

Critically Appraised Topic

Evaluation of new screening protocol for Cervical Cancer screening, one year after implementation, a single center evaluation

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CLINICAL BOTTOM LINE

This Critically Appraised Topic evaluates the first calendar year of primary high-risk HPV screening in UZ Leuven and MCH following introduction of the national algorithm on 1 January 2025. The evaluation covers HPV testing and cervicovaginal cytology from 2022 to 2025, linked at specimen level by patient and sampling date.

Most screening tests (age 30-64) led to an advice to return to routine five-year screening. Approximately 4 per cent required a repeat HPV test after one year and about 3.5 per cent resulted in an immediate recommendation for colposcopy. High-risk HPV positivity showed a clear age gradient and differed substantially between genotype channels. Cytological distributions overlapped between genotype groups, but high-grade findings were most frequent among HPV16-positive women and least frequent in pool B, which contains no genotype covered by the 9-valent vaccine.

Applying alternative triage rules to the same women changed the referral workload considerably, from fewer referrals under cytology-only triage to substantially more under a rule referring every vaccine-covered genotype directly. Compared with a reconstructed pre-2025 estimate, the current algorithm generates more immediate referrals, although differences in available data and in decision rules prevent attributing that contrast solely to the change in primary test.

Laboratory performance was good. Uninterpretable HPV results were rare, turnaround time shortened over the year, and corrections to the requested indication became less frequent after the implementation period. The recomputed advice agreed with the recorded advice in more than 99.5 per cent of comparable tests.

These results describe epidemiology, referral workload and implementation performance. They do not allow the clinical effectiveness of the Belgian algorithm to be evaluated, because histological outcomes are not available for this cohort and a single year of data cannot capture the full screening round.

CLINICAL/DIAGNOSTIC SCENARIO

1. Burden of cervical cancer

Cervical cancer remains the fourth most common cancer among women worldwide. In 2022, approximately 660,000 new cases and 350,000 deaths were estimated (Bray et al., 2024). The burden is unevenly distributed, with large differences in incidence and mortality between countries and most deaths occurring in regions with limited access to HPV vaccination, organised screening and treatment (Bray et al., 2024; Singh et al., 2023).

In Belgium, cervical cancer is less common but remains clinically relevant. In 2023, 610 new cases were registered, while 164 deaths were recorded in 2021 (*Cancer Fact Sheet Cervical Cancer, 2024 / Belgian Cancer Registry, n.d.*). Five-year net survival is 71.0%, ranging from 94.3% for stage I disease to 19.7% for stage IV disease (*Cancer Fact Sheet Cervical Cancer, 2024 / Belgian Cancer Registry, n.d.*). This strong stage gradient illustrates the clinical importance of preventing invasive and advanced disease. Stage-specific survival itself should not be used as evidence of screening effectiveness because of lead-time and length-time bias. Randomised screening trials provide more appropriate evidence for this effect.

2. HPV: virology, oncogenesis and natural history

Most cervical cancers arise after persistent infection with a carcinogenic human papillomavirus (HPV) type. HPV is a small, non-enveloped, double-stranded DNA virus that infects basal epithelial cells. The cervical transformation zone is particularly susceptible to HPV-driven carcinogenesis (Doorbar et al., 2012). Persistent expression of the viral oncoproteins E6 and E7 interferes with the tumour suppressors p53 and Rb and promotes uncontrolled cell-cycle progression (Doorbar et al., 2012).

HPV types differ substantially in their contribution to cervical cancer. In a worldwide study of almost 9,000 HPV-positive invasive cervical cancers, HPV16 and HPV18 accounted for 71% of cases. HPV16/18/31/33/35/45/52/58 together accounted for 91% (de Sanjose et al., 2010).

Most newly acquired HPV infections are transient and become undetectable within one to two years (Kjær et al., 2010; Schiffman et al., 2011). Persistent infection, particularly persistence of the same carcinogenic genotype, is associated with an increasing risk of cervical precancer (Kjær et al., 2010; Schiffman et al., 2011). This risk differs substantially by genotype. In a large longitudinal cohort, HPV16 conferred the highest risk, with a seven-year cumulative CIN3+ risk of approximately 22%. HPV18/45 and, to a lesser extent, HPV31/33/35/52/58 were also associated with higher risks than HPV39/51/56/59/68 (Demarco, Egemen, et al., 2020). Note that HPV35 is the only higher risk (HPV16/18/45/31/33/52/58) genotype not included in the 9-valent vaccine (Gardasil 9, MSD).

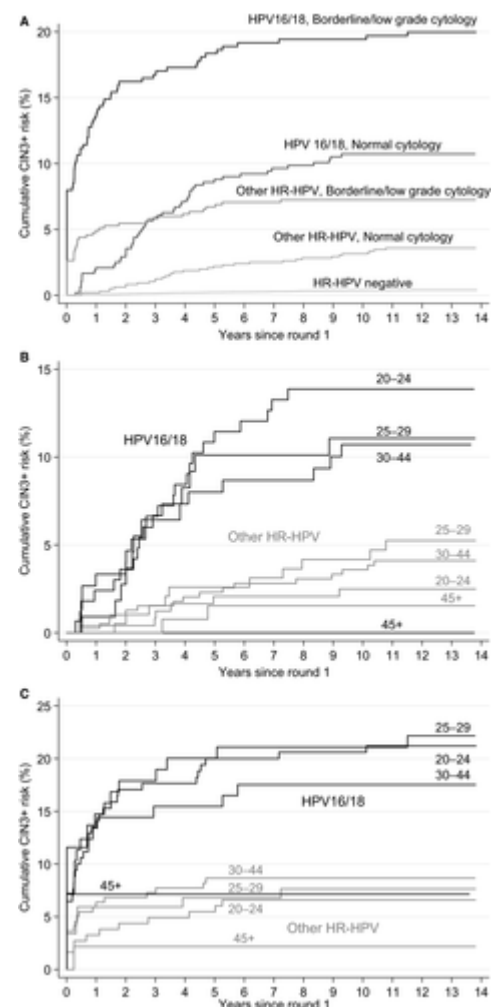


Figure 1: natural history/persistence figure from Gilham et al.

A positive hrHPV test therefore identifies a carcinogenic HPV infection rather than cervical precancer itself. Its clinical significance depends on both genotype and persistence. These characteristics explain both the high sensitivity of HPV-based screening and the need for further risk stratification after a positive result.

3. HPV-based versus cytology-based screening

HPV infection can be detected before clinically relevant morphological abnormalities become apparent, making carcinogenic HPV an attractive primary screening target (Schiffman et al., 2011). HPV-based screening is more sensitive than cytology for the detection of cervical precancer, although this gain in sensitivity is accompanied by lower specificity (World Health Organization, 2021; Yin et al., 2025). In a large multicentre comparison, sensitivity for CIN2+ was 96.9% with HPV-based screening and 79.7% with cytology-based screening, whereas specificity was 88.8% and 92.7%, respectively (Yin et al., 2025). The WHO therefore recommends HPV DNA testing as the preferred primary screening method where appropriate infrastructure is available (World Health Organization, 2021).

The clinical advantage extends beyond cross-sectional detection of precancer. In a cluster-randomised trial in rural India, a single round of HPV screening reduced subsequent advanced cervical cancer and cervical cancer mortality compared with standard care (Sankaranarayanan et al., 2009). In pooled follow-up of four European randomised trials, HPV-based screening provided greater protection against invasive cervical cancer than cytology-based screening, with an overall rate ratio of 0.60 (Ronco et al., 2014). A negative HPV test also provides greater long-term reassurance than negative cytology, supporting longer screening intervals (Ronco et al., 2014).

The higher sensitivity of HPV testing comes with a larger group of screen-positive women. Most HPV infections will not progress to clinically relevant cervical disease (Demarco, Hyun, et al., 2020; Kjær et al., 2010; Schiffman et al., 2011). A positive HPV result can therefore not determine management on its own. The central challenge of primary HPV screening is to distinguish women who require colposcopy from those who can safely undergo surveillance.

4. Triage of HPV-positive women

Triage aims to retain the sensitivity of HPV-based screening while improving specificity for clinically relevant cervical precancer. Cytology provides information on current morphological abnormalities, while HPV genotype and persistence provide additional information on future risk (Bonde et al., 2021; Castle et al., 2009; Demarco, Hyun, et al., 2020; Kjær et al., 2010). Screening programmes combine these factors in different ways.

HPV testing also has limitations beyond its lower specificity. A negative HPV result does not completely exclude cervical cancer. HPV-negative cervical cancers include false-negative results caused by sampling, analytical limitations or misclassification of a non-cervical primary tumour, as well as a smaller group of genuinely HPV-independent cervical carcinomas (Petry et al., 2017; Xing et al., 2021). After histopathological review and additional HPV testing, the proportion of truly HPV-independent tumours is substantially lower than the proportion of cervical cancers initially testing HPV-negative (Petry et al., 2017).

Clinical performance also differs between HPV assays. Assays intended for primary screening should demonstrate adequate and reproducible clinical sensitivity and specificity for cervical precancer. Meijer et al. proposed criteria for clinical validation of HPV assays, while an international scientific framework was created to evaluate, validate and compare HPV assays (VALGENT; Validation of HPV Genotyping tests) (Arbyn et al., 2016; Meijer et al., 2009). The amount of genotype information retained by an assay is a separate consideration from its clinical validation and may become relevant for triage, longitudinal assessment of persistence and epidemiological surveillance.

5. From cytology to primary HPV screening in Belgium

Before 2025, cervical cancer screening in Belgium was primarily based on cytology, and referral to colposcopy was not governed by a single national genotype-based algorithm as introduced with the HPV screening programme in 2025 (Arbyn et al., 2018; *Nomenclature Article 14, g*) / INAMI, n.d.; Smeets et al., 2011; Valérie et al., 2019).

In Flanders, the 2011 primary-care guideline recommended hrHPV testing of ASC-US. hrHPV-positive ASC-US and high-grade cytological abnormalities led to referral for colposcopy, while lower-risk abnormalities were managed with repeat cytology or HPV testing (Smeets et al., 2011). Colposcopy practice was nevertheless not historically uniform. KCE and health-insurance data documented substantial colposcopy use and variation in the relationship between screening cytology and subsequent colposcopy (Arbyn et al., 2015; Valérie et al., 2019). From February 2013, colposcopy was no longer reimbursed as a screening procedure performed together with a screening smear and reimbursement was restricted to defined indications (*Nomenclature Article 14, g*) / INAMI, n.d.).

These historical data are relevant to the comparison with 2022–2024, but they do not establish how referral decisions were made for each woman during that period. The historical comparator in the present study should therefore be considered a cytology-based screening system rather than one uniformly applied referral algorithm. The 2011 Domus Medica guideline is used as the reference for the historical colposcopy referral load, since it was the professional guidance in force before the national programme algorithm took effect in January 2025.

Following the accumulating evidence in favour of HPV-based screening, the Belgian Interministerial Conference for Public Health decided in December 2022 to introduce primary HPV screening from age 30. The new national programme became effective on 1 January 2025 (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België* / RIZIV, n.d.; Simoens et al., 2024).

6. The current Belgian screening algorithm

The current Belgian programme uses different primary tests according to age. Women aged 25–29 years remain eligible for cytology-based screening every three years, with hrHPV testing as triage after ASC-US. Women aged 30–64 years undergo primary hrHPV screening every five years (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België* / RIZIV, n.d.; Simoens et al., 2024). Women aged 65 years and older without reimbursed cervical screening during the preceding ten years can undergo a one-off exit or catch-up screen (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België* / RIZIV, n.d.; Simoens et al., 2024). Self-sampling is currently not possible although non-inferiority has consistently been demonstrated in large studies (Arbyn et al., 2018).

In women undergoing primary HPV screening, a positive result is followed by reflex cytology on the same specimen. HPV16- or HPV18-positive women are referred directly for colposcopy irrespective of the reflex-cytology result. Other hrHPV-positive women are managed according to reflex cytology, with one-year repeat HPV testing after a negative cytological triage where appropriate (Simoens et al., 2024).

The algorithm therefore combines three types of information: the presence of carcinogenic HPV, partial HPV genotyping and cytological morphology. This reduces the number of HPV-positive women referred immediately for colposcopy while retaining direct referral for the two genotypes historically associated with the greatest cervical cancer burden.

