

## 2. PRIMARY SCREENING & REFLEX TESTING (TRIAGE): new test scheme

### 2.1. Sampling technique

The technique to obtain a sample for cytology or an HPV test is equal. As a reminder, to obtain a specimen, a trained healthcare provider visualizes the cervix with a speculum placed in the vagina and performs a scraping of cervical cells (8). Various devices for specimen collection can be used and the manufacturer of the medical devices for in vitro diagnostics (IVD) will generally specify the appropriate collection device. The manufacturer's instructions for use should always be followed. Cervical specimens collected by a healthcare provider are generally placed in a liquid transport medium that is specified by the manufacturer of the IVD (9).

### 2.2. Overview for the different age groups

For the (new) flow and reimbursability of screening, the ages always refer to « **The calendar year in which one turns X years old** ». So also in the period between 1 January and the day one turns X in the same calendar year, one can have a reimbursed screening. Please read all reported ages as such.

**TABLE 1: NEW TEST SCHEME: PRIMARY SCREENING & REFLEX TESTING (TRIAGE)**

Age category	Primary screening	Frequency	Triage - reflex test (after a positive primary test)	2nd triage - repeat testing (in 12 months)
≤24y	No	NA	NA	NA
25-29y (Figure 1)	Cytology	Every 3 calendar years	ASC-US result → HPV test	hrHPV pos. → repeat cytology Primary screening result LSIL → repeat cytology
30-64y (Figure 2)	HPV test	Every 5 calendar years	hrHPV non-16/18 pos. → cytology (not as triage : HPV16/18 pos. → cytology + colposcopy)	NILM result → HPV test
65+ (exit or catch-up screening) <sup>1</sup>	Cotesting (cytology+HPV), on the same sample	Once	NA	hrHPV non-16/18 pos. + NILM → HPV test hrHPV neg. + ASC-US → HPV test

<sup>1</sup> If no screening was reimbursed in the previous 10 years.

- For patients under 25 years of age, screening will no longer be reimbursed.
- For persons between 25 and 29 years of age, a cytological examination every three calendar years remains indicated as a screening test (cfr. 2.3). In this age group, HPV infections are frequent but usually disappear spontaneously. Primary HPV screening would obtain many false-positive results in this group, leading to unnecessary additional examinations and overtreatment (conizations) with possible obstetric consequences (cervical insufficiency) (10, 11).
- For persons aged between 30 and 64 years, primary screening with HPV is done every five calendar years (The list of internationally validated high-risk HPV tests that can be used in cervical cancer screening in Belgium can be found at [National Reference Center \(NRC\) for Human papillomavirus | sciensano.be](http://www.nrc.be/en/cervical-cancer-screening/)). High-risk HPV types 16 and 18 are compulsorily tested and typed. The non-16/18 hrHPV types are also reported. In case of a positive result, additional cytology is performed and the triage algorithm is followed with or without a colposcopic examination (cfr. 2.3).
- After the age of 64 years, a one-off exit or catch-up screening may be performed, if screening has not been reimbursed in the previous ten years (cfr. 2.5). Cotesting is recommended in this cohort to maximize sensitivity (professional opinion).

