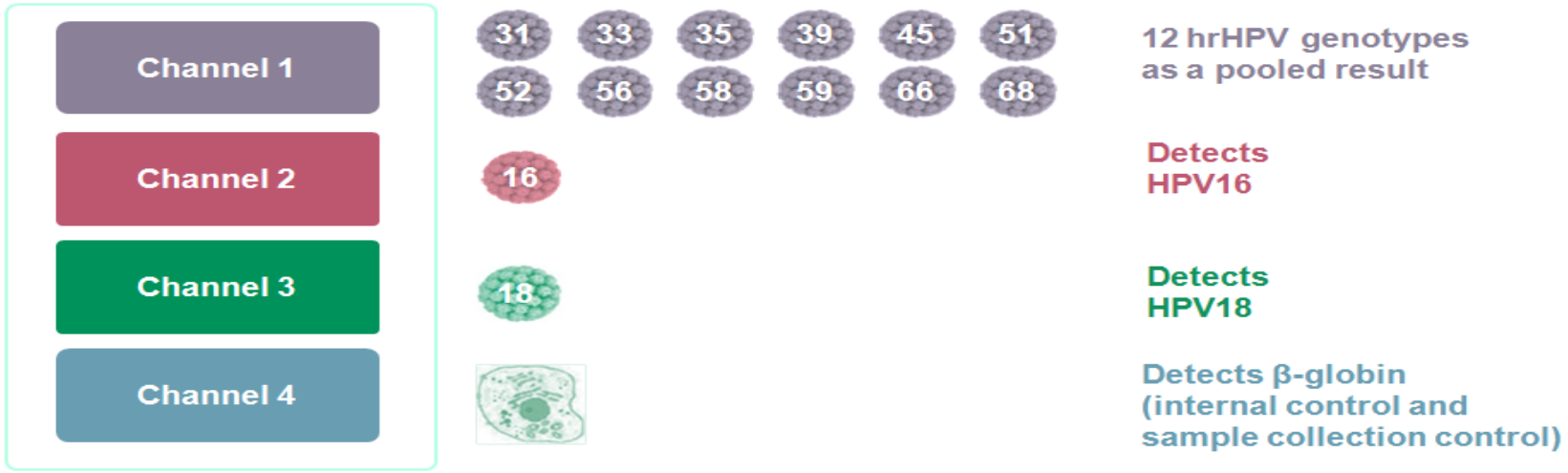
✕ PATIENT / /

PRACTITIONER'S USE ONLY (Reason patient cannot sign):

Cobas® 6800 HPV test detail

cobas® HPV Test

Complies with MSAC recommendations



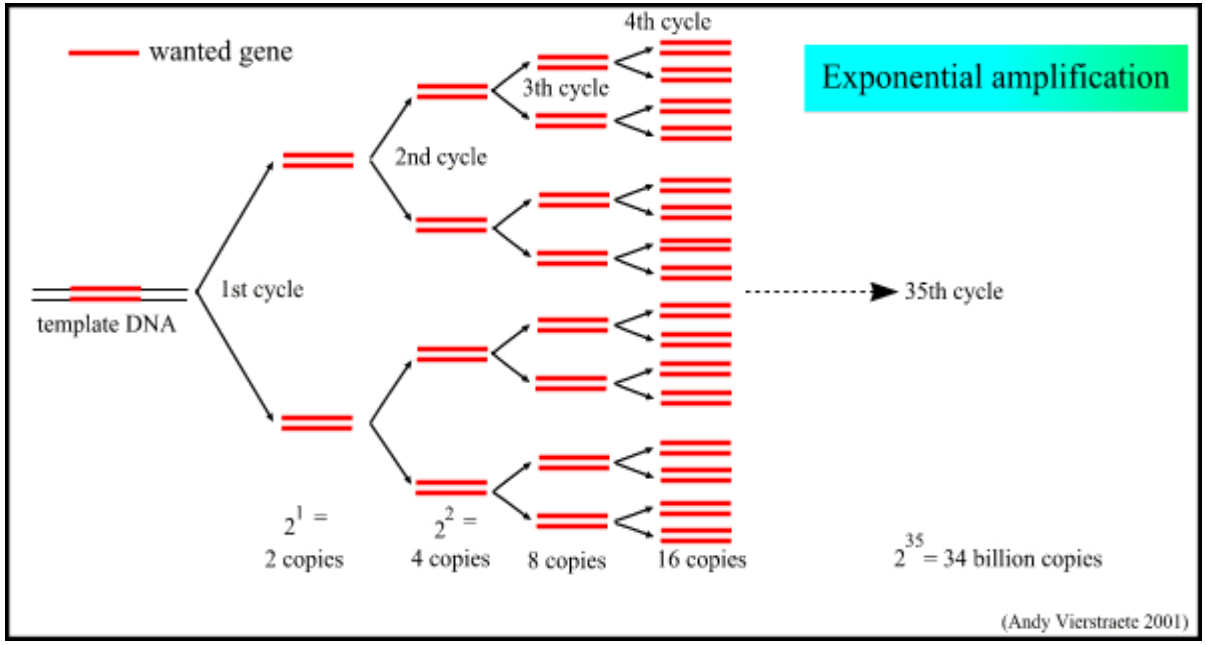
- Produces 3 results in a single test:
  1. Pooled hrHPV results (12 other HR)
  2. HPV 16 genotype
  3. HPV 18 genotype