A Sciensano communication issued shortly after implementation further clarified the classification of HPV66. When HPV66 is measured and identified separately, isolated HPV66 positivity should be reported as hrHPV-negative. This recommendation does not require pooled assays that include HPV66 to infer whether HPV66 is

present in isolation. The implications of this distinction for assay choice and genotype resolution are discussed below (Nationaal Referentiecentrum (NRC) Voor Humaan Papillomavirus / Sciensano.Be, n.d.).

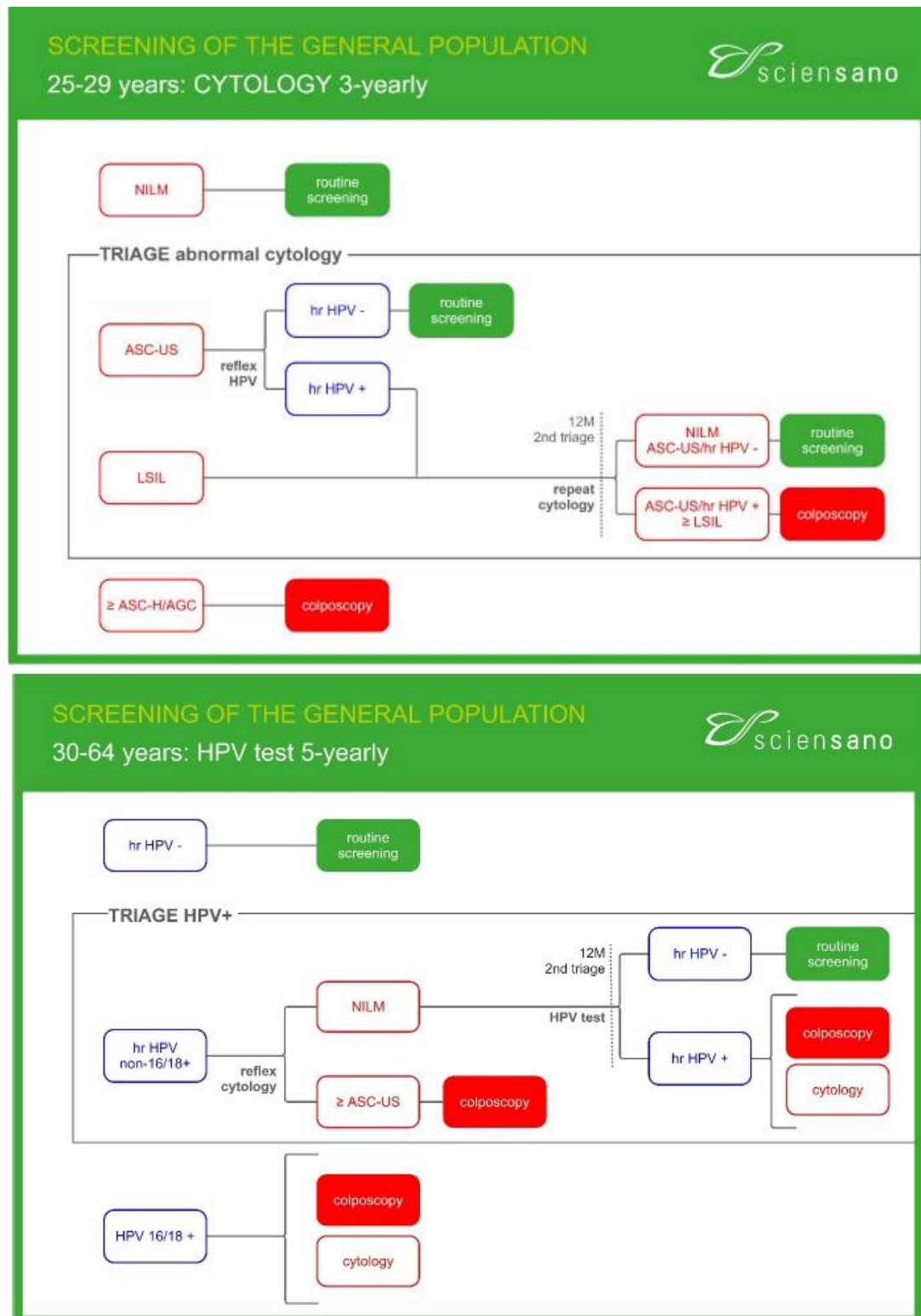


Figure 2: current Belgian screening algorithm.

7. Risk-based management as a framework for interpreting the Belgian algorithm

The Belgian pathway is a fixed national decision tree. It is not an individually calculated risk model. Its individual branches can nevertheless be understood through a broader principle: management intensity should reflect the underlying probability of cervical precancer and cancer.

This principle is made explicit in the 2019 ASCCP Risk-Based Management Consensus Guidelines. Combinations of current screening results and previous screening history are mapped onto estimated CIN3+ risks and corresponding management actions (Egemen et al., 2020; Perkins et al., 2020). An immediate CIN3+ risk of approximately 4% is used as the threshold for colposcopy, while lower immediate risks lead to surveillance intervals determined by longer-term risk (Egemen et al., 2020; Perkins et al., 2020). These numerical thresholds are specific to the ASCCP framework and were not used to derive the Belgian algorithm. However, they are indicative. A screening positive for HPV16 with normal cytology (NILM) is associated with an immediate CIN3 risk of 5.3%, thus above the threshold for direct colposcopy and in line with Belgian recommendations (*ASCCP Management Guidelines Web Application*, n.d.; Demarco, Egemen, et al., 2020).

HPV18 provides an informative example of the limitations of a single risk threshold. In the original ASCCP data, HPV18-positive women with NILM cytology had an immediate CIN3+ risk of approximately 3%, below the conventional colposcopy threshold, but were nevertheless referred because of a disproportionately high cancer risk (Egemen et al., 2020; Perkins et al., 2020). The 2025 Enduring Guidelines continue to recommend colposcopy for HPV16- and HPV18-positive results and use extended genotype information to further stratify several non-16/18 types (Massad et al., 2025).

The HPV16/18 split also has a practical historical basis. Interim guidance for primary HPV screening introduced partial genotyping of HPV16/18 as a method of selecting higher-risk HPV-positive women for immediate colposcopy (Huh et al., 2015). This approach became incorporated into several national programmes and commercial assays. More recent evidence shows that the remaining hrHPV genotypes are not one homogeneous risk group (Demarco, Hyun, et al., 2020; Massad et al., 2025).

8. International approaches to HPV-positive triage

National programmes use the same underlying evidence but differ in how they manage HPV-positive women. The main differences concern the use of cytology, partial or extended genotyping, persistence and previous screening history.

The Netherlands and England rely principally on cytology to triage HPV-positive primary screening results (Aitken et al., 2019; *Bevolkingsonderzoek Baarmoederhalskanker Voor Professionals / RIVM*, n.d.; Rebolj et al., 2019). Belgium uses HPV16/18 for direct referral and reflex cytology for the remaining hrHPV-positive group (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België / RIZIV*, n.d.; Simoens et al., 2024). Australia also incorporated partial HPV16/18 genotyping into primary screening, while current Swedish guidance uses more extensive genotype-based stratification (Brotherton et al., 2023; Creagh et al., 2021). The United States differs mainly in the management of abnormal results, where current results and screening history are translated into estimated risk rather than being managed through one fixed screening decision tree (Egemen et al., 2020; Massad et al., 2025; Perkins et al., 2020). Canada has likewise adopted risk-based principles for colposcopy management, although screening policy remains provincial (Willows et al., 2023).

These differences have practical consequences. In Nordic population data, switching from cytology to HPV-based screening increased colposcopy referral in some programmes but not in others (Partanen et al., 2024; Portnoy et al., 2024). The effect of primary HPV screening on referral burden therefore depends on the triage strategy and the previous screening programme rather than on the HPV test alone.

Country	Programme start age (any test)	Age HPV-primary/co-test begins	Interval (HPV/co-test phase)	Genotyping for direct referral	Triage interval	Self sampling	Algorithm type	Ref.
Belgium	25 (cytology 25-29)	30	5y	Yes (16/18)	12 months, non HPV16/18 hrHPV-normal cytology	No	Fixed decision tree	(Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België RIZIV, n.d.; Simoens et al., 2024)
Netherlands	30	30	5y(<40y) or 10y (≥40y)	No; cytology triage	12 months in case of hrHPV with normal cytology	Yes	Fixed decision tree	(Aitken et al., 2019; Bevolkingsonderzoek Baarmoederhalskanker Voor Professionals RIVM, n.d.)
England	25	25	5y	No; cytology triage	12 months in case of hrHPV with normal cytology	Yes	Fixed decision tree	(Cervical Screening Care Pathway - GOV.UK, n.d.; Rebolj et al., 2019)
Norway	25	25	5y	Yes HPV16	Depending on cytology and hrHPV type	Yes	Fixed decision tree	(Al-Shibli et al., 2026)
Sweden	23	23	5y (23-49); 7y (50-70)	No; cytology triage?	?	Yes	Fixed, genotype	(Fridljung et al., 2025)
Iceland	23	23	3y (23-29); 5y (30-64)	No; cytology triage	12 months, various indications	?	Fixed decision tree	(Cervical Cancer Screening Ísland.is, n.d.)
Italy	25 (cytology 25-29)	30 in transitioned regions	5y	Cytology triage in national model	12 months in case of hrHPV with normal cytology	No?	Fixed decision tree; regional transition	(Gori et al., 2021; Ministerie van Volksgezondheid - Kankerscreening, n.d.)
Germany	20 (annual cytology)	35 (co-test)	3y	Not applicable; co-testing	?	No?	Fixed decision tree	(Xhaja et al., 2022)
Australia	25 (whole population)	25	5y	Yes (16/18)	12 months in case of hrHPV with normal cytology	Yes	Fixed decision tree	(Brotherton et al., 2023; Creagh et al., 2021)
United States (management framework)	21-25 depending screening guideline	30 in commonly used primary-HPV pathways	Guideline-dependent	Genotype contributes to risk-based management			Risk-based management	(Demarco, Egemen, et al., 2020; Egemen et al., 2020; Perkins et al., 2020)

Table I: summarized overview of the international approaches to HPV-positive screening and triage

9. Implementation of primary HPV screening

Changing the primary screening test affects more than diagnostic performance. It alters referral pathways, laboratory workflow, request forms, information systems and communication with requesting clinicians.

The Dutch transition to primary HPV screening produced both higher early detection of cervical precancer and changes in referral burden (Aitken et al., 2019). Across Nordic programmes, the magnitude and direction of the referral effect differed according to the triage strategy (Partanen et al., 2024). These findings underline the importance of evaluating the full screening pathway rather than the primary test in isolation.

Implementation studies also describe operational problems. Australia's transition required major changes in registry infrastructure, communication and laboratory processes (Brotherton et al., 2023). Pathology laboratories reported staffing and IT pressures and specifically identified incomplete or unsuitable request-form information as an implementation problem (Bavor et al., 2023).

These experiences are directly relevant to a first-year laboratory evaluation. Early changes in erroneous requests, turnaround time, invalid results or external workload may reflect implementation of the pathway even when analytical assay performance itself is stable.

10. Rationale for the present evaluation

Belgium's transition to primary hrHPV screening shifted the initial screening signal from cytological morphology to the detection of carcinogenic HPV infection. Subsequent management now combines genotype and reflex cytology. The clinical consequences of this pathway depend on local HPV epidemiology, genotype distribution, cytological findings and the way in which the national algorithm is implemented.

This CAT evaluates the first year of primary HPV screening at UZ Leuven. It describes hrHPV positivity and genotype-channel distribution by age, assesses the association between genotype and reflex cytology, and quantifies the proportion of screening tests assigned to colposcopy, one-year repeat testing or routine screening. Alternative triage rules are applied to the same 2025 cohort to estimate how algorithm design affects immediate colposcopy workload. Historical cytology data from 2022–2024 are used to estimate the corresponding referral burden and cytological yield under the preceding cytology-based screening pathway.

The evaluation also examines practical implementation indicators, including request indications, uninterpretable results, turnaround time and external request patterns. These endpoints address whether the new screening pathway could be implemented reliably in routine laboratory practice.

The present analysis does not include linked histological CIN2+ or CIN3+ outcomes. It can therefore compare epidemiology, cytological phenotype, implementation and referral workload, but cannot determine which triage strategy has the best balance between colposcopy and detection of clinically relevant precancer. Longer follow-up will also be required to evaluate persistence and outcomes after one-year repeat testing.

The dataset is test based rather than a deduplicated population cohort. HPV estimates are therefore reported per screening test and should not be interpreted as population prevalence. In addition, 2025 is a transition year in which eligibility depends partly on previous screening history (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België* / RIZIV, n.d.). The observed population may differ from later steady-state five-year screening rounds.

