Could DNA methylation be used as a primary cervical screening method?

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Current Primary testing methodologies

Cytology

- LBC

- Hologic ThinPrep (20 ml)
- BD SurePath (10 ml)

20 years

HPV Testing

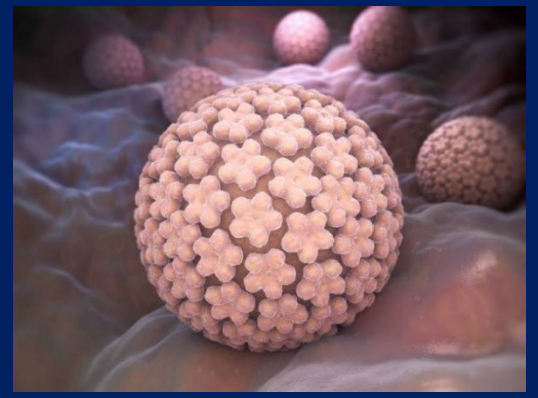
Only 11 approved tests:

DNA

- Qiagen HC2
- Abbott RealTime
- Roche Cobas 4800
- Anyplex II
- BD Onclarity
- Cepheid Xpert HPV

RNA

- Hologic Aptima



Arbyn et al., clinical Microbiology and Infection, 2021

DNA methylation as a primary screening test

Team NO

- Not sensitive or specific enough
- Expensive
- Not ready for clinical implementation
- No evidence based
- No more change

VS

Team YES

- Sensitive or specific enough for specific populations
- Affordable If we reduce the cost to one test with less referral to colposcopy
- Ready for clinical implementation with NGS
- We will bring the evidence

Why?

Which test?

Who?

Where?

WHY?

How can we improve Cervical cancer screening programme?

Current setting

Smear test

Triage test

hrHPV Positive → Cytology

Transformative approach to screening

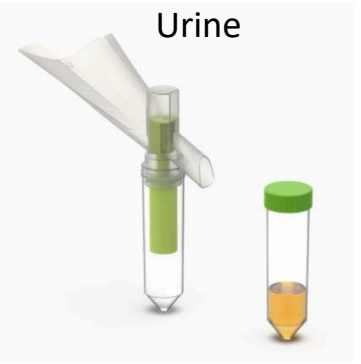
Molecular triage test

Proposed screening setting

Self-sampling

Vaginal

Urine



Triage test

~~hrHPV Positive~~ → DNAmePap Referral → Cytology

Poor specificity of HPV DNA tests

⇡ Increasing referrals numbers (35%)

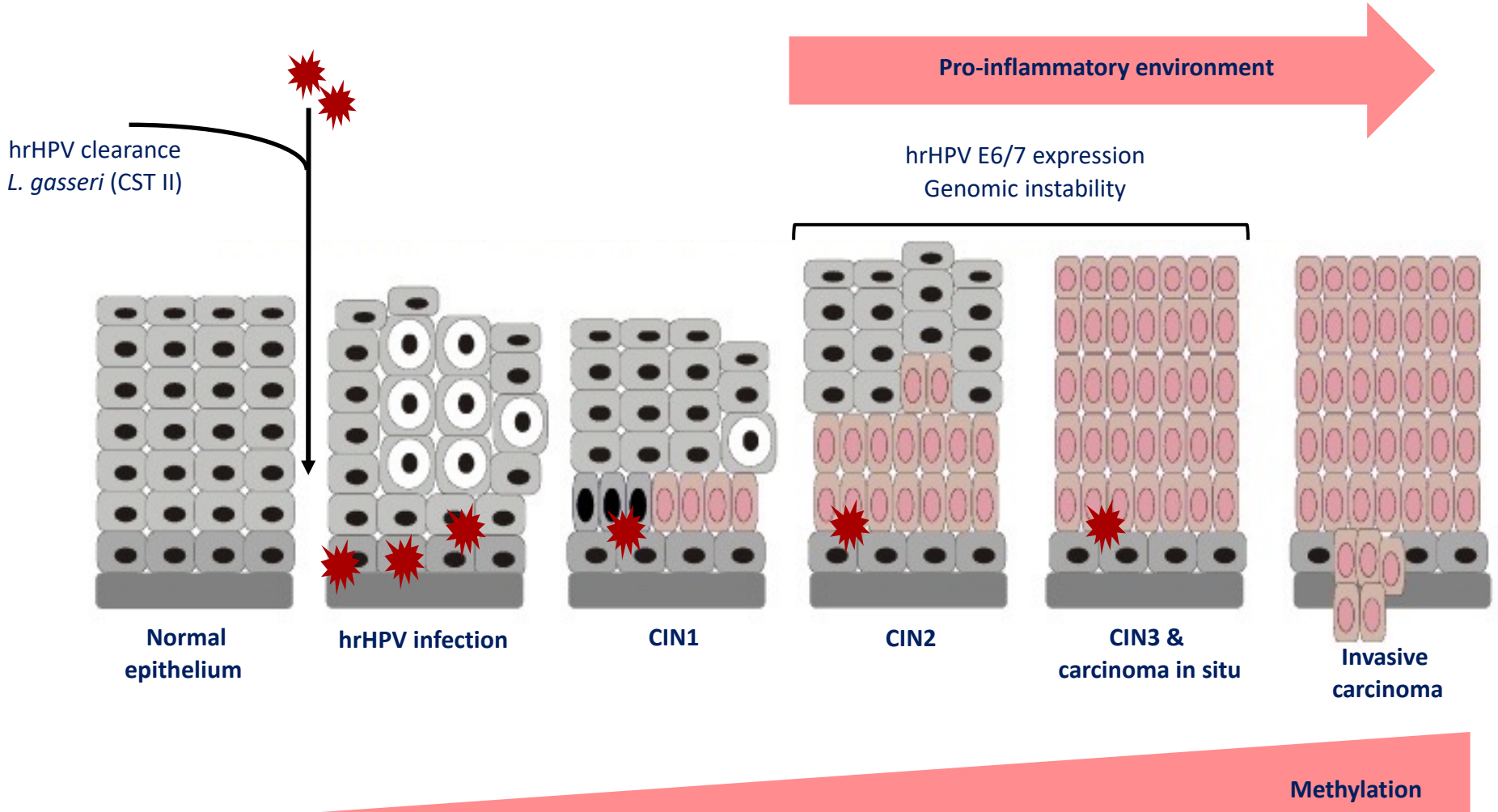
⇣ Decreasing attendance (69.3% in 2021)

⇡ Increasing vaccinated women

Rebolj *et al.*, Cancer Cytopathol. 2022; Pesola, et al BJOG, 2020.