### 2.3. Screening, triage and follow-up in the general population

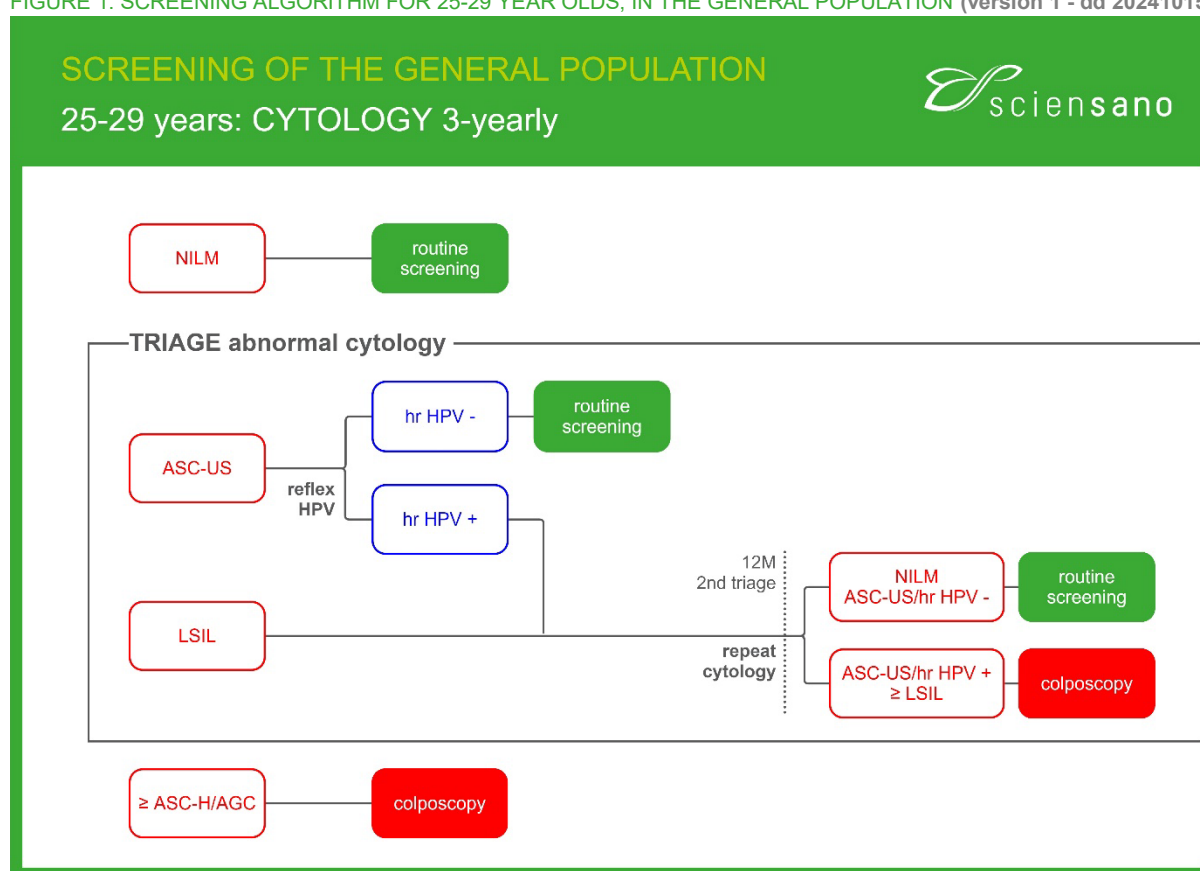
#### 25-29 year: TRIAGE ALGORITHM after a positive cytology screening

A screening test based on or using cytology can only be performed on a liquid based cytology (LBC) sample taken by a physician within the population screening programme. A self-collected sample does not qualify (12).

Women with an initial ASC-US diagnosis should be further triaged using a validated HPV test ([National Reference Center \(NRC\) for Human papillomavirus | sciensano.be](http://NationalReferenceCenter(NRC)forHumanpapillomavirus|sciensano.be)) as the immediate reflex test, followed by a second cytology triage 12 months later if hrHPV positive in the reflex triage step (13).

For women with a LSIL screening result, an initial HPV reflex test is not indicated, but a 12 month repeat cytology will further specify the risk of a high-grade lesion of the cervix (14, 15).

FIGURE 1: SCREENING ALGORITHM FOR 25-29 YEAR OLDS, IN THE GENERAL POPULATION (version 1 - dd 20241015)



≥ASC-H/AGC = ASC-H or a more severe abnormality (HSIL, SCC, AIS, AC) or AGC

Remark: An ASC-US result is always followed by a reflex HPV test (both in primary screening and in the 2<sup>nd</sup> triage 12 months later)