The first female cohort routinely offered HPV vaccination through the Flemish school programme will reach the age of primary HPV screening from approximately 2028 (*Vaccinatieprogramma Vlaanderen Door de Jaren Heen* / Departement Zorg, n.d.). The present evaluation therefore also provides a baseline before routinely vaccinated cohorts begin to enter the 30–64-year primary-screening population in substantial numbers. Changes in genotype

distribution, cytological risk and screening yield will become increasingly relevant as this transition occurs(Cuschieri et al., 2023).

Question(s)

1. Describe the epidemiology of HPV types in the screening and follow-up populations
2. Evaluate whether the new screening protocol changed the colposcopy referral rate
3. Evaluate the association between cytology and HPV status/genotype
4. Describe the implementation indicators associated with the new screening algorithm
5. Evaluate quality indicators.

Methods

I. Data sources

Three laboratory extracts were used. The first contains all HPV requests processed by the UZ Leuven molecular laboratory from May 2023 onward on the Abbott Alinity m platform, with genotype channels, indication fields, integrated advice and, from January 2025, the reflex cytology report. The second contains all HPV requests processed by the same laboratory between January 2022 and May 2023 on the Hologic Aptima assay, for which only a qualitative result is available. The third contains the cervicovaginal cytology requests handled by the pathology laboratory, including the structured lesion code, specimen adequacy and recorded vaccination status. HPV and cytology records belonging to the same pseudonym were linked when their sampling dates differed by no more than seven days. Where several records qualified, preference was given first to records that carried a result and then, among those, to the pair with the shortest interval. Linking is one to one: a record is paired with at most one counterpart.

Quantity	n	note
HPV tests performed on Abbott Alinity (from May 2023)	33909	
HPV tests performed on Hologic Aptima (2022 to May 2023)	9231	<i>after removing duplicates</i>
HPV tests performed on Hologic Aptima removed as duplicates of a LAG record (same woman, same date)	1	
cytology tests (2022-2025)	61365	<i>Which includes 2 double requests and 122 non female cytologies.</i>
linked pairs (HPV and cytology within 7 days, same woman)	30170	
of which with the Abbott Alinity data	20954	
of which with the Hologic Aptima data	9216	
same sampling date (gap 0 days)	30168	
gap 1 to 3 days	2	
gap 4 to 7 days	0	
cytology tests with NO HPV test within 7 days	31195	<i>Cytology-only requests, including the 25-29 primary cytology track.</i>
HPV tests with NO cytology within 7 days	12970	
linked pairs where one of the two tests had more than one candidate	372	<i>The closest pair was kept. These are listed in the ambiguous_links sheet of the merged file for manual checking.</i>
HPV requests dropped: no result and a competing request within the window	552	<i>Duplicate registrations of one order. Most common interlis and manual registration. Excluded from every calculation, including denominators. A lone request without a result is kept.</i>
cytology requests dropped: no result and a competing request within the window	2	<i>Duplicate registrations of one order. Excluded from every calculation, including denominators. A lone request without a result is kept.</i>

Table 2: overview of the raw data, merge and selection.

All three extracts were pseudonymised before release. Request and receipt timestamps are masked where the underlying value was unknown, so the planned sampling time was used throughout as the operative date for period assignment, age and turnaround. Turnaround time was defined as the interval from the planned sampling date to validation of the integrated advice. It therefore covers transport between laboratories, cytological assessment and formulation of the advice, and is not an analytical turnaround time.

2. Study population and definitions

The evaluation covers the first calendar year of the new algorithm, 1 January to 31 December 2025, with earlier data used only where a longer series is informative and explicitly labelled as such. Analyses are performed at the level of the screening test rather than the individual, because the extract is not deduplicated by person. Results are therefore reported as positivity per screening test within the laboratory catchment area, not as population prevalence.

Characteristic	n	%	Note
Overall			
HPV requests in the period	19675	100.0	includes pre 2025 HPV-tests and all 2025 cervical screening requests registered in the laboratory medicine database
with a HPV result	17012	86.5	~ True HPV requests
hrHPV positive	2177	12.8	denominator: "has HPV result"
Age band			
25-29	2457	12.5	
30-34	2594	13.2	
35-39	2395	12.2	
40-44	2338	11.9	
45-49	2196	11.2	
50-54	2052	10.4	
55-59	1904	9.7	
60-64	1794	9.1	
>=65	1782	9.1	
Screening track (age)			
25-29	2457	12.5	
30-64	15273	77.6	
<25	163	0.8	
>=65	1782	9.1	
Indication 2025 cohort			
Organised screening	14089	71.6	
Follow-up	2832	14.4	
Clinical indication	848	4.3	
Other	760	3.9	
Triage (reflex after ASC-US)	697	3.5	
Indication not recorded	248	1.3	
High-risk group	201	1.0	
Reflex testing			
reflex cytology requested	219	1.1	
reflex HPV after ASC-US (ages 25-29)	495	2.5	
Requesting centre			
			<i>indirectly measured: estimated</i>
internal	10748	54.6	
MCH	7851	39.9	

external, unclassified	939	4.8	
Heilig Hart	137	0.7	
Result quality (HPV)			
inconclusive (inhibition)	9	0.0	
unreliable (insufficient material)	4	0.0	
Cytology extract (2022-2025)			
cytology requests (all years)	61241	100.0	
Cytology by year			
2022	20269	33.1	
2023	16292	26.6	
2024	17336	28.3	
2025	7344	12.0	
Cytology in 2025 by source			
reflex or co-test cytology (same specimens as the HPV section)	7261	98.9	do NOT add to the HPV section: these are the same specimens
cytology without an HPV test on the same specimen	83	1.1	includes the 25-29 primary cytology track
Vaccination status (recorded)			
			<i>As reported in the cytology request</i>
not recorded	4044	6.6	denominator: status recorded
not vaccinated	45156	73.9	denominator: status recorded
vaccinated	11876	19.4	denominator: status recorded

Table 3: Characteristics of the tested population

Age follows the national guideline definition: the calendar year in which a woman turns a given age, not her age on the sampling date. The three screening tracks (25 to 29, 30 to 64, and the single exit or catch-up test from 65) are analysed separately and never pooled, because the primary test differs between them. In the 25 to 29 track cytology is the primary test and HPV is a reflex test after ASC-US, so an HPV result in that group identifies a selected subgroup rather than a screening population.

As of May 2023 the assay reports HPV16, HPV18 and HPV45 individually and groups the remaining high-risk types into two pooled genotype channels, 31/33/52/58 and 35/39/51/56/59/66/68 measured by Abbott Alinity m HR HPV. Individual types within a pool cannot be resolved. Where a specimen is positive in several channels, a hierarchical assignment is used for tabulation, with the full combination retained alongside it.

The denominator for genotype rates is valid screening tests, meaning tests with a screening indication and an interpretable HPV result. Inconclusive and technically unreliable results are excluded from that denominator and reported separately as a quality indicator, so that genotype rates are not diluted by technical failure.

3. Derivation of the recommended management

The recommended management for each test was recomputed from genotype, reflex cytology, age and indication using an explicit implementation of the national algorithm, rather than taken from the advice field stored by the laboratory system. This was done for two reasons. The stored advice does not exist before 2025 and is therefore unavailable for any historical comparison, whereas a derived recommendation is comparable across the whole period. In addition, running the derivation over the 2025 tests where the stored advice does exist provides a validation step: agreement confirms the implementation, and disagreement identifies either an error in the implementation or a deviation in practice. Recommendations were compared after normalising equivalent wordings to a common form, so that differences in phrasing are not counted as differences in management. Both the normalised and the raw agreement are reported.

4. The counterfactual model

The counterfactual analysis asks a single question: if a different triage rule had been applied to exactly the same women, on exactly the same test results, how many colposcopy recommendations (~ the referral workload) would it have produced, and which women would have moved?

Four rules were applied to the same 2025 screening cohort aged 30 to 64. Each differs only in which genotypes bypass cytology triage; every other element of the pathway is identical.

- The current Belgian rule, in which HPV16 and HPV18 are referred directly.
- A cytology-only rule, in which no genotype bypasses triage and every HPV-positive woman is triaged by reflex cytology. This mirrors the Dutch programme, which adopted that approach after explicitly considering and rejecting genotype-triggered referral.
- An HPV16-only direct-referral rule, in which only HPV16 is referred directly.
- A wider rule, in which all carcinogenic genotypes covered by the 9-valent vaccine, that is HPV16, HPV18, HPV45 and pool A, are referred directly and only pool B is triaged.

Each rule returns a full management category rather than a referral flag, which allows the results to be presented as a transition matrix against the current algorithm. The matrix shows not only how the totals differ but which women change category and in which direction, since a net total can conceal movement both ways.

The counterfactual model quantifies referral workload only. Histological outcomes are not available for the 2025 cohort, so it cannot establish how many lesions each rule would detect. A higher referral rate is therefore neither better nor worse on these data, and no rule is presented as preferable to another.

Rules that require a second screening round or an individual screening history, such as persistence-based referral or a continuous risk calculation, cannot be simulated on a single year of data and were not attempted.

5. Statistical methods

Proportions are reported with 95% Wilson score confidence intervals, which behave correctly for small counts and for proportions near zero, a situation that arises in several genotype and cytology cells. Comparisons between an indication group and the screening cohort are restricted to ages 30 to 64 and are age-adjusted, because clinical, follow-up and high-risk requests have a different age distribution from routine screening. Three figures are reported for each comparison: the crude rate in both groups, a rate for the comparison group directly standardised to the age distribution of the screening cohort, and a Mantel-Haenszel odds ratio pooled across five-year age strata with its confidence interval. Where a formal test is reported, a chi-squared test is used, with Fisher's exact test when expected counts are small. The evaluation is descriptive by design and was not powered for hypothesis testing; several comparisons are made across genotype, indication and age strata and no correction for multiple comparisons was applied, so p-values should be read as descriptive and confidence intervals remain the primary means of conveying precision.

6. Reproducibility

The analysis was written as a scripted pipeline in R, taking the raw extracts as input and producing every figure and table in a single run, with a fixed random seed and the session environment recorded. Extraction of the free-text cytology reports is exported alongside the original report text so the parse can be verified independently. Mapping of lesion codes to Bethesda categories is held in an editable file rather than embedded in code, so that every classification decision is visible and can be revised without changing the analysis.

I. Principal findings and scope of interpretation

This study provides a first-year evaluation of primary hrHPV screening within a large Belgian laboratory network after implementation of the national screening algorithm in January 2025. The study encompasses over 40000 HPV tests and more than 60000 cytology reports (see **Table 2** and **Table 3**). Several findings emerge. hrHPV positivity showed a clear age gradient (see **Figure 3**) and differed substantially between genotype channels. Most screening tests resulted in an advice to return to routine five-year screening, while approximately 4% required repeat HPV testing after one year and about 3.5% resulted in an immediate recommendation for colposcopy. Referral workload changed substantially when alternative triage rules were applied to the same screening population. The overall cytological distributions overlapped between genotype groups, but the frequency of high-grade or urgent-referral cytology differed markedly. It was highest among HPV16-positive women and lowest in pool B (hrHPV not included in the 9 valent vaccine). At the laboratory level, uninterpretable HPV results were uncommon, turnaround time from collection until validation of the integrated advice shortened during the year, and corrections of the requested screening indication became less frequent after the initial implementation period.

These findings describe epidemiology, referral workload and implementation performance. They do not allow the evaluation of the clinical effectiveness of the Belgian screening algorithm. Histological outcomes were not available

e, so referral strategies cannot be compared according to CIN2+, CIN3+ or cancer detection. Only one year of primary HPV screening is available, which limits evaluation of persistence and longer-term outcomes. The historical comparator comprises three years of cytology-based screening and was not governed by the national genotype-based referral algorithm introduced in 2025 (Arbyn et al., 2015; *Healthcare Professionals / Sciensano.Be*, n.d.; *Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België / RIZIV*, n.d.; Smeets et al., 2011; Valérie et al., 2019). The present study should therefore be interpreted as a descriptive evaluation of the first year of a new screening pathway rather than as a validation of its clinical risk thresholds.

The year 2025 is also a transition year. Eligibility for reimbursed primary HPV screening depends in part on previous screening history (*Introductie van de HPV-Test Als Primaire Screeningstest Voor de Opsporing van Baarmoederhalskanker in België / RIZIV*, n.d.). The case mix may therefore differ from that expected once successive five-year screening rounds are established. This affects comparisons with historical prevalence estimates and with mature primary HPV programmes in other countries.