WHY?

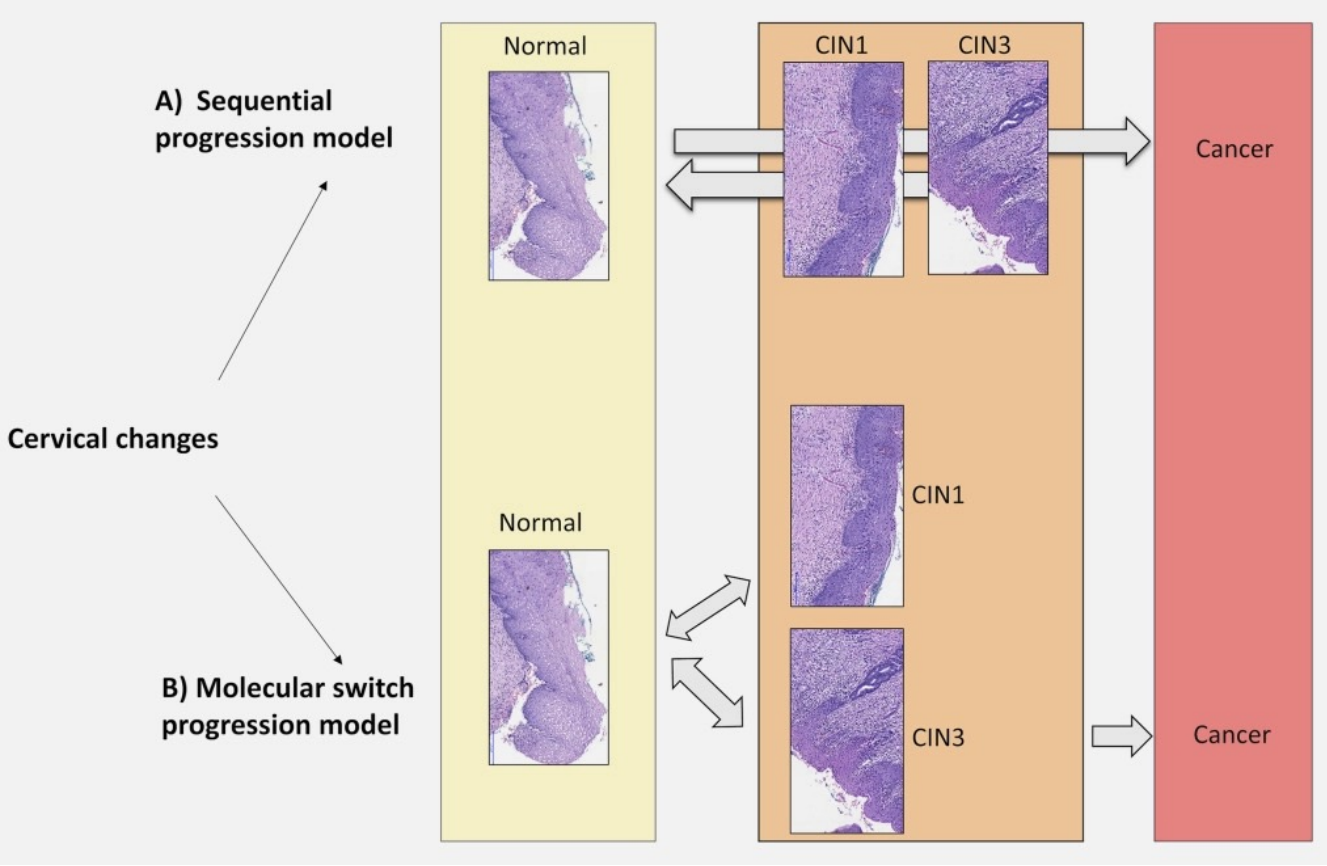
Changes in DNA methylation is characteristic of severe cervical cancer disease



FAM19A4/miR124-2, FAM19A4 alone, EPB41L3, CADM1/MAL/ miR124-2, ASTN1/DLX1/ITGA4/RXFP3/SOX17/ZNF671 and POU4F3, HPV16L1 and L2, HPV18, HPV31 and HPV33

Progression from hrHPV infection to cancer is not linear

WHY?



Nedjai B, et al., Int J Cancer. **2018**

1. Progression from normal epithelium to CIN1 or CIN3 is usually promoted by the same HPV type but occurs via distinct DNA epigenotypes, thus favouring the "molecular switch" model.

2. Methylation predicts CIN2 progression to CIN3:

Louvanto K et al. IJC. 2020.

Kremer WW, et al, J Clin Oncol. 2022

Which DNA methylation test?

Types	Advantages	Disadvantages
DNA Methylation testing **Investigated for triage purposes** QIAsure: FAM19A4 and miR124-2 S5 classifier GynTect®	Pooled 70% set-specificity and 68.6% sensitivity for CIN2+, 71.1% for CIN3+ (Kelly *et al.,* 2019) Distinguish between persistent and transient HPV infections (Clarke *et al.,* 2012) Can be done on self-samples (Nedjai et al, unpublished) Cost-effective if multiplexed or NGS Can be implemented in a clinical lab setting Can be automated	Requires extensive validation in large cohorts Requires large scale validation on screening population

S5 methylation classifier

BACKGROUND

- Pyrosequencing assay for detection of Cervical Intraepithelial neoplasia (CIN2+)
- Developed in a UK referral population
- Combination of host and viral CpGs
- Any starting material
- PCR-based method using bisulfite converted DNA
- Validated in cohorts in US, Canada, Europe, and South America

30.9 ***EPB41L3*** 3 CpGs	13.7 **HPV16L1** 2 CpGs	4.3 **HPV16L2** 5 CpGs	8.4 **HPV18** 6 CpGs	22.4 **HPV31 L1** 2 CpGs	20.3 **HPV32 L2** 4 CpGs

Brentall, et al Cancer Biomark. 2015; Lorincz et al Int J. 2016; Nedjai et al , Int J Cancer. 2018; Cook et al Int J Cancer. 2019; Louvanto et al Clin Infect Dis. 2019

PREDICTOR 5.1

A randomised-assignment comparison study of the performance of self-collected vaginal samples for HPV testing when transported under wet or dry conditions, using different collecting devices (Cadman et al, 2021).

