

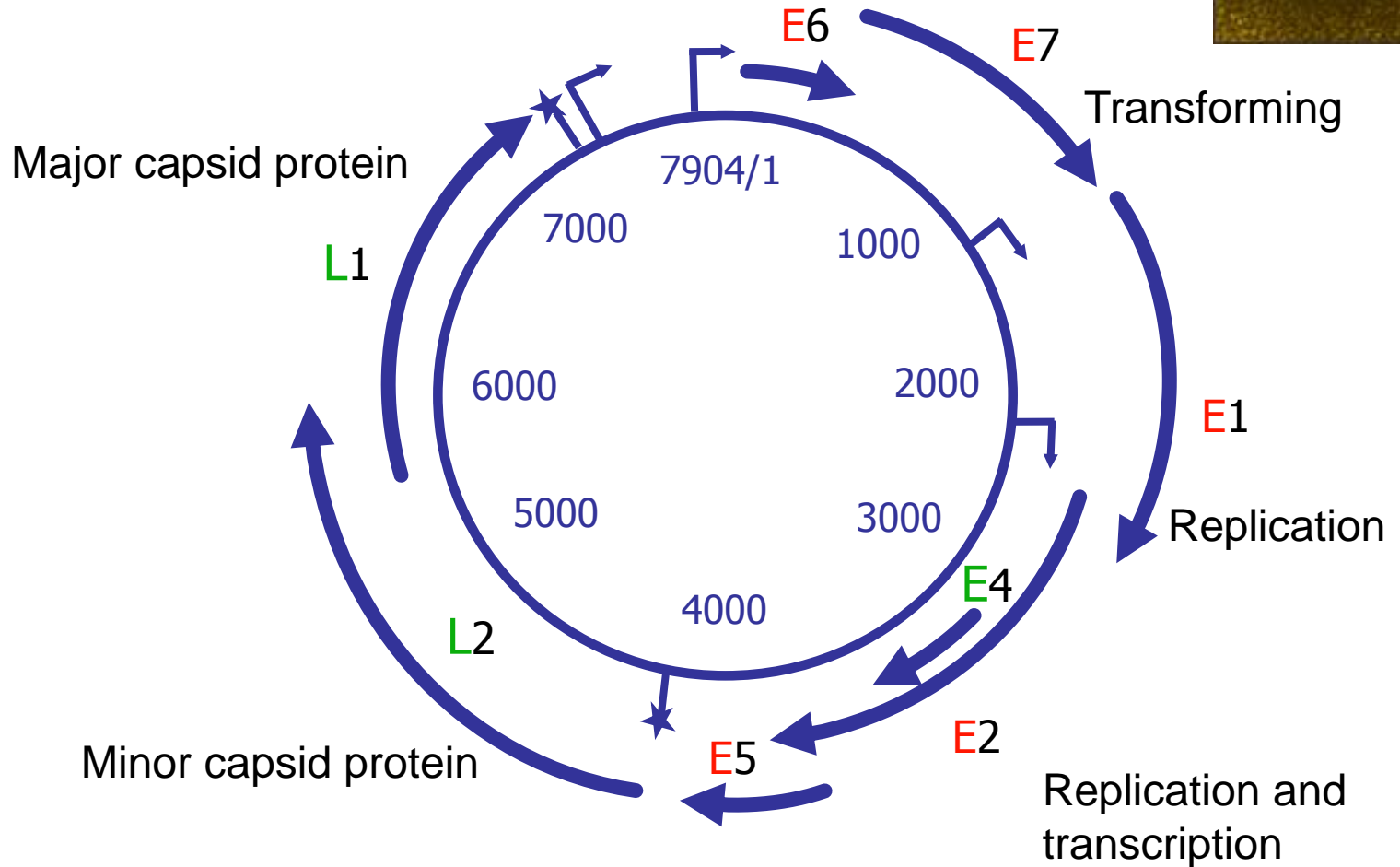
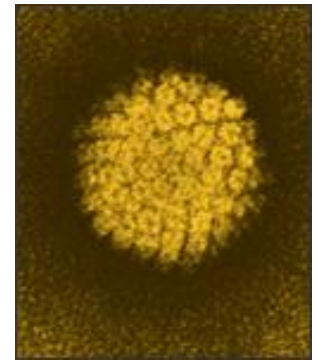


# HPV Technologies update

Margaret Sage  
November 2018

# The HPV genome

is divided into Early and Late regions



# hrHPV Testing: Detecting high-risk HPV subtypes

Test is positive if any of the selected high-risk HPV subtypes are detected

*c.f.* specific genotyping - identifies specific subtype/s

Three PCR-based DNA amplification methods in use in NZ:

## Roche Cobas 4800 HPV Test and Abbott Realtime High risk HPV Assay

- detect the L1 region of the HPV genome
- detect 14 high-risk HPV genotypes: identifies HPV 16, HPV 18 separately and 12 “other” hrHPV subtypes: 31,33,35,39,45,51,52,56,58,59,66,68
- can both be used with either ThinPrep or SurePath

## BD Onclarity HPV Assay

- Detects the E6/E7 region of the HPV genome
- Detects 14 high-risk HPV genotypes: identifies HPV 16,18,31,45,51,52 separately and groups the remainder into three other groups
- Can be used with either TP or SP but optimised for SurePath

# Other hrHPV test methodologies

**Hybrid-Capture 2 (Digene):** was initially the standard test used in research-based clinical trials. Uses a **DNA-RNA hybrid capture technique** for **13** subtypes: HPV 16, 18,31,33,35,39,45,51,52,56,58,59 and 68

## **Aptima® HPV assay (Gen-Probe)**

Identifies **14** high-risk genital HPV types. Detects **E6 and E7 messenger RNA** and so differs from all other currently approved HPV assays, which detect HPV DNA. The test claims to be more specific at identifying cervical lesions *c.f.* the presence of HPV per se.