2. HPV epidemiology in the first year of primary screening

hrHPV positivity showed an overall decline with age, from 99.2 per 1000 valid screening tests at age 30–34 to 58.2 per 1000 at age 60–64, with some variation between intermediate age groups. This pattern is consistent with the established age distribution of cervical HPV infection. Pre-vaccination Belgian studies also found the highest HPV prevalence in younger women, followed by a progressive decline with age (Arbyn et al., 2009; Depuydt et al., 2010).

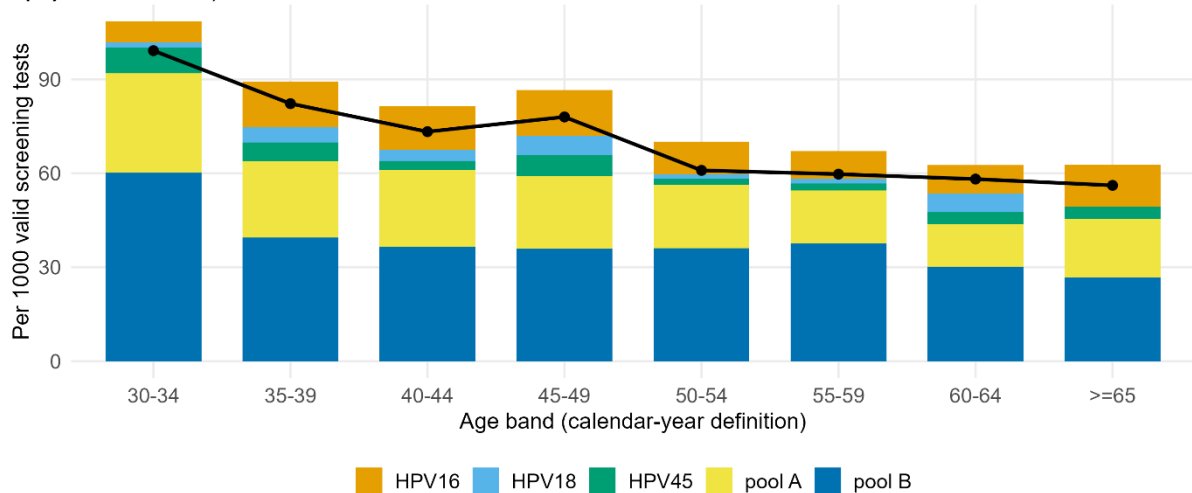


Figure 3: hrHPV and genotype distribution per 1000 screening tests and age group (age 30–64). Age 25–29 is not included as HPV testing is only performed as reflex testing. Age is defined by the guideline definition stating that the calendar year in which the women turns x is used for the algorithm selection. Black dot and line show the total hrHPV positivity, while stacked bars show genotype distribution. As genotypes are not mutually exclusive, the presence of hrHPV/1000 screening tests is lower than the sum of individual genotypes.

The absolute rates should not be compared directly with historical Belgian estimates. Depuydt et al. detected HPV in 15.1% of more than 62,000 consecutive cervical samples collected in Flanders in 2008, with a peak of 26.8% at age 21–25 years (Depuydt et al., 2010). Arbyn et al. similarly found hrHPV in 11% of cytologically normal women in a pre-vaccination Flemish screening population, with substantially higher rates at younger ages (Arbyn et al., 2009). These cohorts differed from the present population in age distribution, assay design, vaccination exposure and screening selection. The current estimates should therefore be interpreted as test positivity within the 2025 primary-screening cohort rather than as directly comparable population prevalence.

More recent Flemish data already show changes after introduction of vaccination. In women aged 20–23 years between 2010 and 2019, Huyghe et al. observed a decline in HPV16/18 prevalence that was not seen in an older control group, alongside changes in several non-vaccine genotypes (Huyghe et al., 2023). Interestingly, subjects aged 30–34 in our cohort had a prevalence of HPV16 of 6.7/1000 screening tests compared to a prevalence of 14.6/1000 in the age-group 35–49 (see **Supplementary Figure 1**). This cohort predates the Flemish school programme, but the vaccine was already available to it and recommended by the Superior Health Council, so opportunistic vaccination is a plausible partial explanation. These findings support continued genotype surveillance, but they also illustrate why historical pre-vaccination prevalence cannot serve as a fixed reference for the present programme.

The genotype distribution in the present study was heterogeneous. Pool B, containing HPV35/39/51/56/59/66/68, was the most frequently detected channel, followed by pool A, containing HPV31/33/52/58. HPV16 was less frequent but remained the most common individually reported genotype. HPV18 and HPV45 were substantially less frequent (**Figure 4**). Frequency and carcinogenic risk should not be equated. Longitudinal studies place HPV16 at the highest risk of CIN3+, while HPV31/33/35/52/58 generally carry greater long-term risk than HPV39/51/56/59/68 (Demarco, Hyun, et al., 2020). The most frequently detected assay channel is therefore not necessarily the channel responsible for the greatest burden of cervical precancer.

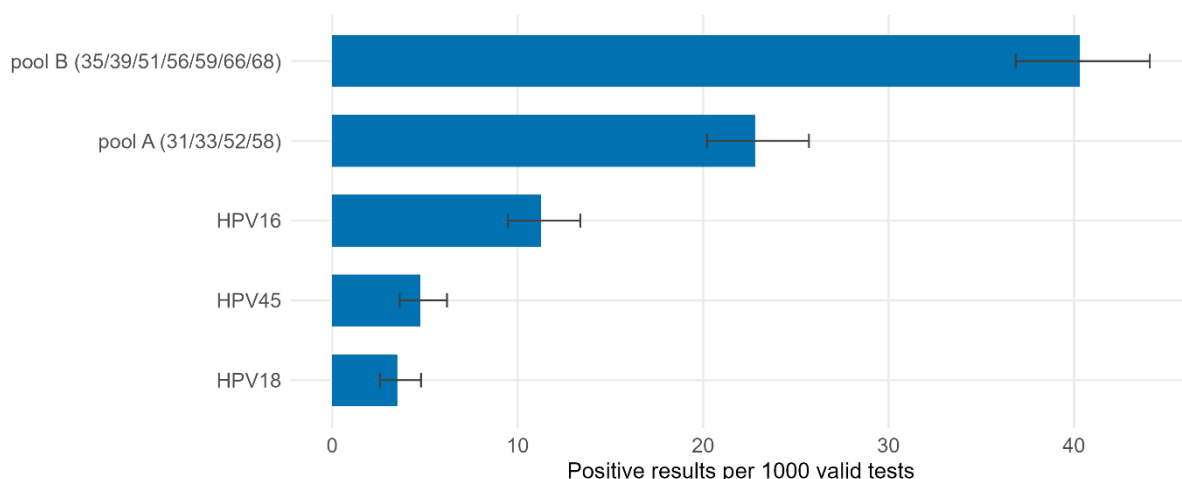


Figure 4: Bar plot of hrHPV genotype channel positivity per 1000 screening tests in age-group 30-64 with 95% confidence intervals based on 11 360 screening tests. Note that genotype channels are not mutually exclusive.

The birth-cohort analysis provides an initial description of how genotype distribution differs across generations, but it cannot yet be interpreted as a direct measure of vaccine effectiveness. More importantly, the first cohort offered organised school-based vaccination in Flanders only entered the programme in September 2010 and will reach age 30 from approximately 2028 onwards (Huyghe et al., 2023; *Vaccinatieprogramma Vlaanderen Door de Jaren Heen* / Departement Zorg, n.d.). The current primary-screening cohort therefore largely predates organised adolescent vaccination.

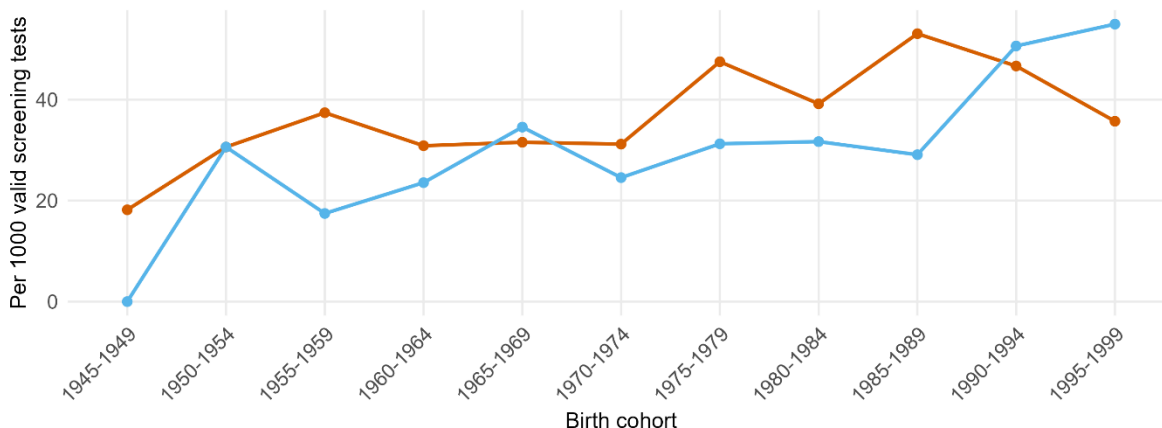


Figure 5: 9-valent vaccine covered vs non-covered hrHPV genotypes by birth cohort.

Under the current Belgian algorithm, HPV testing in women aged 25 to 29 is performed only as a reflex test after an ASC-US cytology result (Figure 2). Unbiased data on hrHPV prevalence and genotype distribution in this age group therefore cannot be obtained. The genotype distribution in this selected cohort nevertheless differs noticeably from that of the 30 to 64 screening population, with a lower proportion of HPV16 and HPV18 (Figure 6). This is consistent with expectation, since the bivalent and quadrivalent vaccines were introduced in Flanders in time to cover these birth cohorts, whereas the older screening population was largely unvaccinated.

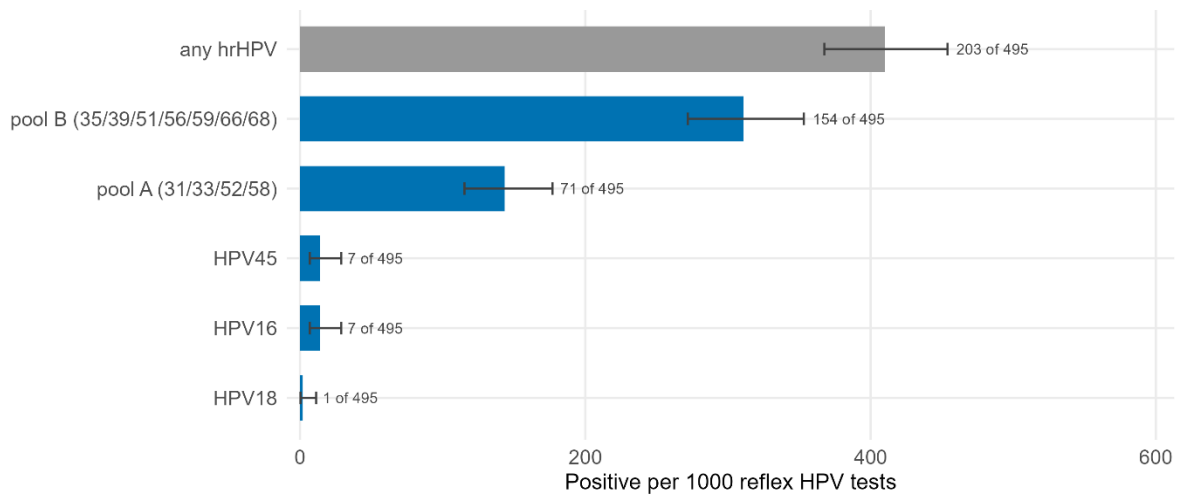


Figure 6: Bar plot of hrHPV genotype channel positivity per 1000 HPV reflex tests in age-group 25-29 with 95% confidence intervals based on 465 reflex tests performed following ASC-US cytology result. Note that genotype channels are not mutually exclusive.

3. Genotype, cytology and clinical risk

Reflex cytology provides a second dimension of risk stratification among hrHPV-positive women. The present data suggest substantial differences between genotype groups. HPV16-positive women had the highest frequency of high-grade cytology, while pool A showed an intermediate pattern and pool B had a lower frequency of high-grade findings. HPV18 was the genotype most frequently associated with NILM cytology, although the total number of HPV18 positive specimens was small (**Figure 8**). These observations are consistent with known genotype-related differences in cervical disease phenotype and risk (Demarco, Hyun, et al., 2020; Gori et al., 2021; Hashim et al., 2020). Odds for detecting HPV positivity and abnormal cytology are significantly higher in the “high-risk” and “clinical-indication” (**Supplementary Figure 2 and 3**) compared to the age stratified screening cohort. Accordingly, the Belgian guidelines subjects these cohorts to a different screening protocol.