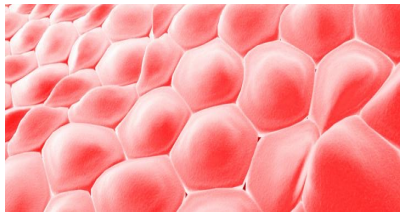
Samples Processing

Samples collection devices

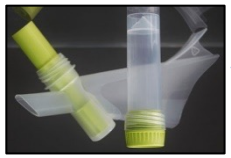
Arm 1 n=300 — *Wet Dacron* / *Dry Flocked Nylon*

Arm 2 n=300 — *HerSwab™* / Qvintip®

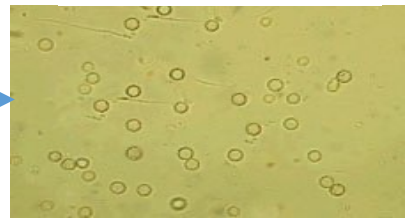
SWAB CELLS n=600



URINE n=503



Colli-Pee



HPV genotyping Tests

BD Onclarity

Genefirst Papilloplex

Qiagen HC2

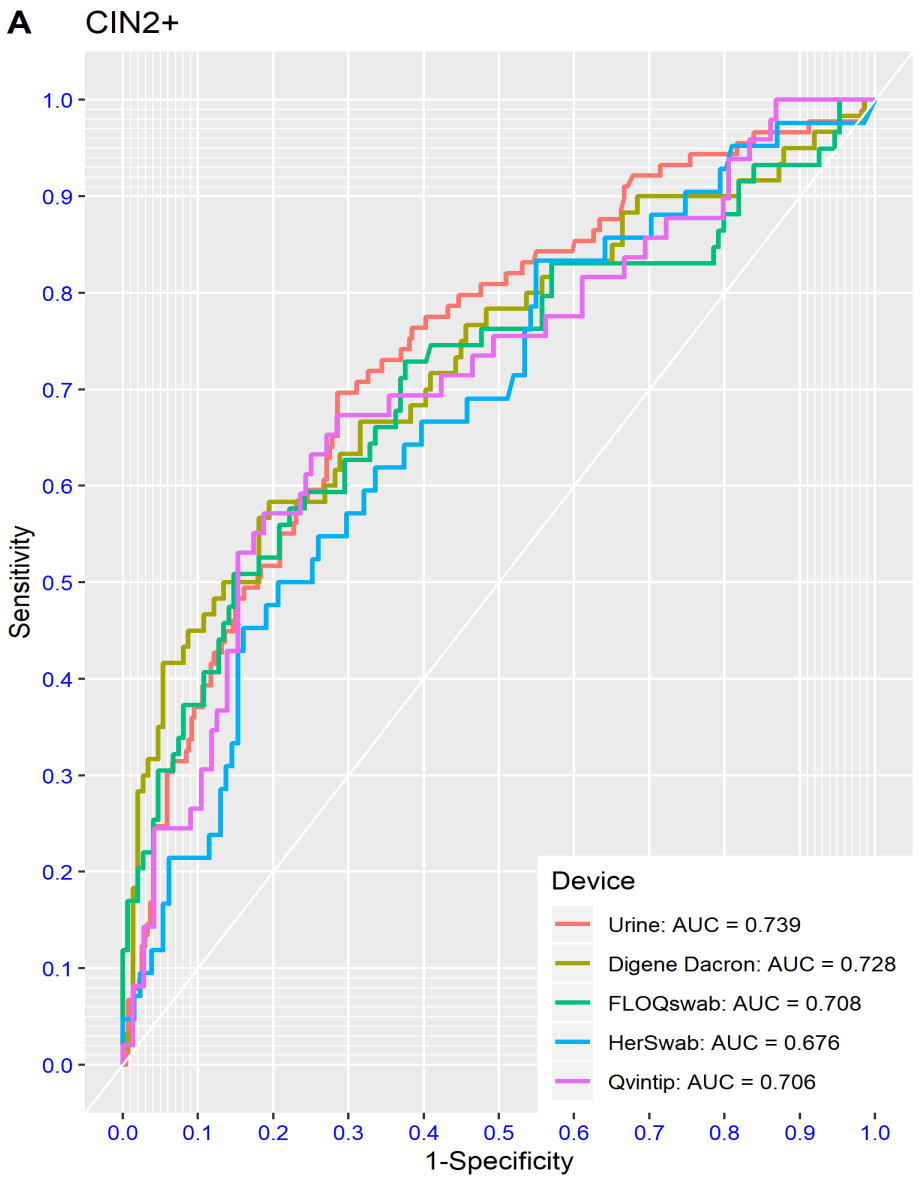
S5 Methylation classifier:

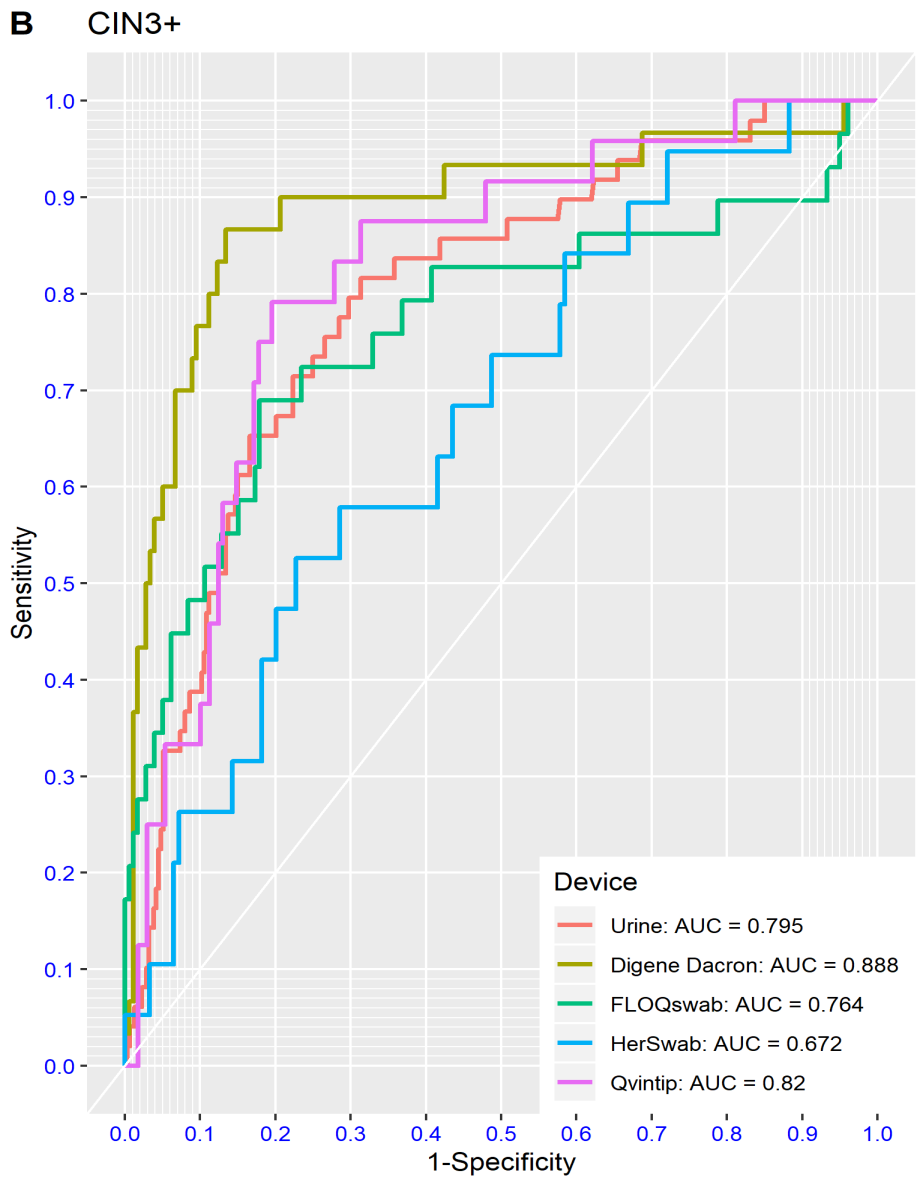
HPV16, HPV18, HPV31 and HPV33

EPB41L3

Results

ROC curves for S5 for detection of CIN2+ and CIN3+ for hrHPV positive women





Nedjai et al manuscript in preparation

ARTISTIC TRIAL

Study Design

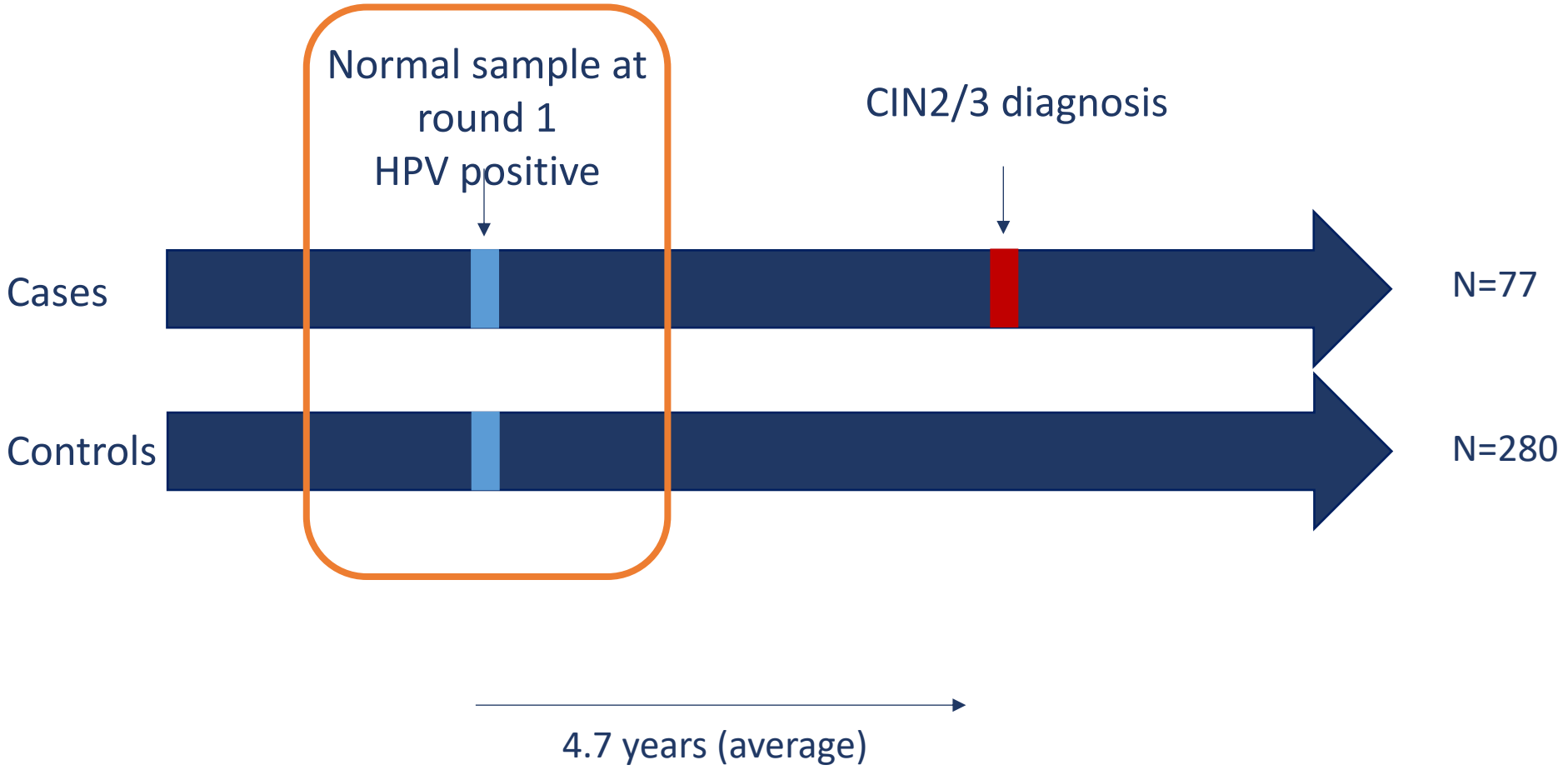
- The ARTISTIC trial cohort was recruited in Manchester in 2001-03 and was followed up for CIN3 and cancer notification through national registration until December 2015.
- Prospective randomised trial comparing routine cytology against routine cytology plus HPV testing in a screening population
- 25,000 women aged 20-64 who attended general practices for routine cervical screening