## **Cervista (Hologic)**

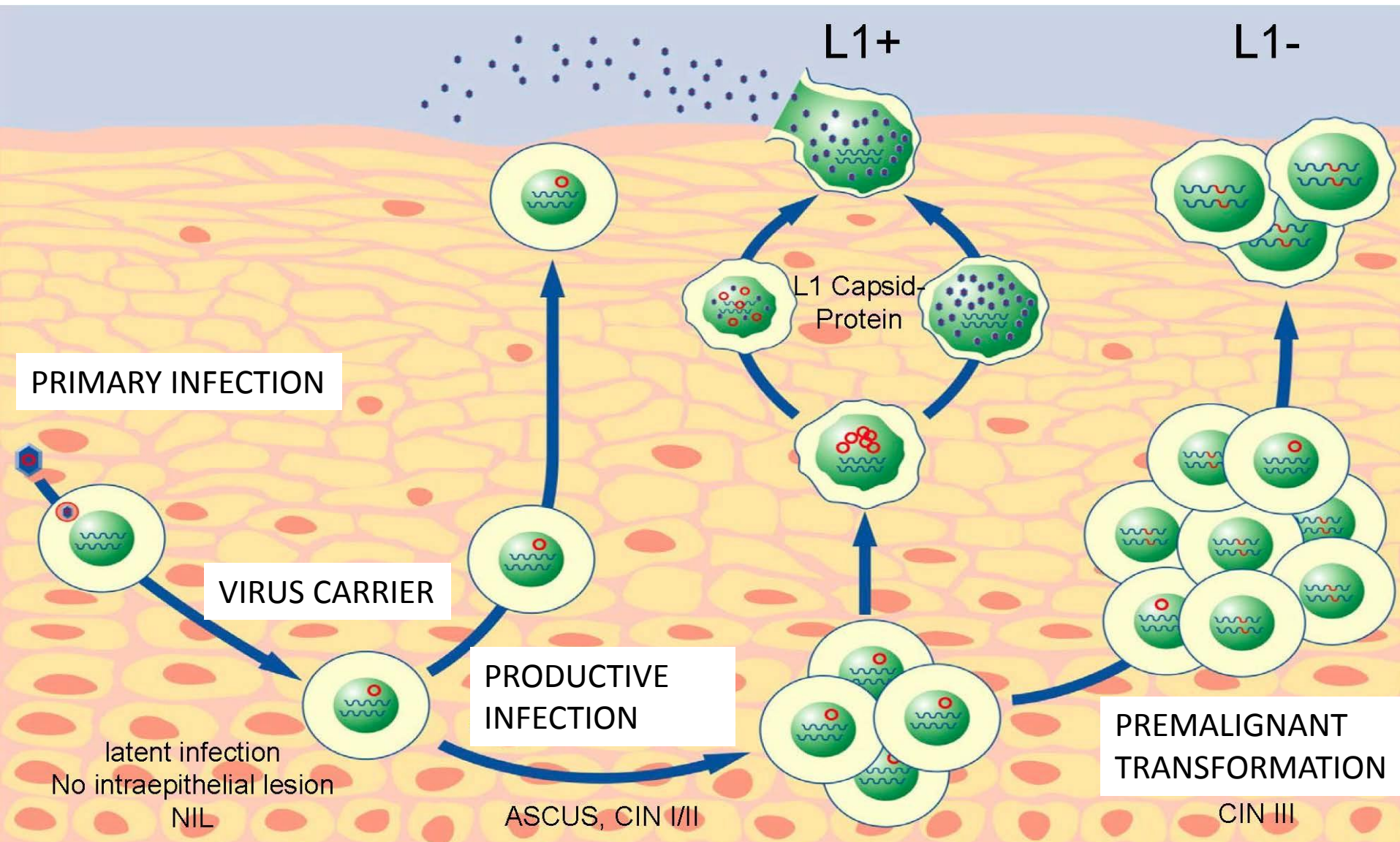
Isolated DNA is mixed with probes that specifically recognise **HPV DNA** for **14** high-risk subtypes. This reaction is detected by another substance that produces light, which measured to determine the presence of HPV.

## HPV Genotyping techniques

Genotyping is performed by amplifying each genotype using primers and probes specific for each HPV type. Differences in nucleotide sequence between HPV genotypes make it possible to amplify and detect each HPV type individually by PCR.

Sequencing is another approach for genotyping. With this method, a woman's HPV DNA is amplified and sequenced. The sequence of the amplified product can be compared with a reference sequences with known genotypes using various data bases.

- Role of genotyping likely to become increasingly important in clinical pathways and to monitor prevalence of HPV subtypes as immunisation rates increase.



"Human Papillomavirus and Related Diseases From Bench to Bedside A Diagnostic and Preventive Perspective"

Ed: Davy Van den Broeck, ISBN 978-953-51-1072-9, Published: April 30, 2013 © The Author(s).

**Chtp 4: HPV L1 Detection as a Prognostic Marker for Management of HPV High Risk Positive Abnormal Pap Smears (Ralf Hilfrich)**

Type-specific oncogenic human papillomavirus infection in high grade cervical disease in New Zealand

Leonardo Simonella, Hazel Lewis, Megan Smith, Harold Neal, Collette Bromhead and Karen Canfell

*BMC Infectious Diseases* 2013, 13:114

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Aim: to measure the pre-vaccination prevalence of hrHPV in 20-69 yrs women with high-grade squamous and glandular lesions

594 women 20-69 years from NCSP-Register 2009-2011

Group 1: histologically confirmed CIN2/3 or AIS

Group 2: possible or definite high-grade squamous or glandular cytology reports

LBC specimens were genotyped for 37 HPV subtypes

# Results

## Group 1: Histologically confirmed lesions

356 women = CIN2/3    6 women = AIS/glandular dysplasia

Any hrHPV infection: 95%

HPV 16/18: 60%

Most common subtypes: 16 (51.2%) 52 (18.9%) 31 (17.1%) 18 (12.1%)

## Group 2: 594 Women with possible or definite HG cytology

Any hrHPV infection: 87%

HPV 16/18: 53%

Most common subtypes: 16 (44.1%) 52 (16.8%) 31 (15.2%)