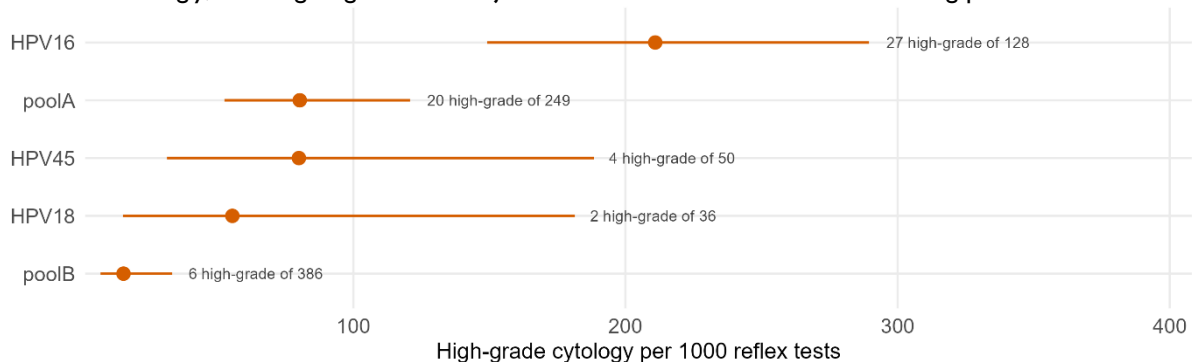


Figure 7: High grade (ASC-H, HSIL, AIS, AC or SCC) cytology per 1000 reflex tests and HPV genotype channel. ASC-US and NILM results are not included. Lines show 95% confidence intervals.

These associations should remain descriptive. Cytological grade is not a substitute for histological CIN3+ risk. The ASCCP and Enduring Guidelines derive management thresholds from CIN3+ and cancer outcomes, together with screening history, rather than from the frequency of abnormal cytology alone (Egemen et al., 2020; Massad et al., 2025; Perkins et al., 2020). A higher proportion of high-grade cytology among HPV16 or pool-A-positive samples therefore supports biological heterogeneity between genotype groups, but does not establish that a genotype group should cross a threshold for immediate colposcopy.

The local HPV-cytology analysis may nevertheless be clinically informative. If pool A remains consistently associated with more severe cytological abnormalities than pool B, this would be compatible with longitudinal

evidence that HPV31/33/35/52/58 and HPV39/51/56/59/68 do not represent equivalent risk groups (Demarco, Hyun, et al., 2020; Massad et al., 2025). Histological linkage and longer follow-up would be required to determine whether this difference translates into a sufficiently large difference in CIN3+ risk to alter management.

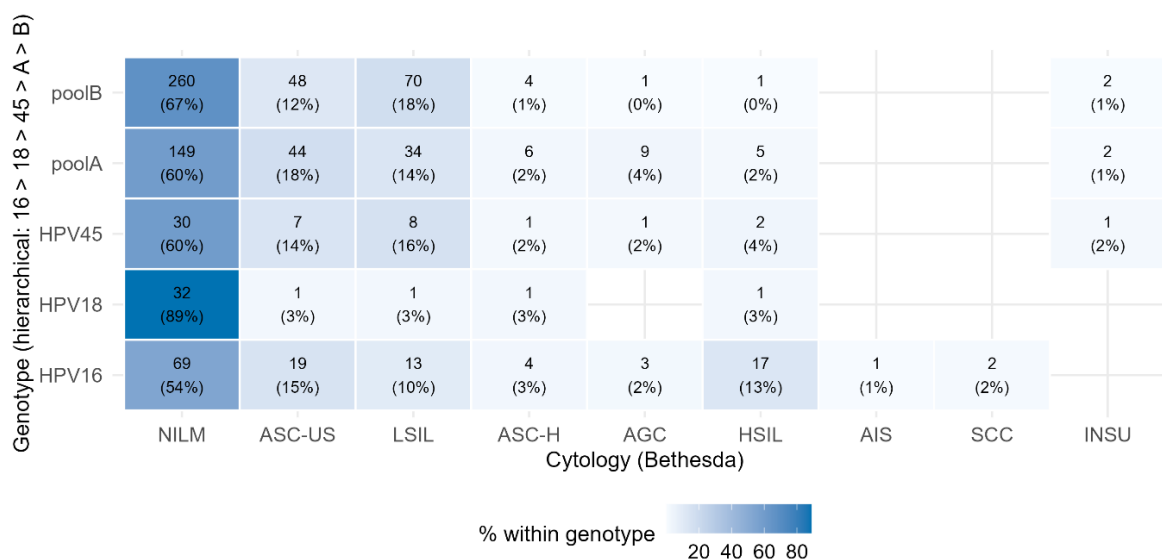


Figure 8: Heatmap of reflex cytology result by HPV genotype channel in ages 30-34.

4. Referral burden and the effect of triage design

The Belgian algorithm generated an immediate colposcopy recommendation in approximately 3.5% of primary screening tests in the present cohort. A further 3.9% were assigned to repeat hrHPV testing after 12 months, while more than 92% returned to routine five-year screening. Monthly referral rates varied during 2025 but showed no clear sustained increase or decrease.

For this study the referral advice was recomputed in R rather than taken from the recorded field, because that field does not exist for the period before 2025 and is not populated for tests that returned to routine screening. The derived and recorded advice agreed in more than 99.5% of comparable cases. The discordant records were reviewed individually. In most the difference was one of wording rather than of management, and in a small number (4) the colposcopy decision itself differed. Those cases suggest that automatic generation of the advice is reliable, but that a second, human assessment remains worthwhile for the combinations in which the algorithm and the reported advice diverge.

Referral rate is not an intrinsic property of primary HPV testing. It depends on HPV prevalence, age distribution, genotype information, reflex testing and the management threshold applied to each result. This is evident from international experience. Introduction of HPV-primary screening increased referral in some European programmes but had a smaller or even opposite effect in others, depending on the previous screening strategy and the triage algorithm (Aitken et al., 2019; Maver & Poljak, 2020; Partanen et al., 2024). The referral burden of one national programme should therefore not be extrapolated directly to another.

The counterfactual analysis (see **Figure 9**) partly removes this problem because each rule was applied to the same 2025 screening cohort. A cytology-only triage strategy generated 304 immediate colposcopy recommendations, compared with 373 when only HPV16 triggered direct referral and 406 under the current Belgian algorithm. Extending direct referral to HPV16/18/45 and pool A increased this to 588 recommendations. This corresponds to an increase from approximately 2.6% under the cytology-only scenario to 5.1% under the widest genotype-based scenario.

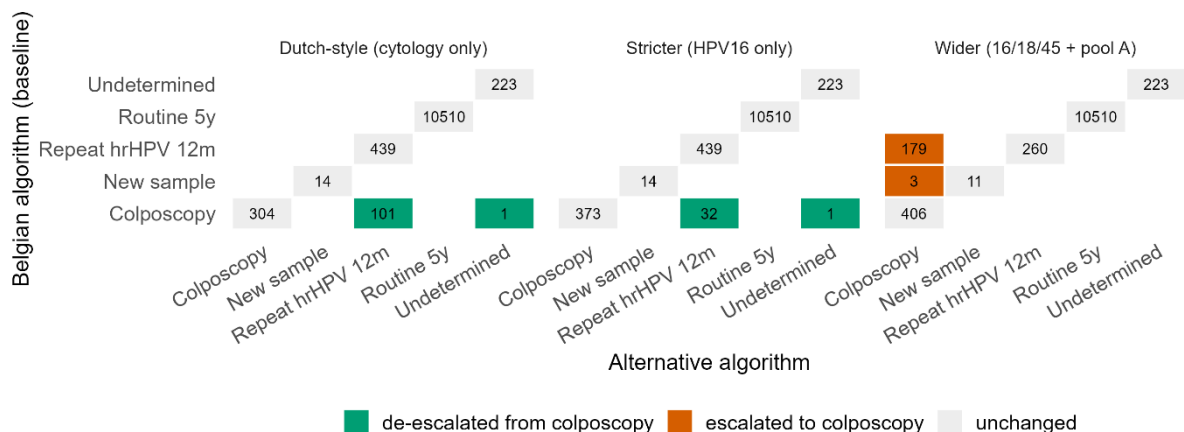


Figure 9. Counterfactual model of the referral workload under alternative triage rules, compared with the current Belgian algorithm (**Figure 2**). Under the Dutch-style rule, colposcopy follows only an abnormal cytology result, irrespective of genotype. Under the stricter rule, colposcopy follows a positive HPV16 result irrespective of cytology, and all other high-risk types are triaged by cytology. Under the wider rule, colposcopy follows any genotype covered by the 9-valent vaccine, that is HPV16, HPV18, HPV45 or pool A (31/33/52/58), irrespective of cytology, and only pool B is triaged.

These results quantify workload, not clinical effectiveness. Histological outcomes were unavailable, so the additional colposcopies cannot be weighed against additional CIN3+ detection. A lower referral rate cannot therefore be considered preferable by itself. The same limitation applies to a cost comparison based only on the number of colposcopies. Rebolj et al. showed in the English primary HPV pilot that adding genotype-based earlier referral increased colposcopy substantially more than CIN2+ detection (+5.9% vs. +1.2%), although earlier referral also reduced loss during surveillance (Rebolj et al., 2019). The balance depends on both disease yield and adherence.

5. Historical cytology-based referral burden

Several authors have described an increase in colposcopy referral burden following the introduction of a partial or full HPV- based cervical screening program. Our reconstructed historical referral estimate was approximately 20 per 1000 primary screening tests, compared with 35 per 1000 tests receiving an immediate referral recommendation under the 2025 algorithm. Differences in available data and decision rules precludes attribution of this contrast solely to the change in primary test. It should however be noted that before 2025 there was reimbursed national programme algorithm/guideline. The referral rate was estimated based on high grade cytology or ASC-US with or without a positive HPV test. Follow-up and triage as currently performed following LSIL cytology was not included.

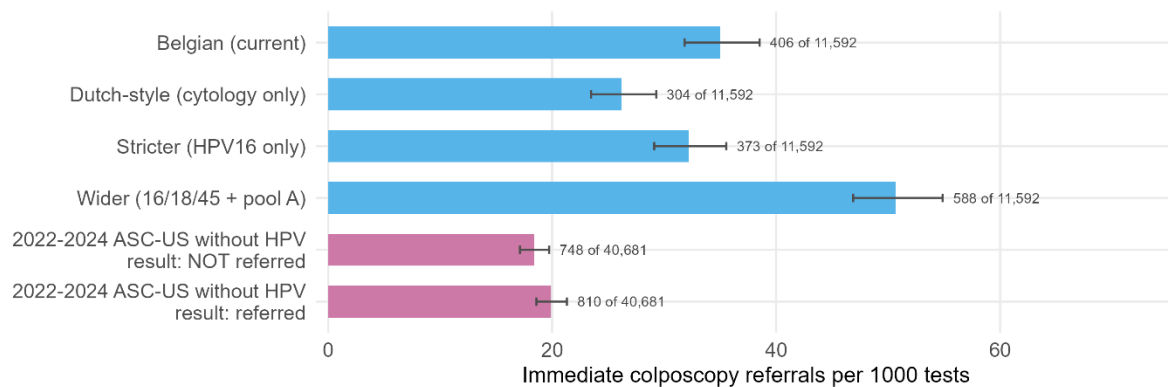


Figure 10: Counterfactual model assessing the referral burden under several alternative rules compared to the Belgian approach (see **Figure 2**) compared to *estimated* historical referral burden by high-grade cytology counts and ASC-US with HPV positivity counts for ages 30-64 and samples collected in the context of screening. Referral burden with and without HPV testing after ASC-US result is shown, because reflex HPV testing after ASC-US was not performed routinely before 2025.

6. Genotyping resolution, persistence and assay design

The clinical value of extended genotyping depends on the purpose for which genotype information is used. Under the current Belgian algorithm, HPV16/18 are separated because they trigger colposcopy irrespective of reflex cytology. Other hrHPV-positive results are mainly managed according to reflex cytology (Simoens et al., 2024). Full individual genotyping would therefore change initial management for relatively few women under the present algorithm.