Gilham C, Sargent A, Peto J, *BJOG.* 2019

Kitchener HC, et al Br J Cancer. 2006

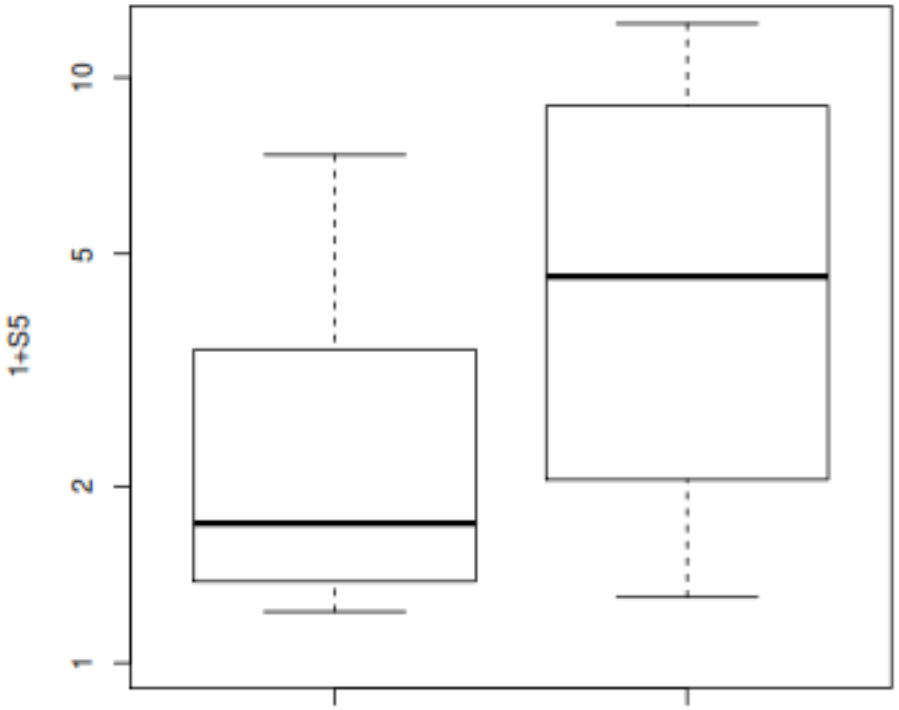
AIM 1

Prediction of progression



S5 Classifier can predict future CIN3

RESULTS

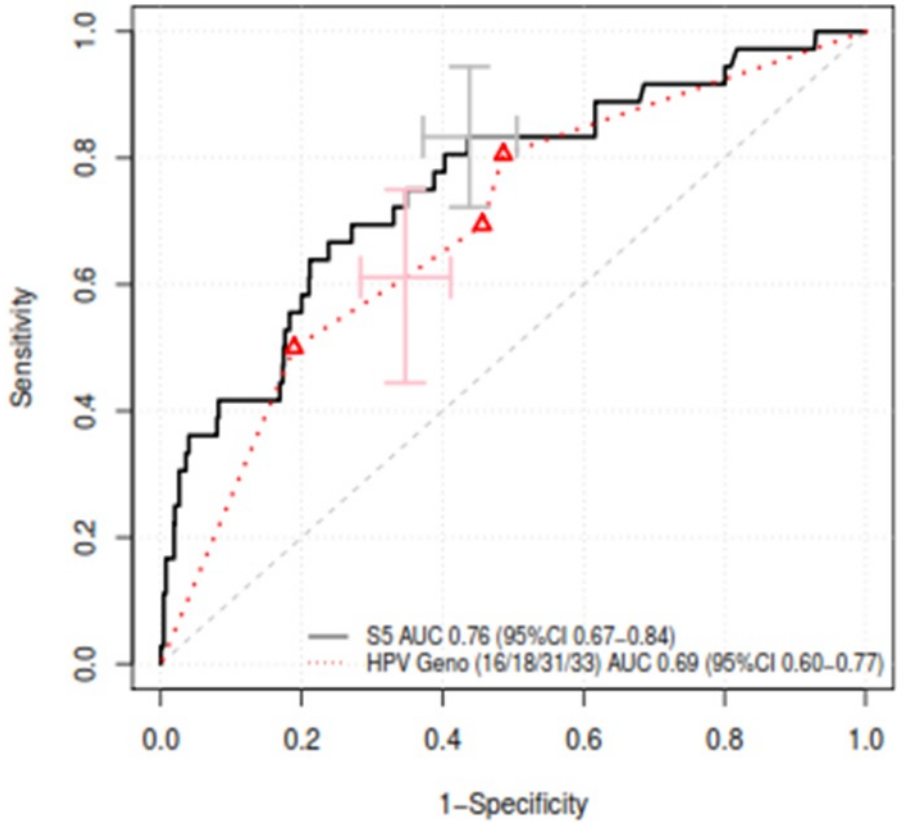


Normal sample at round 1

N=280

Normal sample at round 1 that develop CIN3 within 5 years

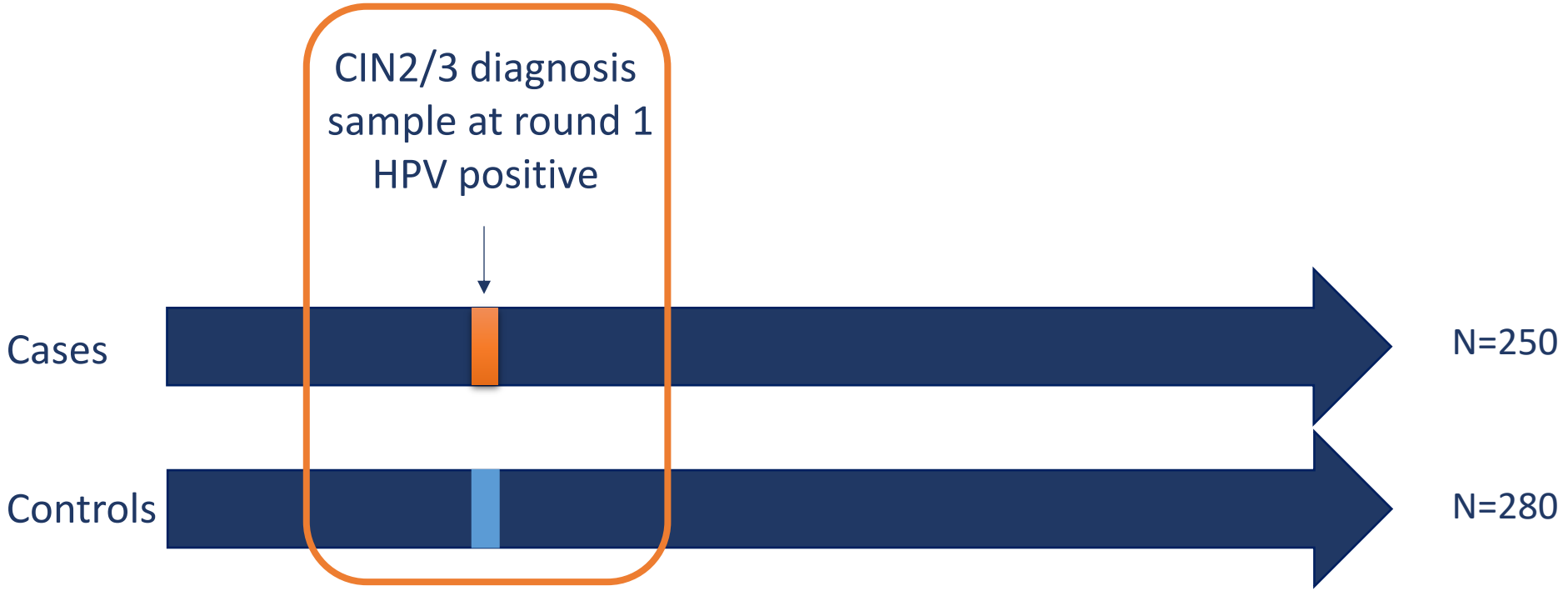
N=36



P value of 0.0001

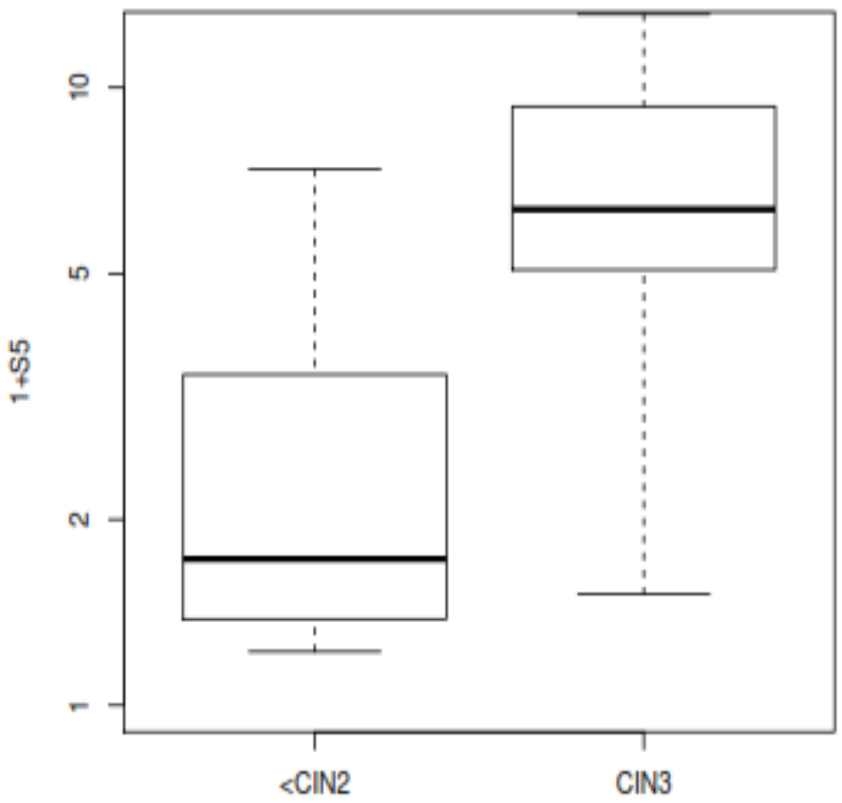
Detection of prevalent CIN2/3 and CIN3

AIM 2

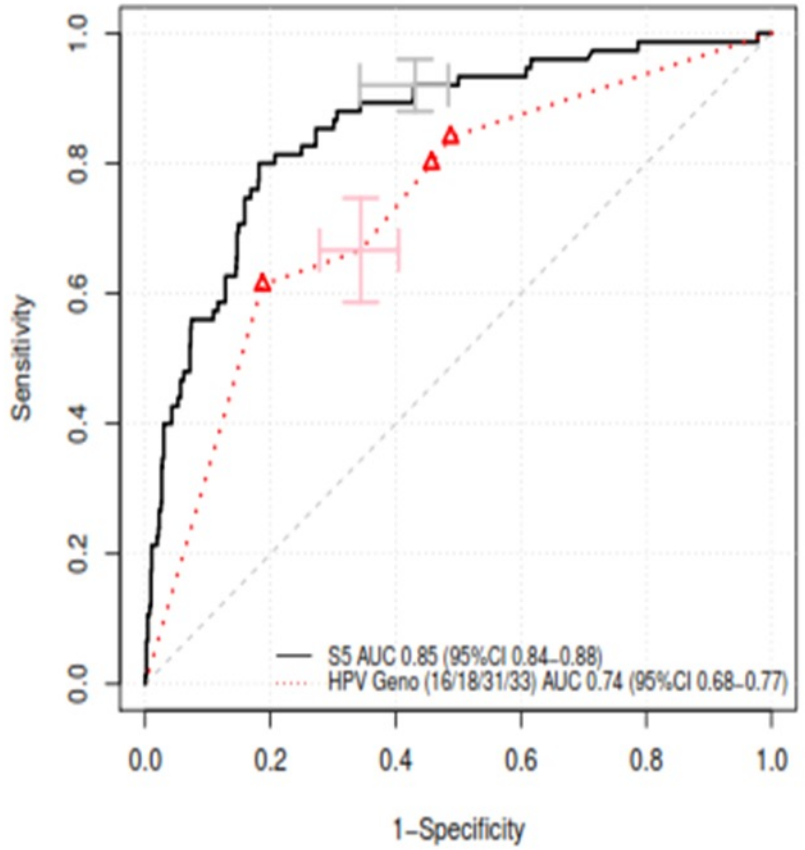


Detection of prevalent high-grade disease at round 1

RESULTS

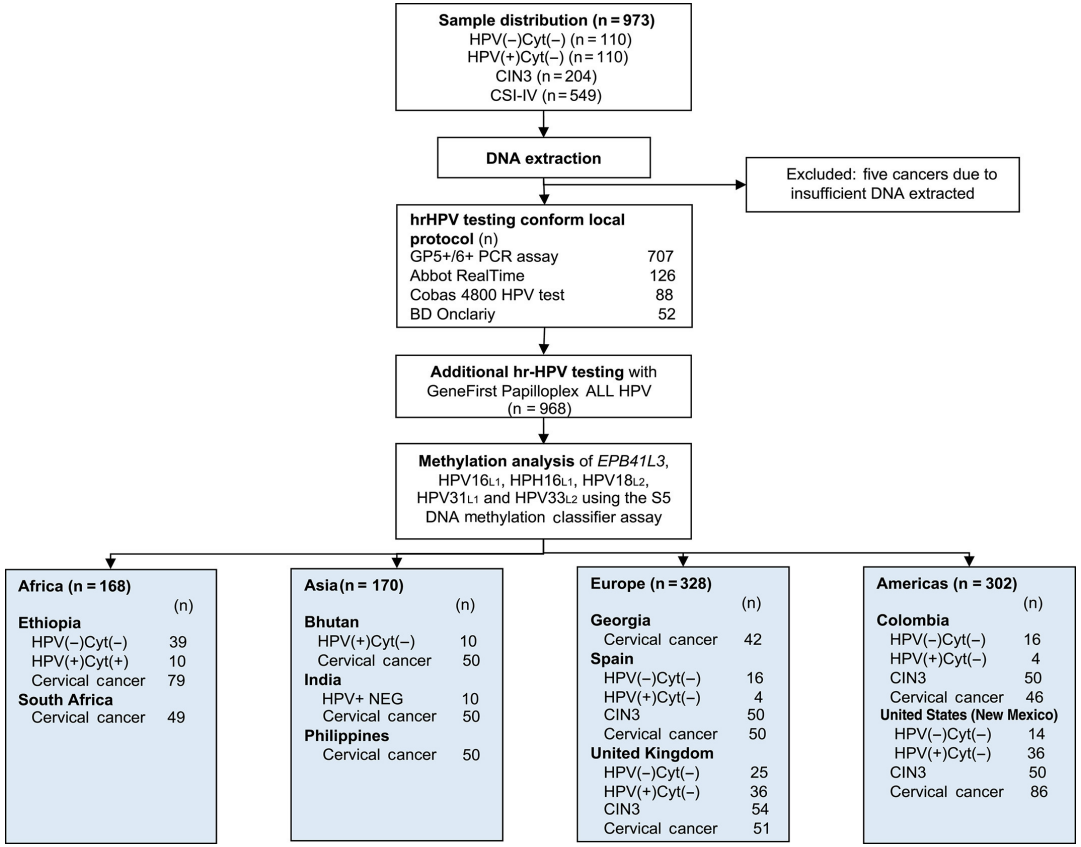


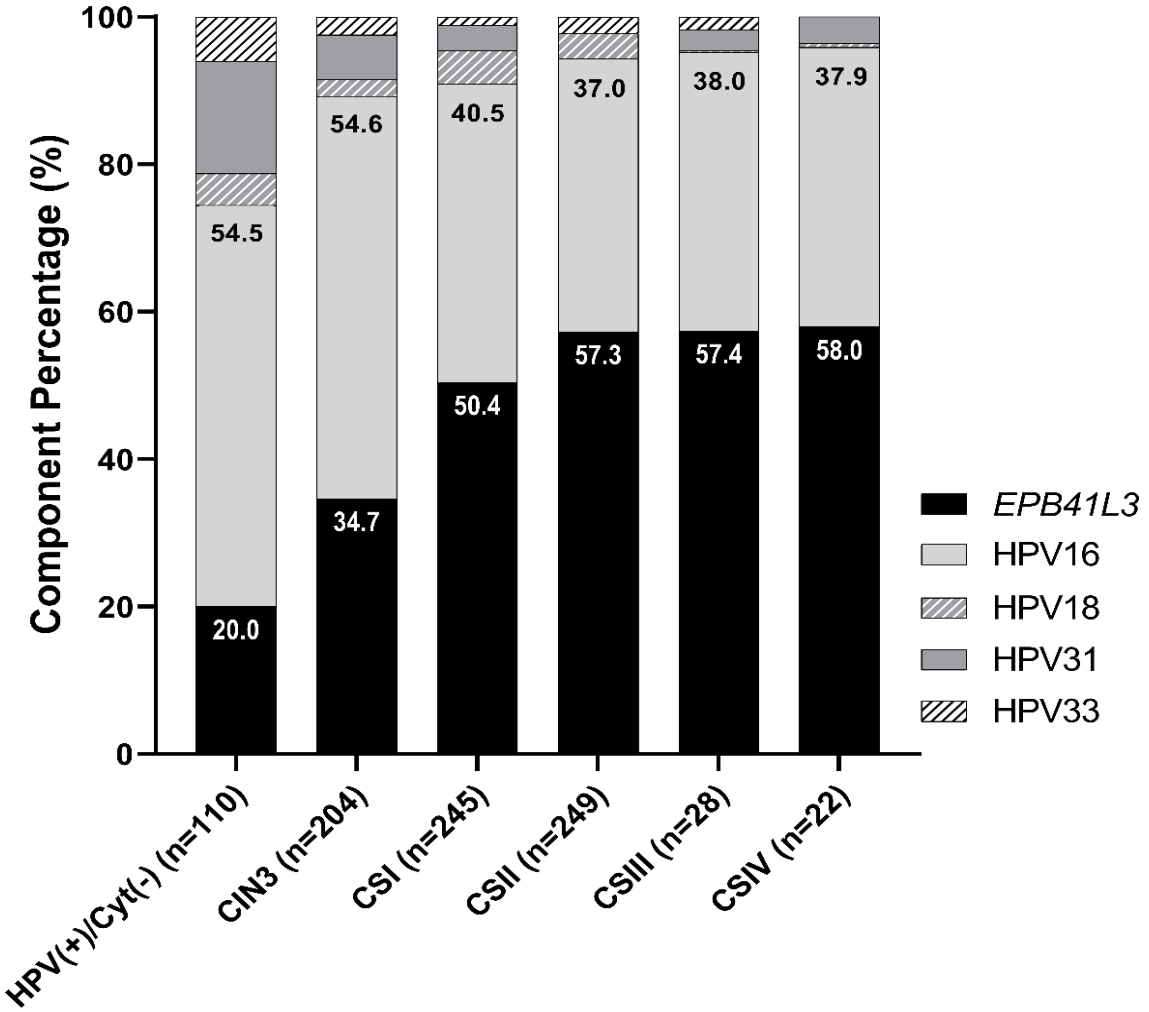
N=280 N=250



P value of 0.0001

Clinical performance of methylation as a biomarker for cervical carcinoma in situ and cancer diagnosis: A worldwide study