Highest relative prevalence of HPV 16/18 in confirmed CIN3 was seen in women 20-29 years of age



# International comparisons

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- The prevalence of **HPV 16 in CIN2/3** was broadly consistent with that in Australia and Europe (about 50%) but higher than that reported for North America, Asia and South/Central America (about 40%)
- The prevalence of **HPV 18 in CIN2/3** was broadly consistent with Australia and North America but higher as that reported for Asia, Europe and South/Central America
- The prevalence of **HPV 52 was higher** than that reported from other regions

# Type distribution of human papillomavirus among adult women diagnosed with invasive cervical cancer (stage 1b or higher) in New Zealand

Peter Sykes, Kusuma Gopala, Ai Ling Tan, Diane Kenwright,  
Simone Petrich, Arico Molijn, and Jing Chen  
*BMC Infectious Diseases* 2014,14:374

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- Women  $\geq 18$  years of age with ICC FIGO stage 1b or higher diagnosed 2004 - 2010 from five NZ hospitals
- Stored paraffin embedded cervical specimens were used, with consent.
- Cervical specimens underwent histopathological review and assays were performed for HPV genotyping: **227 Invasive cancer cases**
  - 159 (70.0%) = squamous cell carcinoma (SCC)
  - 61 (26.9%) = adenocarcinoma (ADC)
  - 7 (3.1%) = adenosquamous carcinoma (ASC)

Results: HPV was detected in 88.5%

93.1% = SCC

77.9% = ADC/ASC

# Results: HPV Subtypes

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For the 227 ICC cases: HPV 16 and 18 were the most frequent

HPV 16 = 51% : 56% of SCC	40% of ADC/ASC
HPV 18 = 21% : 15% of SCC	35% of ADC/ASC

For the 201 HPV positive cases:

HPV 16 + 18 = 81.1%

Frequent non-16/18 types were types 31, 45, 52, 59 and 33

Two low-risk HPV types (11,70) = one SCC each

Multiple subtypes

5.5% had multiple subtypes present (Both SCC and ADC/ASC)

# Results: Ethnicity

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- 15% of the women (n=34) with invasive cervical carcinoma were of Maori ethnicity
- There was no significant association between ethnicity and either HPV detection rate, histological cancer type or stage of disease at diagnosis
- Subtype prevalence was similar:
  - HPV 16: 58.8% Maori 49.7% non-Maori
  - HPV 18: 11.8% Maori 22.3% non-Maori
  - Minor variations in non-16/18 subtype distribution

# International Comparison

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HPV infection and subtype distribution rates are comparable with other international studies

Supports the evidence that the biology of HPV infection and its role in cervical pathology is consistent internationally and across different ethnicities

# Use of hrHPV testing in New Zealand

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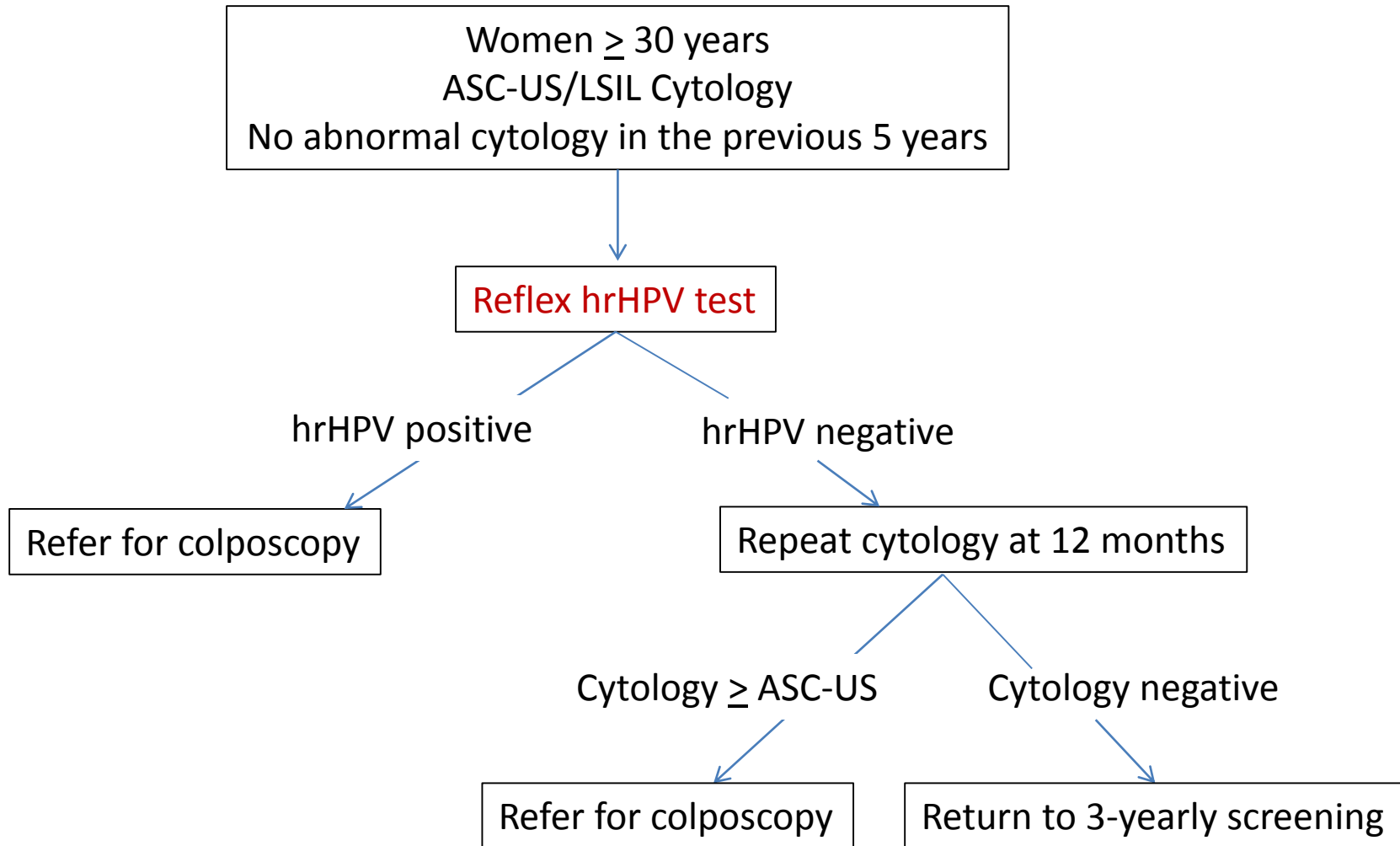
Introduced nationally on 1 October 2009