The biological information contained in the residual hrHPV category is more complex. Longitudinal data show substantial differences in CIN3+ risk between individual non-16/18 types (Demarco, Hyun, et al., 2020). The 2025 Enduring Guidelines already use extended genotype information to separate several of these risk groups (Massad et al., 2025). Genotype resolution may therefore become more clinically relevant if future Belgian management becomes more explicitly risk based.

A second potential advantage is measurement of persistence. Persistent detection of the same HPV genotype carries more specific risk information than repeated positivity for a pooled group (Bonde et al., 2021; Castle et al., 2009). With a pooled assay, a woman who is HPV31-positive at baseline and HPV58-positive at follow-up will be pool-A-positive at both visits. This appears as persistence at the channel level but is not same-genotype persistence. In the Guanacaste and ARTISTIC cohort, repeated carcinogenic HPV positivity was attributable to a genotype switch in 12-18% of cases (Castle et al., 2009; Gilham et al., 2019). Literature has consistently reported that same-genotype persistence generally improved specificity and positive predictive value for high-grade CIN compared with pooled repeat positivity (Bonde et al., 2021; Stoler et al., 2023).

This distinction becomes particularly relevant for the one-year repeat branch of the Belgian algorithm. A positive pooled result at both time points confirms repeated detection of carcinogenic HPV within that channel, but cannot establish that the original infection persisted. Individual genotyping could therefore add information even if it did not alter the initial screening decision (Stoler et al., 2023). In our cohort 409/439 cases for which a reassessment after 12 months was advised, concerned pool hrHPV genotypes. For these patients, we will not be able to definitively conclude on persistence when retested.

The interpretation of a newly detected genotype also requires caution. Detection of a different genotype at follow-up does not prove recent sexual acquisition. In mid-adult women, apparently incident HPV detection can reflect new acquisition, reactivation or intermittent detection of a previous infection (Fu et al., 2016). For screening purposes, the most robust observable distinction is therefore same-genotype detection versus a different genotype, rather than assumed timing of acquisition.

7. HPV66

The Sciensano communication of 16 January 2025 requires isolated HPV66 positivity to be reported and followed as hrHPV-negative when HPV66 is identified separately (*Nationaal Referentiecentrum (NRC) Voor Humaan Papillomavirus / Sciensano.Be*, n.d.). This is narrower than reclassifying every positive pooled channel that contains HPV66. Sciensano specifically addressed assays that can identify an isolated HPV66 result.

Alinity m reports HPV35/39/51/56/59/66/68 as one pooled channel (Valenčák et al., 2019). A pool-B-positive result therefore cannot establish whether HPV66 is present alone or together with another genotype. The isolated-HPV66 rule cannot be applied within this channel, but the Sciensano communication does not require a pooled assay to infer HPV66 when it is not measured separately. In 2009, HPV66 was responsible for an estimated 1.5% of all HPV infections in Belgium and represented 17% of the current pool B genotype group (Arbyn et al., 2009). The limitation is loss of genotype resolution, not non-compliance of the pooled result with the national algorithm.

This episode illustrates a broader issue. Screening guidelines can change more rapidly than assay architecture. An assay that preserves individual genotype information allows retrospective regrouping when classifications change. A pooled assay may remain clinically suitable but cannot recover information that was never separately measured.

The same principle applies to HPV35. HPV35 is combined in Alinity pool B with several types that have lower longitudinal CIN3+ risk, despite cohort data grouping HPV35 with higher-risk genotypes descent (Demarco, Hyun, et al., 2020; Valenčák et al., 2019). This currently has little effect on Belgian initial management, but would matter if future management adopted finer genotype-specific risk categories.

8. Assay selection and clinical validation

Assay selection should distinguish analytical genotype resolution from clinical validation. A test can provide extensive genotyping without having adequate clinical performance for primary screening. Conversely, a clinically validated assay may report only the genotype information required for a particular screening algorithm. The Meijer criteria established comparative requirements for clinical sensitivity, specificity and reproducibility, while VALGENT provides a standardised framework for clinical evaluation and genotype agreement (Arbyn et al., 2016; Meijer et al., 2009). More recent reviews and the current Sciensano overview identify assays with sufficient international clinical validation for primary screening (*Nationaal Referentiecentrum (NRC) Voor Humaan Papillomavirus / Sciensano.Be*, n.d.). Sciensano explicitly describes its current list as internationally clinically validated tests considered suitable for Belgian screening.

Table 4 gives an overview of those tests. With exception of Xpert assay, all tests are high-throughput. While some tests are “sample-to-result”, others use distinct extraction and analysis platforms. The assays differ in which genotypes are pooled or evaluated separately. However, not all assays comply with the direct requirements of the guideline as Xpert and Aptima do not allow a distinct evaluation of HPV18 and HPV45. Laboratories using those platforms should send positive HPV18/45 samples to the reference centre for definite genotyping. The Allplex and Anyplex assays are the only assays that allow to distinctively evaluate HPV66.

We should be very cautious comparing costs. There are no reliable European/Belgian benchmarks available of the reagent cost/product cost. Cost estimates in **Table 4** are based on a 2024 Unitaaid review. They show rather low variation among the assays.

As such, the availability of existing platforms for other PCR tests might well be the major consideration for evaluating HPV assays.

Assay	Genotype	public price/test	public instrument price	Type	sources
Abbott Alinity m HR HPV	16 +18 + 45 + 31/33/52/58 + 35/39/51/56/59/66/68	USD6.24 reagent-only; USD8.49	~USD 299 000	high-throughput	(Affordability of Screen and Treat Tools for Cervical Cancer, n.d.; Alinity m Instrument Abbott Molecular Molecular Diagnostics at Abbott, n.d.; Arbyn et al., 2021)
Roche cobas HPV, 5800/6800/8800	16 + 18 + 12 hrHPV	USD 7.90	~USD 120000-600000	high-throughput	(Affordability of Screen and Treat Tools for Cervical Cancer, n.d.; Cobas® 5800 System, n.d.; Arbyn et al., 2021)
BD Onclarity HPV	16 + 18 + 31 + 45 + 51 + 52 + 33/58 + 35/39/68 + 56/59/66	?	?	high-throughput	(Arbyn et al., 2021; BD ONCLARITY HPV Test Ready-to-Use (RTU) Buy Online BD Fisher Scientific, n.d.)
Seegene Allplex HPV HR Detection	Individual genotyping 16 + 18 + 31 + 33 + 35 + 39 + 45 + 51 + 52 + 56 + 58 + 59 + 66 + 68	?	?	high-throughput	(Oštrbenk Valenčák et al., 2024)
Seegene Anyplex II HPV HR	Individual genotyping 16 + 18 + 31 + 33 + 35 + 39 + 45 + 51 + 52 + 56 + 58 + 59 + 66 + 68	?	?	high-throughput	(Arbyn et al., 2021; Oštrbenk Valenčák et al., 2024)
Hologic Aptima HPV + Aptima 16/18/45 GT	pooled 14 hrHPV primary assay 16+18/45 as "second tier"	USD 9.00–11.28	?	high-throughput	(Affordability of Screen and Treat Tools for Cervical Cancer, n.d.; Arbyn et al., 2021; Nth et al., 2020; White et al., 2024)
Cepheid Xpert HPV	16 + 18/45 + 31/33/35/52/58 + 51/59 + 39/56/66/68	USD14.90	~USD 19 000	Single-sample	(Affordability of Screen and Treat Tools for Cervical Cancer, n.d.; Akbari et al., 2018; Arbyn et al., 2021; Denny et al., 2023)

Table 4: brief overview of commonly used HPV assays with genotyping abilities and pricing.

9. Implementation performance and laboratory quality

The first year of a screening programme also tests the processes surrounding the analytical assay. Errors in the requested indication were most frequent early after implementation, with a marked peak in February and lower rates during the later months of 2025. Interestingly, corrections were uncommon for primary screening requests but substantially more frequent for follow-up and other indications, compatible with the higher complexity of those flowcharts. Similar problems have been reported elsewhere. During implementation of primary HPV screening in Australia, pathology laboratories identified incomplete or unsuitable request information, new IT requirements, staffing pressure and workflow redesign as important barriers (Bavor et al., 2023; Brotherton et al., 2023).

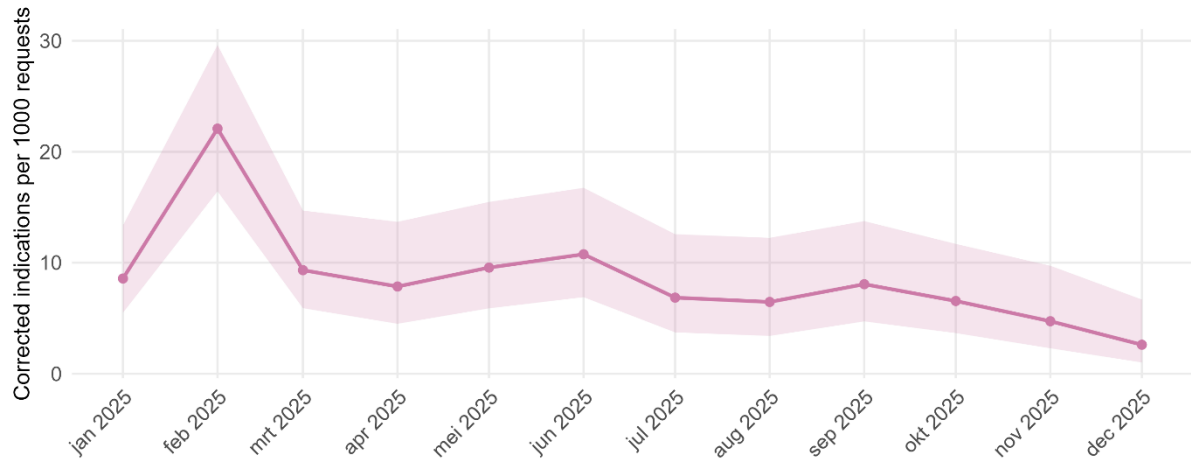


Figure 11: Requests which required indication correction per month and normalised to 1000 requests.

Analytical failure of HPV PCR was rare, with nine uninterpretable results among 14089 screening requests (0.6 per 1000). This suggests that inhibition and insufficient cellularity were uncommon causes of failure in routine use. Comparison with a formal quality target will require a predefined institutional or external benchmark.

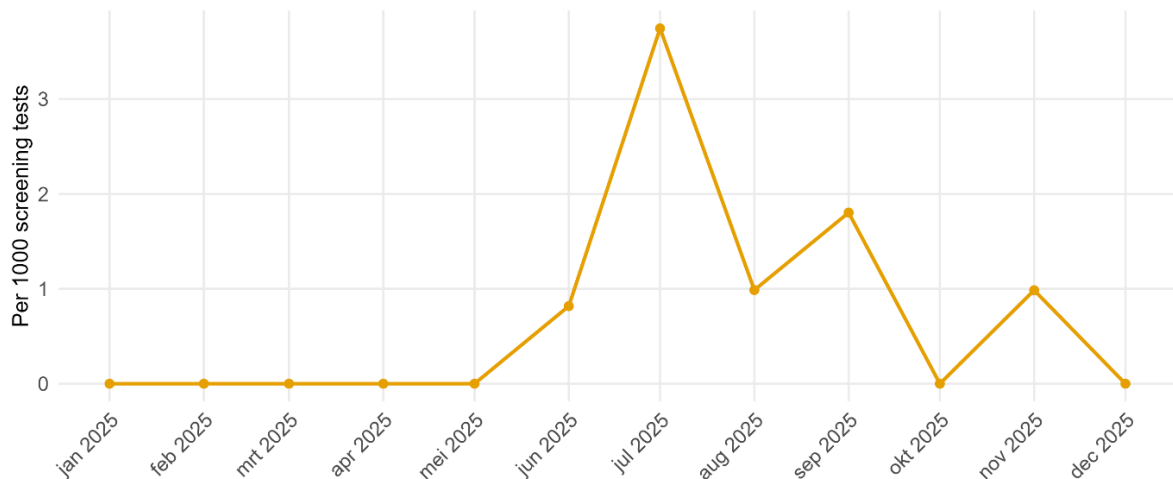


Figure 12: Uninterpretable HPV results per month and normalised to 1000 requests.

In 1.9% of cytology samples the specimen was recorded as not representative, so no Bethesda category could be assigned. This proportion remained stable across the year. Separately, of the 3,527 specimens in 2025 that carried a quality statement, 1,272 (36.1%, all indications) lacked endocervical or transformation-zone cells and are therefore suboptimal according to the Belgian guideline.