A total of 543 out of 544 cancer patients tested positive for S5 at 0.80, yielding a sensitivity of 99.81% (95% CI = 98.34-99.96)

Results

Adjustment of the S5 cut-off for better

Triage scenario	S5 Cut-off	Sensitivity for CIN3+ (95%CI)	Specificity for CIN3+ (95%CI)
HPV(-)/Cyt(-) → estimated vaccinated population	0.80	**99.81** (98.56 – 99.99)	**65.12** (54.59 – 74.31)
	3.70	**93.26** (90.89– 95.05)	**100** (95.72 – 100)
HPV(+)/Cyt(-) → estimated current triage population	0.80	**99.81** (98.56 – 99.99)	**50.60** (43.11 – 58.06)
	3.70	**93.26** (90.89– 95.05)	**83.33** (76.97 – 88.21)

Increasing the S5 cut-off:

- **Large increase** in specificity with a small decrease in sensitivity →
- Decrease in colposcopy referrals

Results

S5 cut-off 3.70 has a better diagnosis potential than HPV testing

	Variables	OR	95% CI	Z value	P value
CIN3	HPV 16/18	2.86	1.77 - 4.62	4.30	Reference
	S5 0.80	4.50	2.71 – 7.46	5.83	<0.0001
	S5 3.70	6.42	3.67 – 11.24	6.52	<0.0001
	*HPV 16/18 and S5 0.80	3.26	2.01 - 5.30	4.79	<0.0001
	*HPV 16/18 and S5 3.70	5.01	2.82 - 8.90	5.49	<0.0001
Cervical Cancer	HPV 16/18	4.80	3.13 - 7.36	7.19	Reference
	S5 0.80	20.94	7.89 - 51.71	7.22	<0.0001
	S5 3.70	45.55	24.67 – 73.38	13.49	<0.0001
	*HPV 16/18 and S5 0.80	6.32	4.08 - 9.80	8.25	<0.0001
	*HPV 16/18 and S5 3.70	14.90	8.69 - 25.56	9.81	<0.0001

Who?

Vaccinated women → Host methylation and other hrHPV methylation will be more informative

Women LWHIV→ methylation will inform about a need for further referral

Where?

HICs-> reduce the number of test = cost effective

LMICs-> only refer women with an increased risk

Conclusions

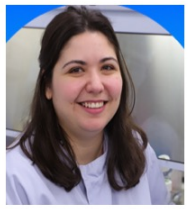
YES but …

- DNA methylation tests need improvement to be evaluated as screening test.
- We need to redefine the screening endpoint to identify only the women with CIN2 who will progress to CIN3. Improved clinical sensitivity for ≥CIN 2,
- We need new Meijer guidelines for the use of DNA methylation tests as triage and as primary screening test?
- In low resource settings can we design an affordable Point of care test test reliable, cheap and mobile?
- In IHCs is it worth testing women with a hrHPV DNA test against the vaccine types?

Molecular Epidemiology Laboratory

Acknowledgements

Dorota Scibior-bentkowska



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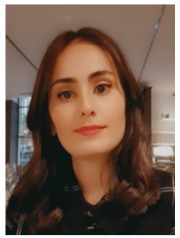

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