Funded for use in three clinical situations

1. Triage of ASC-US and LSIL in women 30+ years (with no abnormal cytology in the last 5 years)
2. Test of Cure after treatment of HSIL
3. Discordant results at colposcopy

# 1. ASC-US and LSIL triage

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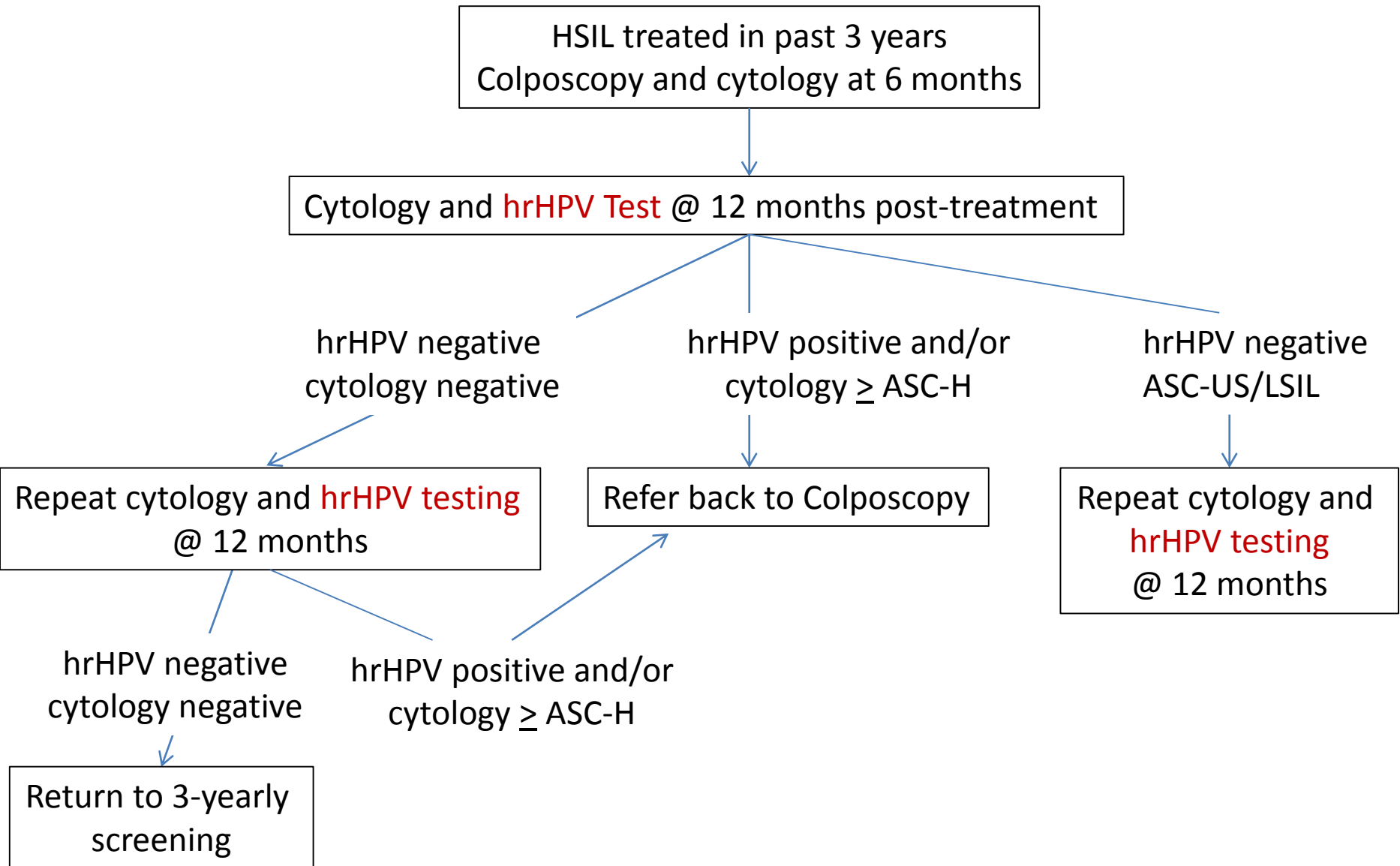
## 2. Test of cure after treatment of HSIL

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- Used to identify women who have been successfully treated for **high-grade squamous lesions**.
- involves **cytology plus hrHPV testing** performed on **two occasions 12 months apart**
- If all four results are negative, women can return to three yearly screening instead of remaining on annual screening for life.