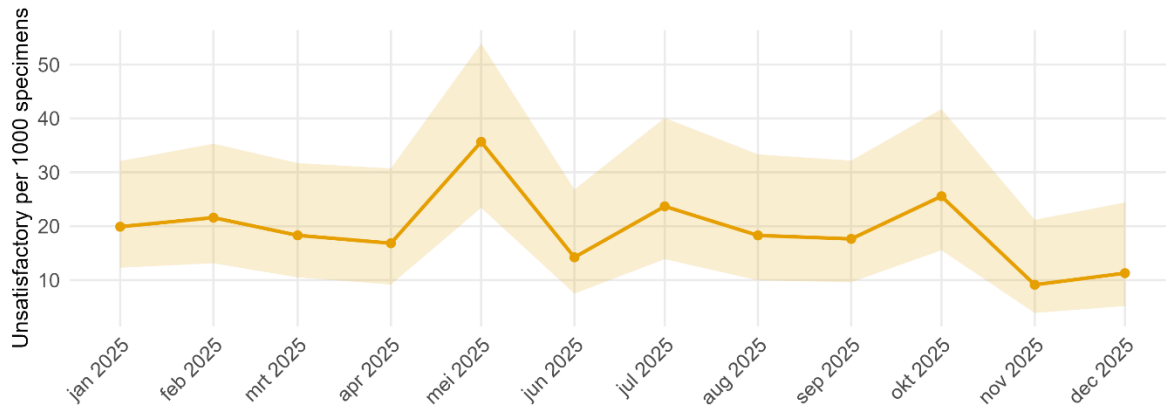


Figure 13: Cytology samples recorded as of “insufficient quality” and for which no Bethesda score could be obtained. Normalised to 1000 requests.

At the result level, reflex triage was complete: all 845 hrHPV-positive primary screening tests had a cytology result recorded.

Turnaround time (TAT) improved substantially during the year, with the longest median interval observed in January and shorter intervals towards the end of 2025. The available TAT measure runs from recorded sampling time to validation of the integrated HPV advice. It therefore includes pre-laboratory transport and cannot be interpreted as analytical laboratory turnaround time alone. The long outlying intervals should remain included in summary statistics even when omitted from the figure for readability.

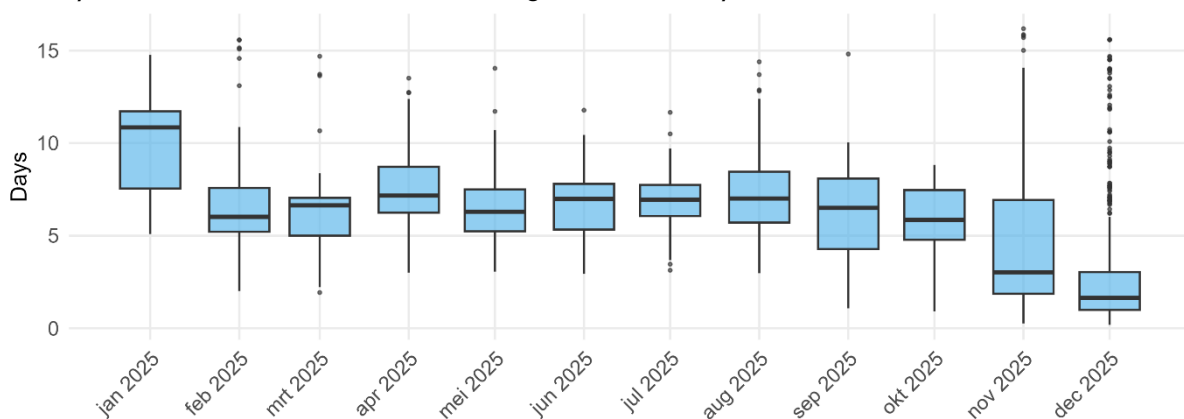


Figure 14: Boxplots showing turnaround time from sampling to integrated advice per month. Note, that this is not representative for the turnaround time of individual tests.

10. Requests outside the guidelines.

Physicians can request screening fully outside the programme or add tests to the existing programme on indication or patient request. These tests are not eligible for reimbursement.

Requests placed fully outside the organised screening programme were present throughout the year, varying between approximately 26 and 55 per 1000 HPV requests. They should not automatically be regarded as inappropriate: some represent testing that is clinically justified but falls outside the reimbursed population programme, and abnormal findings were present in roughly one in ten of them. Their frequency did not decline after the first months of implementation, so they cannot be explained as a transitional effect of the new algorithm. Whatever their justification, their number is a useful measure of the interaction between the national programme, requesting clinicians and laboratory administration.

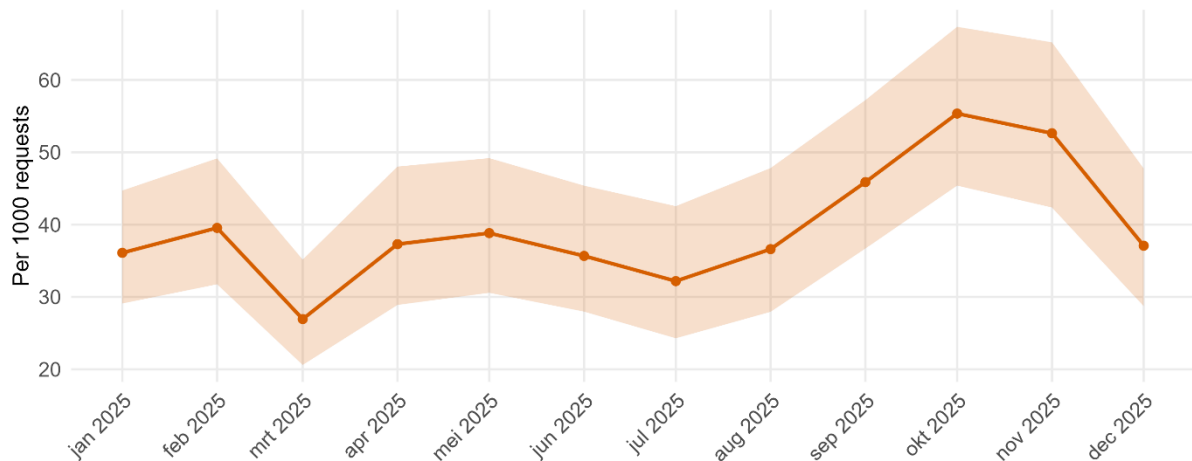


Figure 15: Requests fully outside organised screening programme per month and 1000 requests in 2025. Remark data concerning cytology might be incomplete.

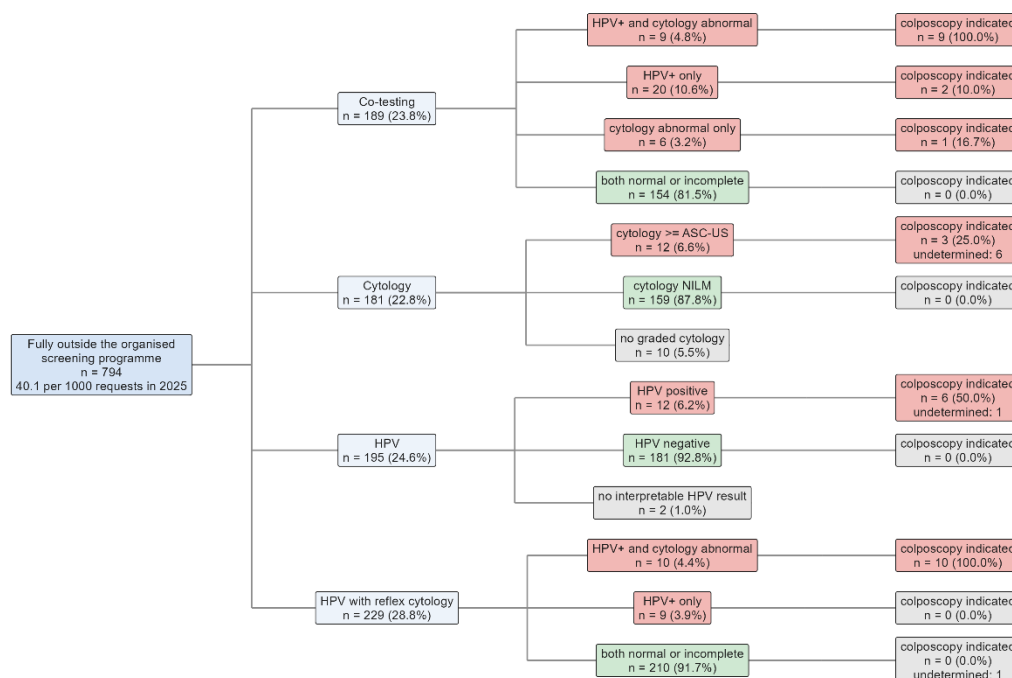


Figure 16: Results of request fully outside organised screening programme per month and 1000 requests in 2025. Note that data concerning cytology might be incomplete.

Physicians can also request supplementary tests that the reimbursement does not provide for. Administratively these requests reveal inconsistencies: the same additional test is sometimes ordered on a request that was already placed outside the organised programme, and tests are occasionally duplicated, as in Figure 17, where cytology was added on top of a co-test under a clinical indication. Additional tests requested in the screening setting rarely lead to a colposcopy referral.

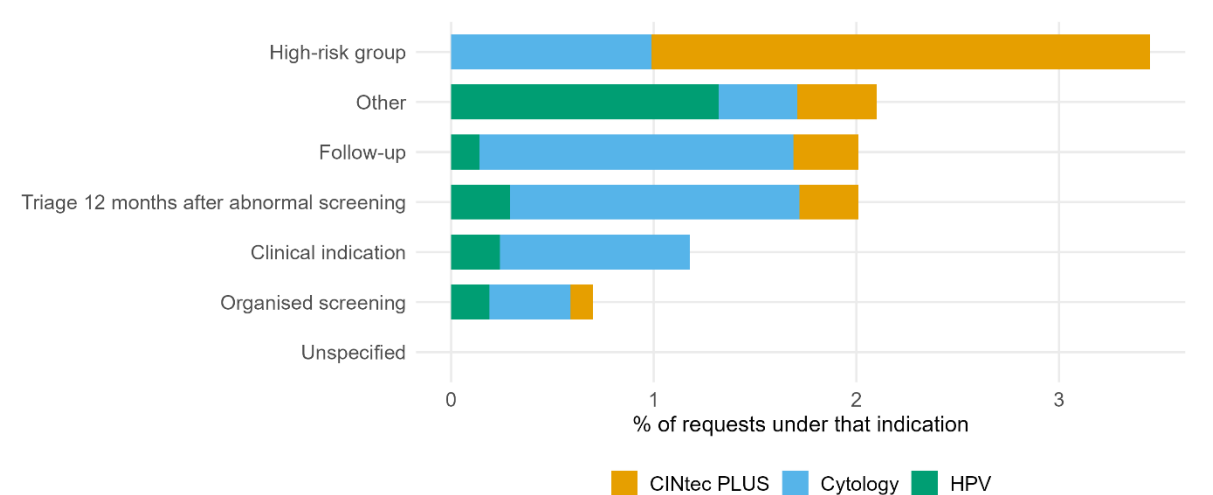


Figure 17: Additional tests requested outside the primary available indication.

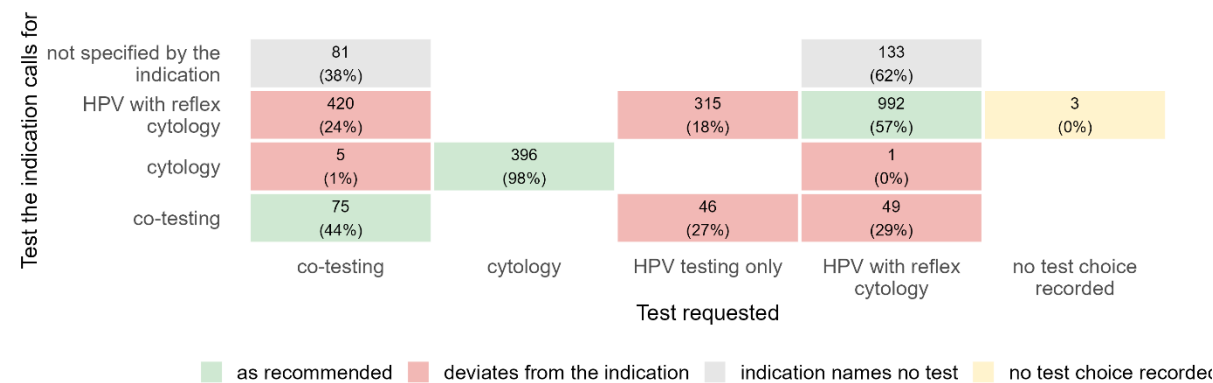


Figure 18: Follow-up requests with indicated (y-axis) and requested test in 2025. Note that “HPV testing” is available as separate option, though reflex testing is always performed.