Roche

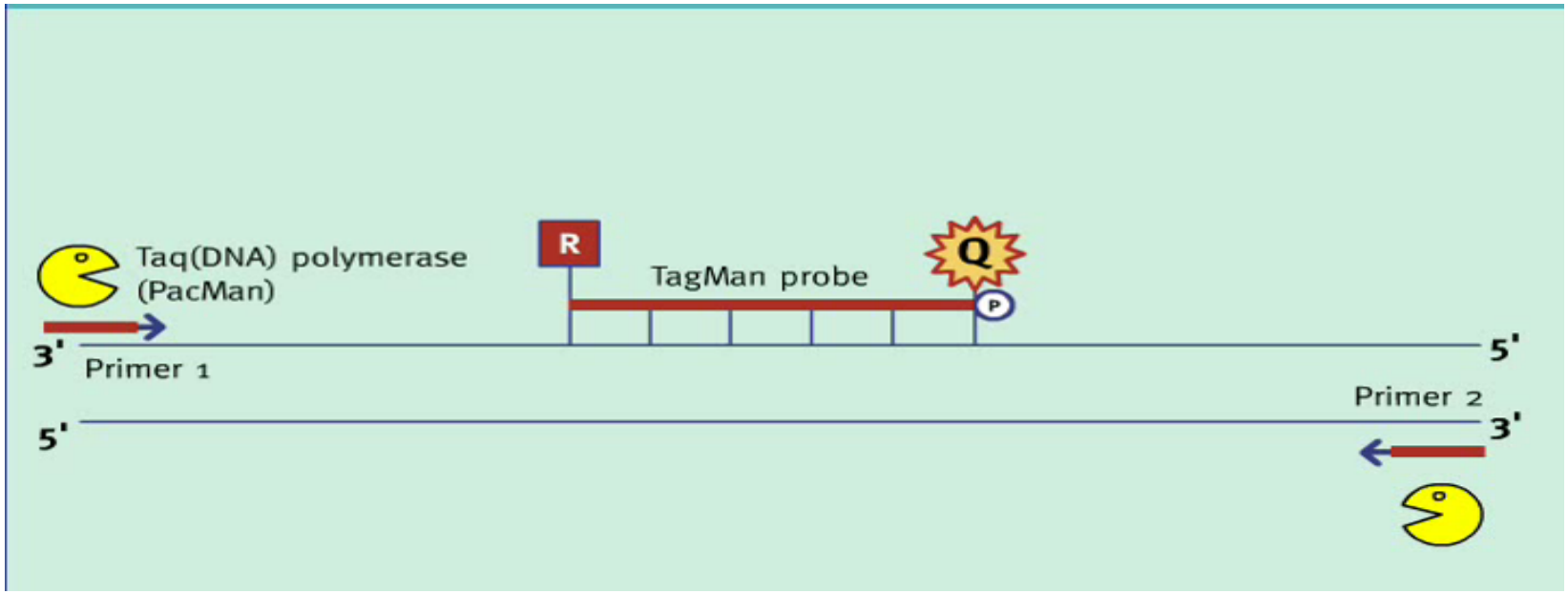
cobas®
Life needs answers

PCR amplification process

- Exponential amplification: 2^n ,where n = number of cycles



‘Real-Time’ PCR – Taqman



Analytic sensitivity vs clinical sensitivity

- Analytic sensitivity = ability to detect HR HPV DNA
  - Nucleic acid amplification tests are very good at analytic sensitivity, i.e. detecting HPV DNA
  - Design features and concurrent typing helps temper the true analytic sensitivity
- Clinical sensitivity = clinically relevant infections due to HR HPV ≥ CIN2/3,HSIL

PCR – Why such a good analytic?
Both sensitive and specific (extremely so)

- Sensitivity
  - Theoretically one molecule
  - Due to amplification 1 copy becomes 34 billion (2^{35}, 2x2x2…..) across 35 cycles of PCR temperature
- Specificity
  - A:T and C:G
  - Sequences must match for amplification to happen
    - At each location of a primer or probe there is 4 bases to choose from e.g. HPV16 Forward Primer

HPV16	HPV16 E2 Forward primer	AACGAAGTATCCTCTCCTGAAATTATTAG
	HPV16 E2 Reverse primer	CCAAGGCGACGGCTTTG
	HPV16 E2 Probe	(TAMRA)-CACCCCGCCGCGACCCATA-(BHQ)