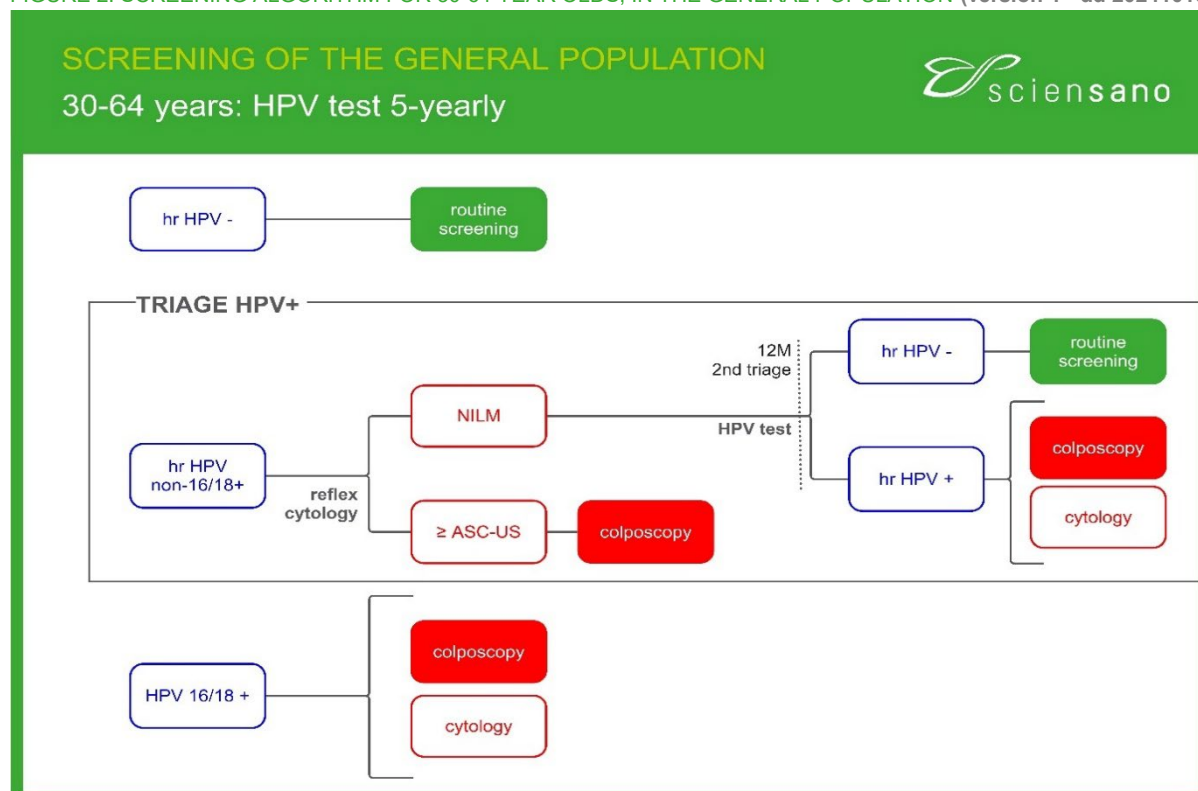
- Women from the general population (aged 25-29 in the initial screening round) with a negative cytology result (NILM = negative for intraepithelial lesion or malignancy) are called for the next screening after three years (16). The screening test offered varies according to the woman's age in the next screening round: cytology if under 30 years, an HPV test if the woman is 30 years or older in the meantime.
- Women with an ASC-US result (atypical squamous cells with undetermined significance) are immediately triaged using a reflex HPV test:

- ➔ If negative for hrHPV, the woman is referred back to the routine screening schedule (3-yearly).
- ➔ If positive for hrHPV, a repeat cytology after 12 months is advised:
  - If this result is normal (NILM), or shows ASC-US again with a negative hrHPV reflex test result, she can rejoin routine screening (3 years after the second triage cytology)
  - If the result is ASC-US again but with a positive hrHPV reflex test result, or LSIL or a more severe lesion, the women shall immediately be referred for colposcopic examination (13).
- For women with a LSIL result (Low-grade Squamous intraepithelial Lesion) a 12 month repeat cytology is indicated:
  - If this result is normal (NILM), or shows ASC-US with a negative hrHPV reflex test result, she can rejoin routine screening (3 years after the second triage cytology)
  - If the result is ASC-US but with a positive hrHPV reflex test result, or LSIL or a more severe lesion, the women shall immediately be referred for colposcopic examination (14, 15).
- Women with a cytological result of ASC-H/AGC or a more severe lesion (HSIL, SCC, AIS, AC) are immediately referred for colposcopic examination (17).