The effect on screening should therefore become progressively stronger over the next decade. Real-world data show large reductions in vaccine-type HPV infection and high-grade cervical disease after vaccination (Huyghe et al., 2023; Kavanagh et al., 2017). The relative contribution of non-vaccine genotypes among remaining HPV-positive women may consequently increase. This should not be interpreted as evidence of biological type replacement without evidence that the absolute incidence of those genotypes increases (Kavanagh et al., 2017).

The age threshold for primary HPV screening deserves reconsideration as vaccinated cohorts enter the screening population. The 2015 KCE assessment supported cytology below age 30 because HPV prevalence is high in younger women, many infections are transient, and the pooled European randomised trials had not demonstrated superior protection against invasive cervical cancer below age 30 (RR 0.98, 95% CI 0.19–5.20) (Arbyn et al., 2015). Increased detection could therefore translate into more colposcopies and excisional treatment without demonstrated cancer benefit, with particular concern for subsequent obstetric morbidity. This reasoning remains reflected in the current Belgian guideline for women aged 25–29 years (Simoens et al., 2024). As vaccination reduces vaccine-type HPV infections in cohorts entering screening, the balance of benefits and harms of screening at ages 25–29 may change. Whether a different starting age would improve outcomes in Belgium requires outcome and programme data. The biological and economic cost/benefit will change considerably over time presumably for both cervical screening in general as well as specifically in the 25–29 age group.

The present birth-cohort analysis should be considered an early surveillance signal rather than evidence of vaccine effectiveness. The routinely vaccinated cohorts have not yet entered the 30–64-year primary-screening population. Continued surveillance of genotype distribution will become more informative once they do.

STRENGTHS AND LIMITATIONS

The main strength of this study is its real-world coverage of the complete first year of the new screening pathway within a large clinical laboratory. The dataset combines primary HPV results, assay genotype channels, reflex cytology, derived management advice and several implementation indicators. Counterfactual referral strategies were applied to the same population, avoiding differences in HPV prevalence and age structure that complicate comparisons between countries.

Several limitations define the scope of the conclusions. Histological CIN2+ and CIN3+ outcomes were not available. Recommended colposcopy could not be distinguished from colposcopy actually performed. Adherence to the one-year repeat branch could not yet be assessed, because none of the 439 women (screening, 30–64 year) assigned to this pathway had reached the scheduled repeat window by the end of follow-up. However, we know based on historical data, that the adherence to cervix cancer screening is estimated to be at around 60% in general (Arbyn et al., 2015). The assay reports several HPV types in pools, preventing individual genotype attribution within these channels. The dataset is test based rather than a deduplicated population cohort. Individual vaccination status was unavailable. Birth cohort is therefore only an indirect proxy for possible vaccine exposure.

The historical comparison also requires caution. Cytology was the primary screening test in 2022–2024, whereas cytology in women aged 30–64 years in 2025 is predominantly reflex testing in an HPV-positive population. Cytological distributions from these periods cannot therefore be compared as if they represented the same test population. The planned historical referral analysis should use primary screening tests as the denominator and reconstruct the management rules that were applicable at that time.

Finally, 2025 represents the first transition year rather than a stable five-year screening round. The observed prevalence, referral rate and workload may change as women enter the programme according to their previous screening history.

RECOMMENDATIONS AND FUTURE EVALUATION

The current data support continued monitoring rather than immediate modification of the national management algorithm. The laboratory can improve the quality of future evaluation by retaining the greatest feasible analytical genotype detail, even when the clinical report uses the simpler categories required by the national guideline. Clinical reporting resolution and stored analytical resolution do not need to be identical. More detailed data would permit same-genotype persistence analysis, retrospective regrouping after guideline changes and surveillance of genotype shifts as vaccinated cohorts enter screening.

Linkage with colposcopy and histology should be the main next step. CIN3+ outcomes would allow calculation of genotype-specific absolute risk, positive predictive value, number of colposcopies per CIN3+ detected and the clinical consequences of alternative referral algorithms. Longer follow-up will also permit evaluation of adherence to the one-year repeat branch and of same-genotype persistence.

A locally calibrated CIN3+-risk model is not justified after one year. Such a model would require substantially more outcomes, longitudinal screening history, robust histological ascertainment and validation in an independent or later cohort. Vaccination status will become increasingly important once routinely vaccinated cohorts enter primary HPV screening.

Assay selection should be reviewed in the same context. Additional genotype resolution has potential value for persistence, future risk stratification and vaccine surveillance, but this should be balanced against clinical validation, cost, throughput, hands-on time and the number of patients whose management would actually change.

CONCLUSION

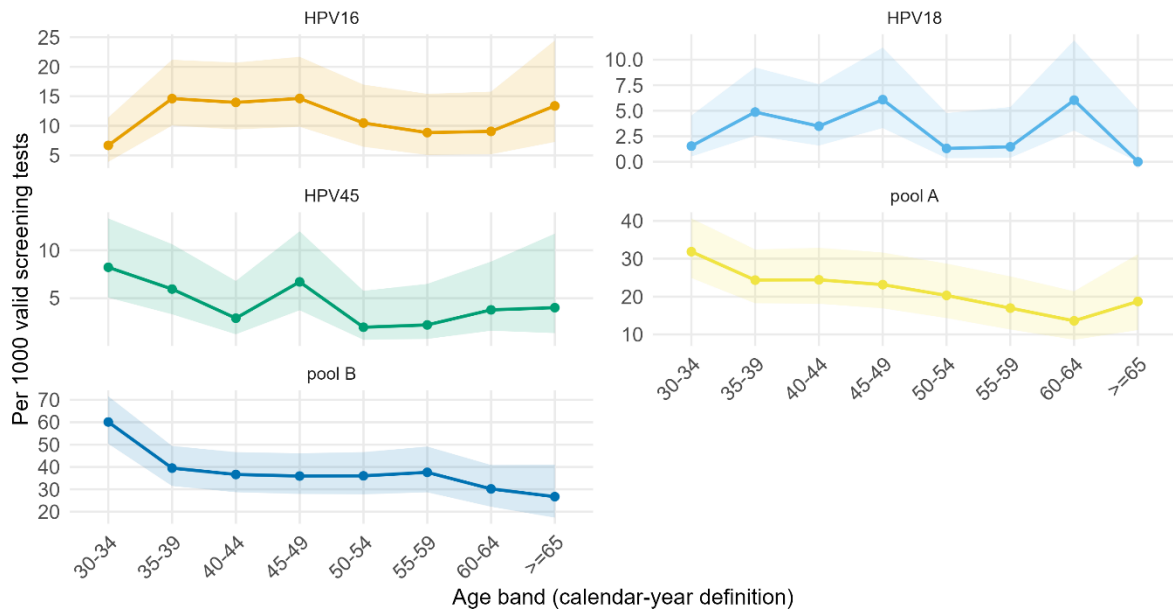
The first year of primary HPV screening shows that the Belgian algorithm can be implemented at scale and that only a minority of screening tests require intensified follow-up. HPV positivity and reflex cytology differ substantially between genotype groups, while the choice of triage rule materially changes colposcopy workload. These differences cannot yet be ranked according to clinical effectiveness because histological outcomes are unavailable. Longer follow-up linking genotype, same-type persistence, colposcopy and CIN3+ will be required to determine whether current flowchart is optimal for the Belgian screening program.

COMMENTS

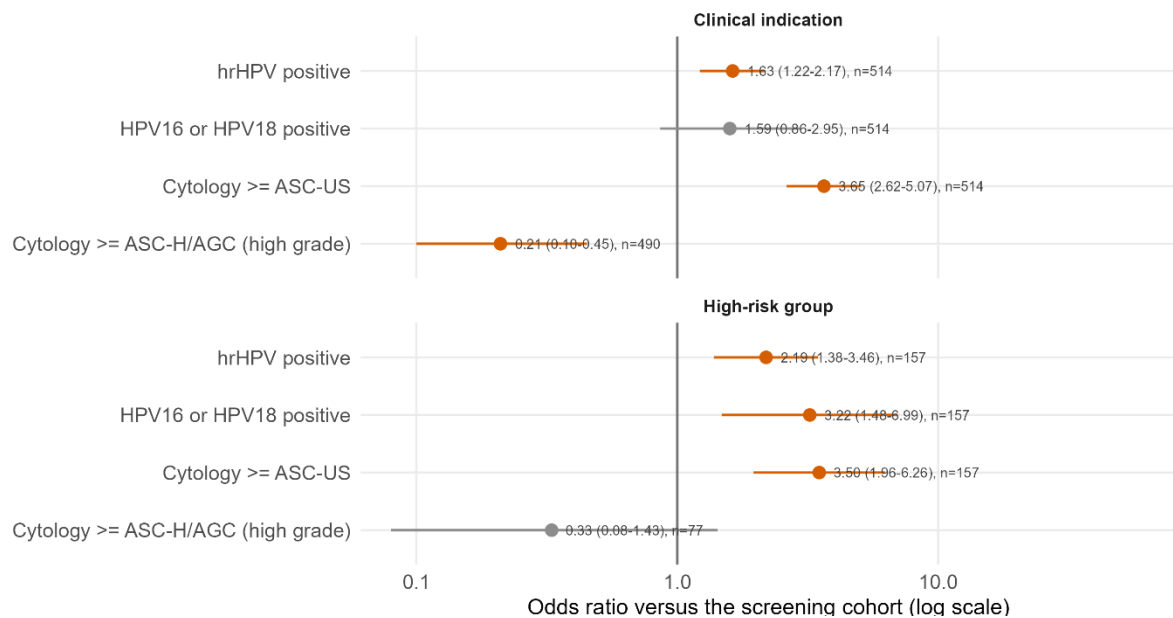
AI declarations:

AI (ChatGPT, Claude) was used to support literature searching, language correction and for coding; all cited sources and factual claims were verified.

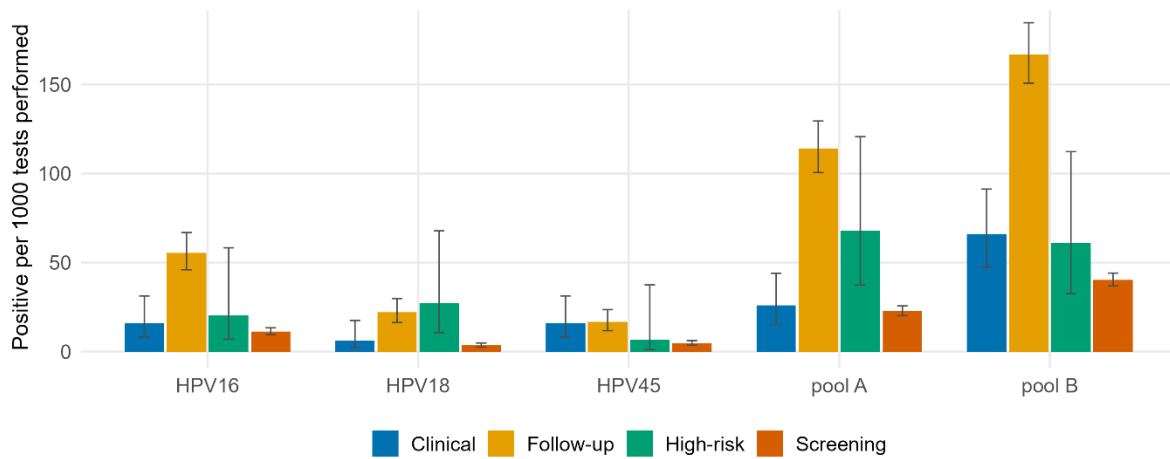
SUPPLEMENTARY MATERIAL



Supplementary Figure 1: HPV genotype channel positivity with 95% confidence intervals per age group.



Supplementary Figure 2: Age-stratified Mantel Haenszel odds ratio in the age-group 30-64 showing odds ratio for HPV positivity and abnormal cytology compared to the screening cohort per 1000 tests. Orange lines represent 95% confidence intervals per age group.



Supplementary Figure 3: Prevalence of HPV genotype channel in age-group 30-64 by indication and per 1000 tests with 95% confidence intervals.

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