AACGAAGTATCCTCTCCTGAAATTATTAG

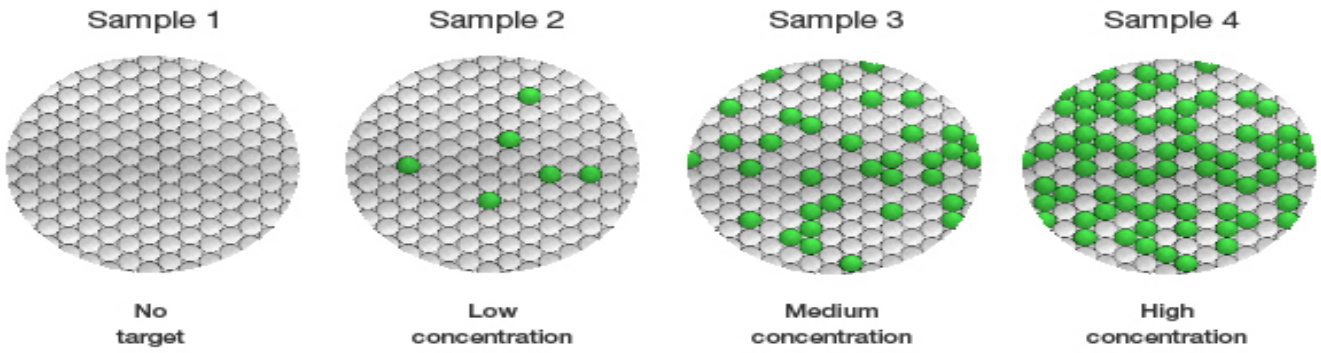
    - 4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4x4
    - 1 in 288,230,376,151,711,744 chance of random occurrence of those 29 DNA bases in that order
    - 2 primers, one probe = ~60-90 bases = additive specificity

Realities of PCR test sensitivity

- PCR – has a theoretical sensitivity of detecting one single copy of a DNA target
  - **IF** you get that target in the sample volume that you extract the DNA from (400 uL from 20 mL PreservCyt that becomes 100 uL of buffer with DNA in it)
  - **AND IF** the DNA molecules in that 100 uL then make it into the PCR reaction vessel (25 uL into 25 uL reagents)
  - *In practice sensitivity* is between 1 and 10 copies of target in the PCR reaction tube volume for real-time PCR
- Why are self collects more sensitive?
  - All material in 5 mL, not 20 mL – more chance at lower viral concentrations, and lower Cts in general (more sensitive)

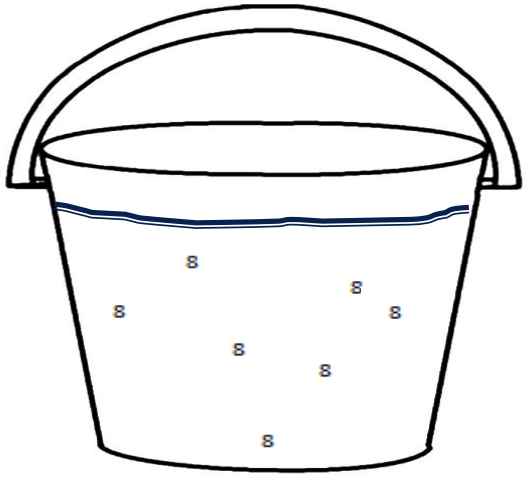
Poisson distribution effects at low volumes

- Copies of PCR targets vary in the test volume



Sampling and Poisson

Bucket represents the DNA extraction out of 400 uL PreservCyt. “A bucket with several molecules of HPV DNA in it”



The cup represents the liquid volume sampled from the “DNA elution bucket” which is then added in to the PCR reaction/test well

- The “cup” might get some HPV DNA “s” from the bucket at low DNA concentrations
- LOD quoted for a PCR assay is the amount in the bucket where the cup would get some target 95% of the time
- PCR can still be positive at concentrations lower than LOD even down to one copy – the lower you go the less chance your “cup” picks up some DNA to go into the PCR reaction – randomness

False negative rates and causes

- “Screening” – screening assays are optimised to minimise false negatives; how: Maximisation of sensitivity minimises false negatives (also why no longer using microscopy as screen)
  - BUT can result in increases in false positives
- Rates of false negative
  - 0.12% (1 in 865 Compass Trial) to 0.2%
- Causes of false negatives
  - Tiny amounts of HPV and those not making it to PCR reaction
  - Inhibition of PCR enzyme/process
  - Reduction of DNA extraction efficiency
  - Total failure of DNA extraction

} Internal control detects these -> “Invalid”

Appreciate the subtlety?