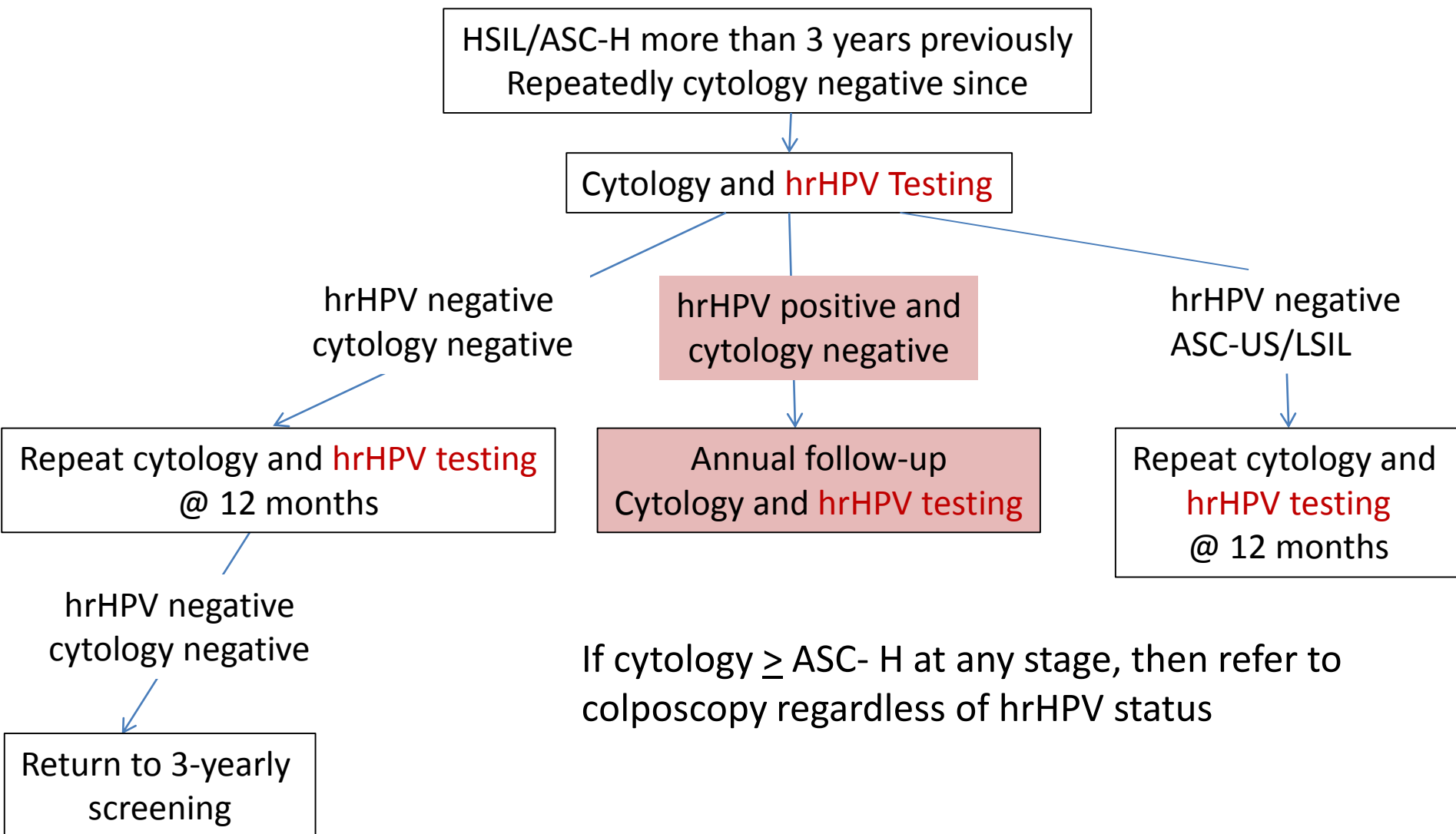


# High-grade lesions treated in the past 3 years



# High-grade lesions > 3 years previously, with subsequent repeated negative cytology

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## 3. Specialist testing

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hrHPV Testing is ordered by colposcopists to assist with case management, particularly when there are discordant results (colposcopy/histology/cytology)

In practice, laboratories perform any hrHPV tests ordered by colposcopists

# Glandular lesions

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Using hrHPV testing as part of a Test of Cure regimen is not recommended after treatment for high-grade glandular lesions

Reason: The hrHPV positivity rate in high-grade glandular lesions is lower than that of high-grade squamous lesions

## Using hrHPV testing for primary screening

- More sensitive than cytology for detecting high-grade disease but less specific because the test detects infection, not the presence of a lesion
- UK ARTISTIC trial data: of **18,386** women (revealed arm, screening)
  - Cytology +ve = 2,061
  - Cyto -ve, HPV +ve = 1,675
- At any given time, **8-10% of women in a screening population will be cytology negative and hrHPV+ve** but this rate varies considerably with age

The ARTISTIC Trial: HPV primary screening trial in the screening porigr

hrHPV +ve rates: 20-24 years = 40%

25-29 years = 28%

30-39 years = 15%

40-49 years = 9%

50-64 years = 7%

- 80% of sexually active women are estimated to have an HPV infection at some time in their lives: young women particularly, acquire lesions readily and clear them quickly.

# A negative HPV test gives greater reassurance than a negative cytology test

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Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow up of four European/UK randomised controlled trials comparing HPV screening and cytology-only screening