‘Not detected’

vs

‘Negative’

Potential inhibitors

- Other than high concentrations of HR HPV causing competitive inhibition
- As per pack insert

18. The presence of PCR inhibitors may cause false negative or invalid results.
19. Cervical specimens often show visibly detectable levels of whole blood as a pink or light brown coloration. These specimens are processed normally on the **cobas**® 4800 System. If concentrations of whole blood exceeds 2% (dark red or brown coloration) in **cobas**® PCR Cell Collection Media, PreservCyt® Solution or SurePath™ Preservative Fluid, or above 4% in SurePath™ Preservative Fluid treated with cobas Sample Prep Buffer, there is a likelihood of obtaining a false-negative result. See Interference results for details.
20. Use of the vaginal moisturizer Replens® has been associated with false-negative results in SurePath™ Preservative Fluid.
21. Removal of red blood cells from PreservCyt or SurePath specimens through treatment with glacial acetic acid (GAA) has not been validated with the **cobas**® 4800 HPV Test. Any use of GAA treatments with the **cobas**® 4800 HPV Test must be validated by the testing laboratory.

Endogenous interference/inhibition

- Sample-specific potential inhibitors/interfering substances

Table 21
Interference Testing Results with Endogenous Interferents

Interferent Tested	Concentrations Tested	Interference Observed		
		PreservCyt	SurePath	SurePath treated w/ cobas Sample Prep Buffer
Whole Blood	1%, 1.5%, 2%, 3%, 4%, 6%, 8% v/v	Above 2%	Above 2%	Above 4%
PBMC	10^4, 10^5, 10^6 cells/mL	None	None	None
Cervical Mucus	Mucus obtained from standard cervical cleaning procedure	None	None	None

Exogenous interference/inhibition

- Substances of exogenous origin present in sample potentially interfering/inhibitory

Table 22
Interference Testing Results with Exogenous Interferents

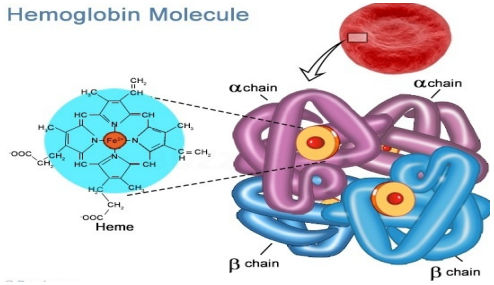
Interferent Description	Interference Observed
Contraceptive Gels, Foams	None
Vaginal Lubricants	*Yes
Vaginal Douche	None
Anti-fungal creams containing 1% clotrimazole, Phenazopyridine Hydrochloride, 1% Hydrocortisone, 2% Miconazole nitrate, 6.5% Tioconazole Ointment, 20% Benzocaine	None

* Replens® (topical anti-dryness gel) produced negative results in replicates of the SurePath™ Preservative Fluid 3 x LOD pool. This interference was also seen when SurePath material was treated with cobas Sample Prep Buffer.

β-globin gene – internal control

Channel 4

Detects β-globin (internal control and sample collection control)




```
Translation     MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFE
                SFGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPE
                NFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH

SEQUENCE (5' to 3' sequence of the coding strand)
     1 ccctgtggag ccacacccta gggttggcca atctactccc aggagcaggg agggcaggag
    61 ccagggctgg gcataaaagt cagggcagag ccatctattg cttacatttg cttctgacac
   121 aactgttgtt actagcaacc tcaaacagac accatggtgc acctgactcc tgaggagaag
   181 tctgccgtta ctgccctgtg gggcaaggtg aacgtggatg aagttggtgg tgaggccctg
   241 ggcaggttgg tatcaaggtt acaagacagg tttaaggaga ccaatagaaa ctgggcatgt
   301 ggagacagag aagactcttg ggtttctgat aggcactgac tctctctgcc tattggtcta
   361 ttttcccacc cttaggctgc tggtggtcta cccttggacc cagaggttct ttgagtcctt
   421 tggggatctg tccactcctg atgctgttat gggcaaccct aaggtgaagg ctcatggcaa
   481 gaaagtgctc ggtgcctttа gtgatggcct ggctcacctg gacaacctca agggcacctt
   541 tgccacactg agtgagctgc actgtgacaa gctgcacgtg gatcctgaga acttcagggt
   601 gagtctatgg gacccttgat gttttctttc cccttctttt ctatggttaa gttcatgtca
   661 taggaagggg agaagtaaca gggtacagtt tagaatggga aacagacgaa tgattgcatc
   721 agtgtggaag tctcaggatc gttttagttt cttttatttg ctgttcataa caattgtttt
   781 cttttgttta attcttgctt tctttttttt tcttctccgc aatttttact attatactta
   841 atgccttaac attgtgtata acaaaaggaa atatctctga gatacattaa gtaacttaaa
   901 aaaaaacttt acacagtctg cctagtacat tactatttgg aatatatgtg tgcttatttg
   961 catattcata atctccctac tttatttttct tttattttta attgatacat aatcattata
```

- 'In-Situ' control – actually in sample
  - Detecting it indicates:
    - Sufficient human cells in collection (but ?HPV infected)
    - PCR enzyme/reaction did work
    - DNA extraction did work and at right efficiency (by Ct value range)
    - Proves 'Not Detected' results not due to analytic failure
  - Only time it's ok for this IC to be negative is if HPV is detected

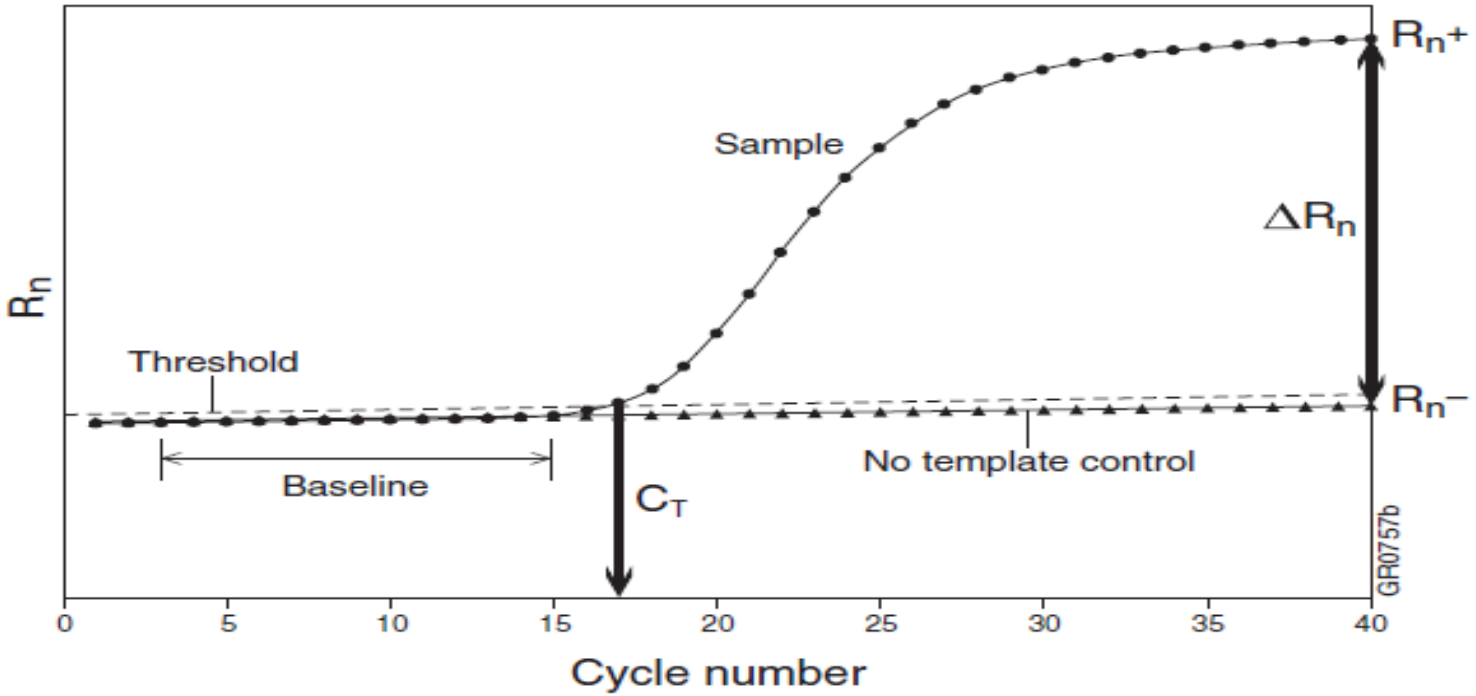
What is a Ct/Cp value

- Cycle threshold Ct = Crossing Point Cp
- Describes the thermal cycle where the fluorescent signal from a positive sample first becomes exponential/doubles
- More target = smaller/earlier Ct e.g. 17
- Less target = bigger/later Ct e.g. 35

Sample rack ID/Position	Sample ID/Pool ID	Type	Flags	Internal control result	QS result	Valid	Test result	Target 1 result	Target 2 result	Target 3 result	Target 4 result	Target 1 Ct value	Target 2 Ct value	Target 3 Ct value	Target 4 Ct value	Internal control Ct value	QS Ct value
1142/2	HPV170320	PreservCyt®		Valid	Not applicable	Yes	Reactive	Other HR HPV Positive	HPV 16 Negative	HPV 18 Positive	-	23.65	-	27.15	-	24.23	Not applicable