Italy - NTCC

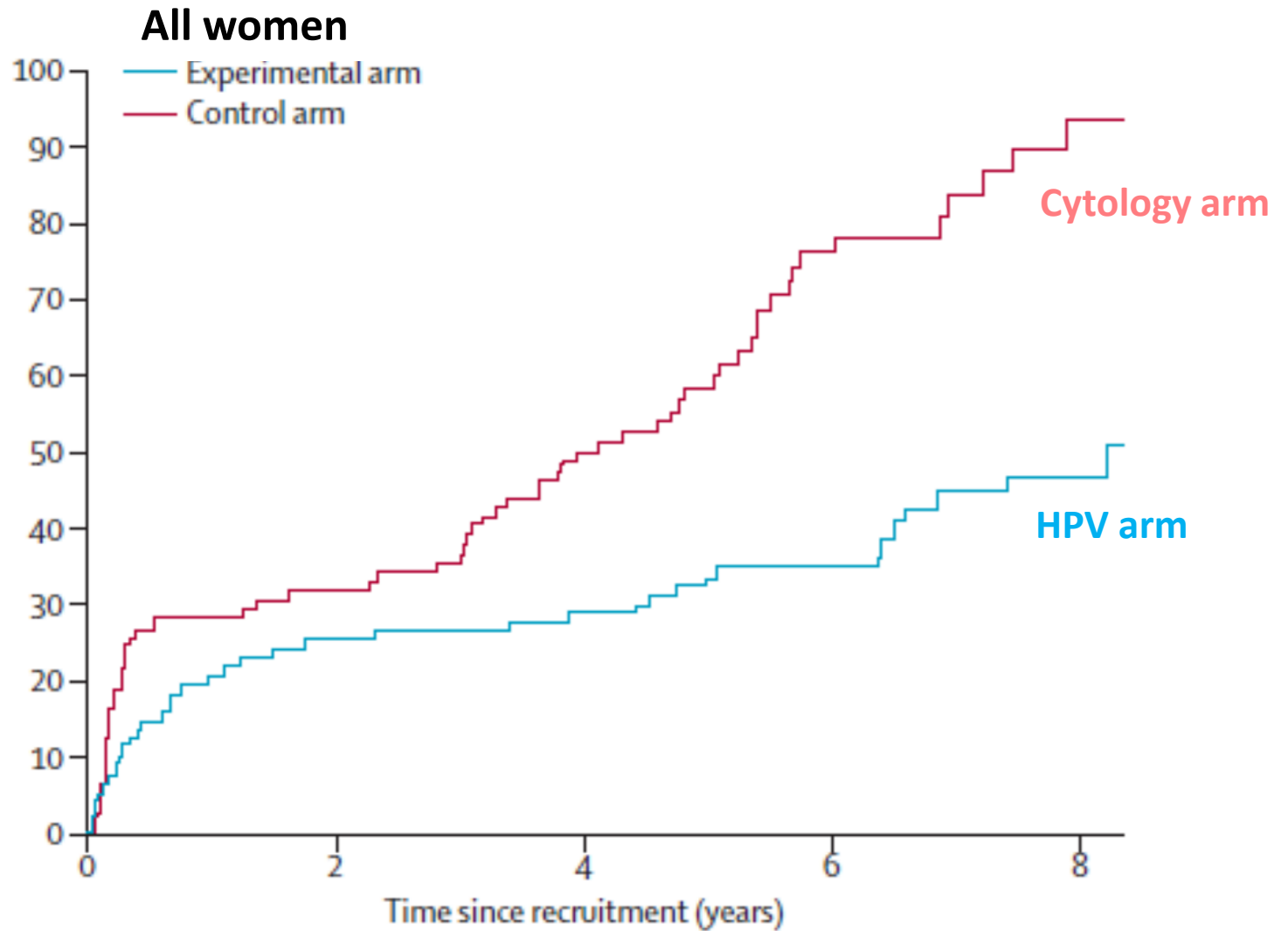
Netherlands - POBASCAM

Sweden - Swedescreen

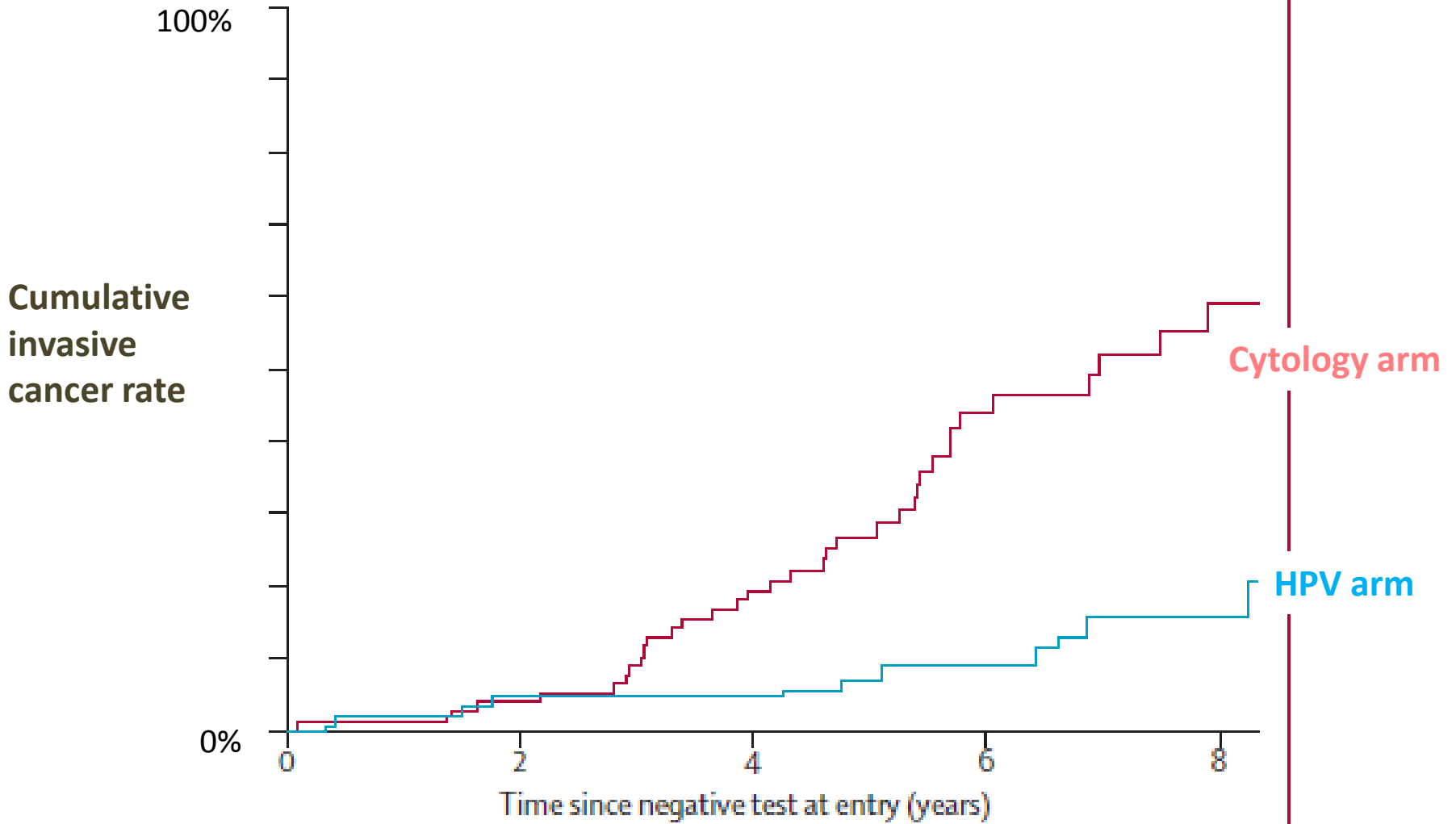
England – ARTISTIC

- Detection of invasive cancer was similar for the first 2.5 years but was significantly lower in the HPV screening arm thereafter
- HPV-based screening provided 60-70% greater protection against invasive cancer compared with cytology