Quantitative PCR

Real-Time PCR amplification curve

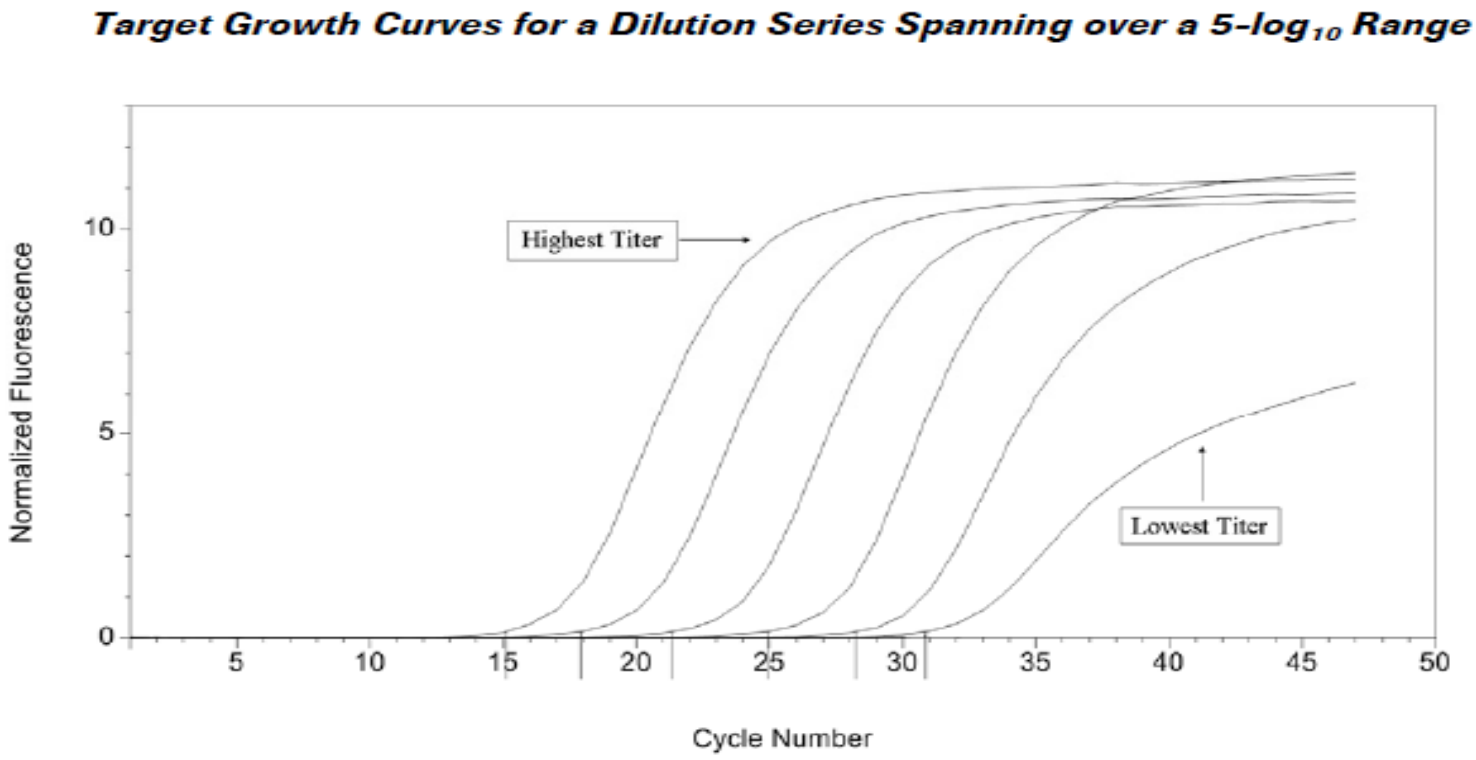


- These ‘curves’ are all behind the scenes in 6800 and 4800
- All we see are Ct values

C_T also known as C_P = Crossing Point = copies first double

Quantitative PCR

Curves for different concentrations of target



Patient MM

Sullivan Nicolaides PATHOLOGY
Quality is in our DNA

21 August 2023

[redacted]

I send this letter regarding Ms [redacted] and your enquiries regarding the changes in her HR-HPV testing results through 2018-2023.

We have retested the most recent CST specimen (671719597 14/08/2023), collected by yourself. The repeat result is no HPV detected. We have not retested the self collect specimen from 30/06/2023 (680698702) as we believe the result would not settle the matter. Whilst we cannot report a quantity of HPV virus present in the self collect sample, the PCR testing produced a cycle threshold "Ct" value of 36.15. These values around Ct 30 are close to the limit of virus detection. The assay can report out to Ct values in the region of 40 cycles, with each 3.2 cycles representing a ten fold decrease in target quantity. As such a Ct value of 30 is about 1000 fold more concentrated than the limit of detection possible with the HPV assay.

Screening test outcomes rely heavily on assay sensitivity; all pathology tests, and in particular screening tests which are biased to maximise sensitivity, are not 100% specific meaning that there is an associated rate of false positives. The parameters of the Roche 6800 HPV assay for detecting the presence of HPV DNA in a 20mL sample by testing 400uL of it (noting this is detecting HPV DNA not detecting clinical disease) are: Sensitivity 97.99% and Specificity 98.35%. This of course means that 2 in every 100 potential true positive test results might be false negative on sensitivity grounds and 1.7 in every 100 true negative cases might be false positive on specificity grounds. The 97.99% and 98.35% performance observations are primarily lower than 100% due to the uneven distribution of virus and cells in the collection media and the comparative molecular methods used to establish the data each sampling a different fraction of the collected volume, but these claims do reflect the expected performance of a 'one off' test result. As a comparison, the Pap smear investigation as a 'one-off' test result has a sensitivity of approximately 50% for clinical disease, but had effective screening outcomes in the pre-vaccination setting due to the higher prevalence of disease, the 2 year frequency of investigations and the ongoing progression of lesions providing increased chances of observing cellular changes for those that didn't regress naturally.

As we have now moved to very sensitive molecular technology for the new screening program we will continue to observe biological variation that has previously gone unnoticed when the molecular based testing was only applied in the test of cure scenario. One of those aspects is the rare observation of HPV infections (transient or progressive) where an individual considers they have had no sexual partners in a specific interval of time.Exposure to HPV without transmission from sexual partners has been described in a number of studies and we have attached some relevant papers. HPV is very robust and can survive in the environment and on fomites for periods of time. Fomite derived and incidental environmental genital contact (i.e. non partner based/sexual activity) have been implicated as transmission routes from persisting environmental reservoirs of HR HPV. In regards what could constitute 'persisting environmental reservoirs' – these can be many and varied but could for instance include sharing living spaces or community locations with individuals who may themselves have HPV infection. Exposure to HR HPV that has been shed to environmental locations could theoretically occur from things such as: shared washing, shared clothing or towels, shared bedding, shared surface contact areas such as bath tubs, chairs or treatment beds, and hand based contamination from any source later transmitted to the genital tract directly or indirectly. An interesting finding has also been the prevalence of HR HPV on fomites such as lamps, glove boxes, and specific gynaecological implements in the clinic setting even with normal forms of disinfectants. We are of course not suggesting that the previous HPV16 detection from [redacted] as come from any particular source let alone your clinic and indeed the origin of any possible inoculation will likely never be known, but we thought it useful to provide reference

Sullivan Nicolaides PATHOLOGY
Quality is in our DNA

information that having no 'partners' in a classical sense does not absolutely preclude contact with HR-HPV and then a detectable infection.

Of course the laboratory setting is indeed also a potential reservoir of HR-HPV positive material. Each test run contains a negative control test to ensure that we are not observing any environmental contamination of our testing processes. We also perform thorough decontamination of our laboratory surfaces and instrumentation with reagents that degrade DNA, and we periodically swab surfaces in the lab and test for HR-HPV as 'sentinel' swabs to see if we have any unrecognised HR-HPV present in/on the laboratory environment. Patient sample to patient sample contamination is avoided by the use of instrument automation equipment of molecular grade with barrier tips which prevent molecular carryover, and the p480 pre-analytic sample handling equipment we utilise also ensures barcode matching between a patient sample and any aliquot prepared so that we avoid any patient sample mix ups.

There are 2 possibilities in this particular patient. The first possibility involves contamination of the self collect specimen. Ms [redacted] self collect specimen was the only one in it's batch to have a HPV16 positive result, therefore likely does not represent contamination from other specimens in the laboratory. Fomite contamination at the time of self collect is possible. The second possibility is that the specimen represents a true positive with low quantity of virus. The concept of fluctuating/silent/recurrent HPV infection is well known in the molecular community. There is however no way to determine whether an HPV result is acute or one of the above.

With respect to negative results in general, and consideration if the current clinician collected specimen could have been a false negative outcome, it is an important distinction that molecular results are reported as 'Not Detected', and not as 'Negative'. We specifically report in this fashion as HPV DNA may still be present but could be below the detectable threshold of the assay or at a level that by distribution in the 20mL PreservCyt vial collected did not make it into the 400uL tested. As such any prior Not Detected result could indeed be a false negative on that basis, however the sensitivity of molecular technology based results is superior to any other current technology.

Finally, you queried the possibility of cross contamination of the self collect specimen from a head and neck cancer (?). We have no record of that diagnosis for this patient on our system. I am assuming this lady has had a diagnosis of an HPV associated head and neck carcinoma (HPV-HNSCC) made elsewhere. HPV isn't a systemic viral process in that it can't seed from one part of the body to another by itself, but it can infect other parts of the body by fomite ', as described above. HPV-HNSCC is thought to be caused in a similar way to cervical SCC through HR-HPV infection of the oropharyngeal tract via oral sex.

I hope this helps. Please feel free to call me for any further questions.