Cumulative  
invasive  
cancer rate



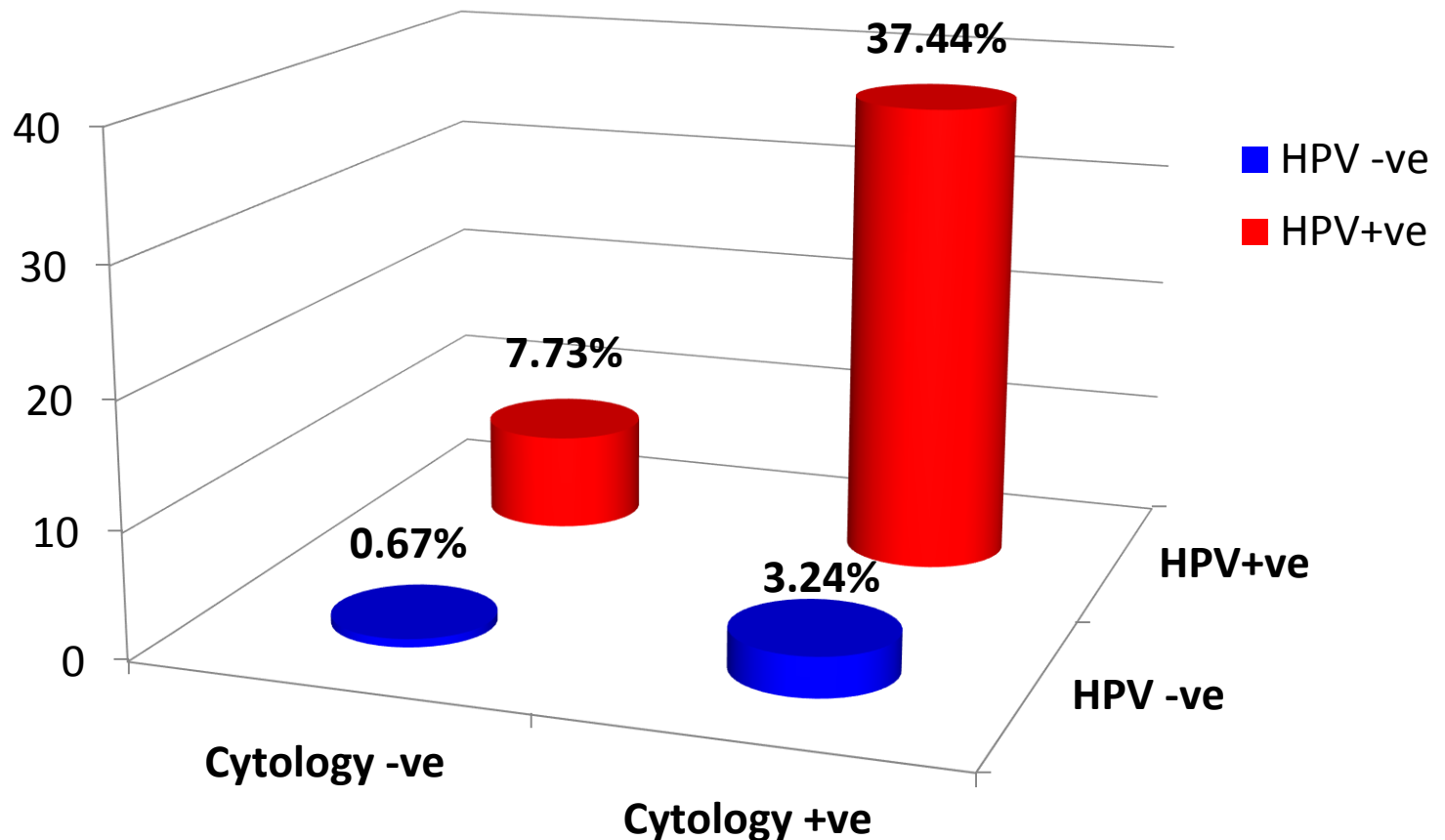
## Women with a negative test at entry





# The ARTISTIC Trial results

Cumulative % CIN 2+ outcome by cytology and HPV status at entry



Cervical cancer risk for 330,000 women undergoing concurrent HPV testing and cervical cytology in routine clinical practice at a large managed care organisation *Lancet Oncology* 2011;12(7):663-72 Katki et al

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**Prospective study** analysing the cumulative CIN3+ and invasive cancer rates for 331,818 women aged 30+ who were cotested and **followed for 5 years.**

# Findings for invasive cancer

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- 5-year cumulative incidence was extremely low for  
315,061 HPV-ve women: 3.8/100,000
- only slightly higher than for  
306,969 co-test -ve women: 3.2/100,000
- and half that of the rate for  
319,177 cyto -ve women: 7.5/100,000

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HPV+ve cyto -ve women had 29% of the cancers and 63% of the adenocarcinomas