Regards

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Case 1

GG (Dr SL)

- March 2018 HPV non16/18+ (641021905) - then ‘no partners’
- April 2019 HPV18 (647426781)

- Possibilities:
  - Sample-handling error
  - Contamination from other sample
  - Other HR competitively inhibited HPV18 3/2018
  - Contamination from fomites if no partners

Non-sexual transmission of HPV

ORIGINAL ARTICLE

Human papillomavirus (HPV) contamination of gynaecological equipment

Caroline Gallay,[1] Elodie Miranda,[1] Sonja Schaefer,[2] Rosa Catarino,[2] Martine Jacot-Guillarmod,[3] Pierre-Alain Menoud,[4] Frederic Guerry,[4] Chahin Achtari,[3] Roland Sahli,[5] Pierre Vassilakos,[6] Patrick Petignat[2]

CSIRO PUBLISHING
Sexual Health, 2016, **13**, 10–21
http://dx.doi.org/10.1071/SH15089

Review

Penises not required: a systematic review of the potential for human papillomavirus horizontal transmission that is non-sexual or does not include penile penetration

Zhiyue Liu[A], *Tasnuva Rashid*[A] *and Alan G. Nyitray*[A,B,C]

THE JOURNAL OF
Obstetrics and Gynaecology Research
doi:10.1111/jog.13248
J. Obstet. Gynaecol. Res. Vol. 43, No. 3: 429–435, March 2017

Possible non-sexual modes of transmission of human papilloma virus

Sasidharanpillai Sabeena[1], Parvati Bhat[2], Veena Kamath[3] and Govindakarnavar Arunkumar[1]
[1]Department of Virus Research, [2]Dr T.M.A. Pai Hospital and [3]Department of Community Medicine, Kasturba Medical College, Manipal University, Manipal, Karnataka, India

Case 2

KZ (Dr EM)

- 2016 HPV16 + Other HR HPV + (633304896)
- 2017 Not Detected (639146296) – then ‘no partners’
- 2018 Other HR-HPV (7/11/18 retest 10/11/18) (644262085)
  - 13/11/18 Other HR-HPV (648683699)

- Possibilities:
  - 2017 was a false negative
    - IC was OK – rules out inhibition/interference
    - Leaves - ?low levels of HPV, sample homogeneity <6.4 copies of HPV DNA
    - If tested 10 times ? positive in some
  - 2017 true negative and 2018 = picked up Other HR (new) from fomites
  - 2018 is a false positive
    - repeat test xVial same though
    - 2018 repeat collection also confirmed result

Case 3

JB (Dr LO)

- 5 March 2019 Other HR HPV (646646053)
- 8 March 2019 Not Detected (646646358)

- Possibilities:
  - 5/3/19 False positive
    - Ct of Globin: 22.94
    - Ct of Other HR HPV: 30.14
    - Unable to re-test (discovered October 2019)
  - 8/3/19 False negative
    - IC was OK – Ct of Globin: 24.72: rules out inhibition/interference
    - Leaves - ?lowered levels of HPV, sample homogeneity <6.4 copies of HPV DNA
    - If tested 10 times ? positive in some
  - 5/3/19 was true positive, 8/3/19 false negative
    - recollection interval too short impacting HPV levels below detection limit / sample homogeneity impact
    - Surface epithelium disturbed – 6–12 week interval usual not 3 days
    - Globin differences
      - + 3.3 Ct = 10 fold less
      - 24.72-22.94 = 1.78 = ~5x less cells
    - Cytology cellularity assessment agreed

HPV-negative cervical cancer

A word about HPV-negative cervical cancer

Can we be a bit more specific?

- HPV-independent SCC estimated to represent ~7–11% all cervical SCCs
- HPV-independent adenocarcinoma estimated to represent ~15–38% all cervical adenocarcinomata

HPV-negative cervical cancer

Gets less and less common depending on the histologic type

- Initially, HPV detection was not sensitive
- Increasing sensitivity and ways to detect HPV
- Adenocarcinoma much more likely to be truly HPV negative
- More accurate classification of cervical tumours

Three plausible reasons for a negative HPV result

1. Real HPV-negative cervical tumour
2. False HPV-negative case
3. Incorrect classification of a non-cervical cancer

Reasons for negative HPV in tumours

Limitations of HPV tests

- Sampling errors. Low cellularity tumours; tumours with lots of necrosis; obscuring blood or lubricant; poor fixation or cytolysis
- Low sensitivity of some HPV testing methods applied to formalin-fixed paraffin-embedded tissues
- Histologic misclassification – inclusion of endometrial neoplasms
- Low HPV DNA content (viral load) in tumour. Usually loss of targeted HPV DNA fragment
- Cancer caused by low-risk HPV strains
- Dedifferentiation and subsequent loss of HPV within the tumour may also alter HPV assay results

P16 immunohistochemistry

- P16 immunohistochemistry is used as a surrogate marker for HPV
- Most HPV positive tumours show strong diffuse staining
- HPV negative tumours may also be positive
- HPV positive tumours may also be negative

Questions