# Cervical cancer prevention strategy

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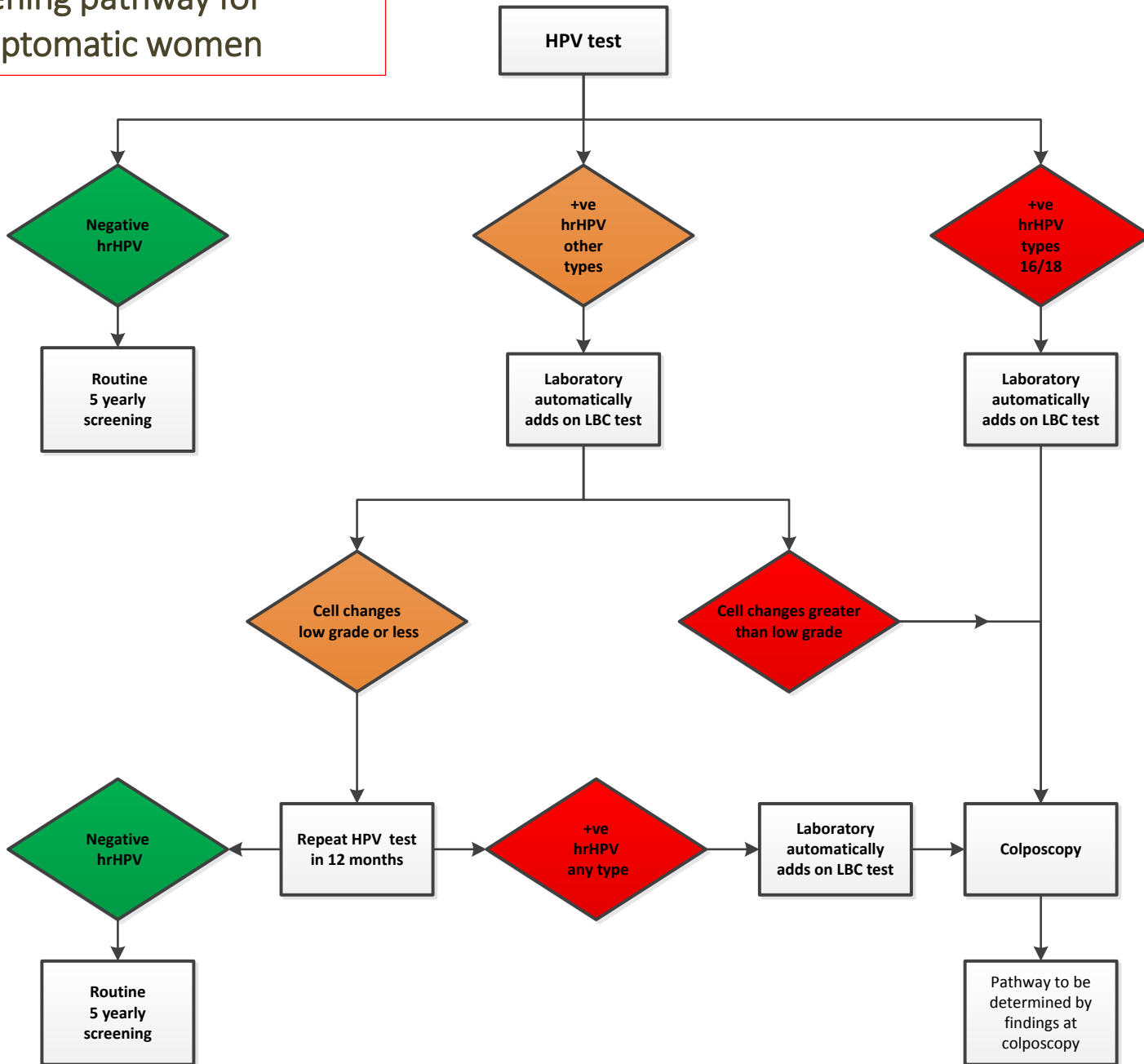
Primary prevention: HPV Immunisation

Secondary Prevention: Screening tests

Currently: LBC cytology every 3 years

2018: hrHPV Testing with partial genotyping and  
cytology triage every 5 years

# Screening pathway for asymptomatic women



Key	
Low	Risk of developing cervical cancer
Medium	
High	

## Potential for self sampling for hrHPV screening

- A sample for cytology needs to be taken from the transformation zone so that abnormal cells are sampled
- hrHPV testing doesn't require such as specific sample –a high vaginal swab can be used
- Opens up the possibility for self-sampling, particularly as a way to increase screening coverage for women who aren't currently being screened
- If the hrHPV test is positive, the woman will need to have a speculum examination for a clinician-taken sample for cytology
- Self-sampling has recently been shown to be as sensitive and specific for HPV testing as a clinician-taken sample as long as DNA-based PCR tests are used.
- NZ research projects are underway to investigate the acceptability of this option for NZ women

# HPV immunisation currently

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- Initially offered free to 12-13 year girls from 2008 (with a catch-up programme for women up to 20 years) using Gardasil-4.
- Mainly a school-based programme. Also available through GP's, local health centres and some Family Planning clinics. Primary care can follow up girls who were not vaccinated in the school based programme
- Extended on 1 Jan 2017 to **males and females free up to 27 years of age**, with 2-doses up to age 14 then three doses for 15+ using **Gardasil-9**
- protects against hrHPV types 16 and 18 (70% of cervical cancers) and low-risk types 6 and 11 (90% of genital warts)
- HPV immunisation coverage currently approximately 70% for both males and females

# Immunisation against HPV: Current programme

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Since 1 Jan 2017 immunisation against HPV has been funded for:

- Girls and boys
- school based programme at 12-13 years of age with free vaccination up to the age of 26 years
- Gardasil-9 includes 7 high-risk types plus 6 and 11
- Two doses at least 6-months apart will assist with coverage
- Protects against 90% of invasive cervical cancers

Immunised or not, women still need to participate in regular cervical screening. The vaccine does not protect against all hrHPV types.

Non-immunised women and men also gain considerable benefit because of herd immunity.



# The future is bright for cervical cancer prevention

- HPV primary screening, effective 9-valent vaccination and the possibility of self-sampling to increase coverage will combine to have a **major impact** to further reduce invasive cervical cancer rates
- **Coverage remains as important as ever**: women who aren't screened or immunised don't get the benefits of all the research and development that has occurred in the last 20 years
- **Disparities in coverage** between different ethnic groups in NZ **remains a major challenge** for the NCSP. Self-sampling may help considerably with this.