### 30-64 year: TRIAGE ALGORITHM after a positive HPV screening

The triage of HPV-positive women is based on HPV type and reflex cytology outcome as a threshold for colposcopy. In 30- to 64-year-olds, triage of hrHPV non-16/18 positives consists of an initial reflex cytology, followed by another HPV test 12 months later if the first step demonstrated NILM.

FIGURE 2: SCREENING ALGORITHM FOR 30-64 YEAR OLDS, IN THE GENERAL POPULATION (version 1 - dd 20241015)



- Women from the general population (30-64y) who test negative on the initial hrHPV screening should only undergo a new routine screening after five years (18).
- Women who test positive for another hrHPV type than 16 or 18 are further triaged via reflex cytology. Based on the outcome of the cytological examination:
  - ➔ women with a NILM result (negative for intraepithelial lesion or malignancy) are advised for another hrHPV test after a maximum of 12 months:
    - if this test is positive, the woman will be referred for colposcopy. Besides colposcopy, a reflex cytology will be performed.
    - if negative, she can rejoin routine screening (5-yearly hr HPV test: five years after the 2nd triage test) (19).
  - ➔ women with a result of ASC-US (atypical squamous cells with undetermined significance) or a more severe lesion will be referred for colposcopy.
- Women who test positive on the initial hrHPV test for the most high-risk HPV types 16 or 18 are immediately referred for colposcopy (19). Besides colposcopy, a reflex cytology will be performed.

#### GENERAL REMARKS ON THE FULL ALGORITHM OF 25 till 64 year

- ***Please adhere to the above screening algorithms, even if the NIHDl nomenclature allows broader testing possibilities.***
- By "immediate referral for colposcopic examination" is meant **within 3 months or faster**, according to the severity of the screen-positive result (cfr. 5.3.1: Target waiting time for colposcopy after screen-positive results).
- If cells from the transformation zone (endocervical cells) are not present, this is listed as being a **suboptimal sample**.
- If cytological examination finds **normal** endometrial cells in a sample from a woman older than 45 years, this will be reported. The treating physician will consider (for post-menopausal women) whether this is of clinical significance for the woman in question and whether additional investigation is required (19, 20).
- If on cytological examination **abnormal** endometrial cells are found at any age, the treating physician will get the additional advice: 'Exploration to exclude endometrial pathology' (19, 20).

#### 2.4. Integrated advice from the lab to the physician

##### **25-29 year: sample and results flow**

###### Flow from cytology to molecular laboratory

The cytology laboratory provides the molecular laboratory with the remainder of the primary LBC sample or at least an aliquot (in addition to administrative data from patient and sample) for performing a reflex HPV test after a cytology result indicating ASC-US.

###### Results flow from molecular to cytology laboratory

The molecular laboratory provides the cytology laboratory with the result of the reflex HPV test for incorporation of the HPV result into the integrated advice, to be send to the requesting physician (and to the Belgian Cancer Registry, in the future).

### 30-64 year: sample and results flow

#### Flow from molecular to cytology laboratory

The molecular laboratory provides the cytology laboratory with at least both the primary sample or an aliquot thereof and the obtained HPV test result (administrative data from patient and sample, type(s) of HPV).

#### Results flow from cytology to molecular laboratory

The cytology laboratory provides the reflex cytology result following positive primary HPV testing to the molecular laboratory for incorporation of the cytology result into the integrated advice; to be send to the requesting physician (and to the Belgian Cancer Registry, in the future).

### INTEGRATED ADVICE – 25-29 AND 30-64y in the general population (ANNEX 1, for a bigger format)

Age range: 25-29 years					
Result cytological examination	Result reflex hrHPV test	(Integrated) advice	Result 2 <sup>nd</sup> triage (in 12 months)	Result reflex hrHPV test	(Integrated) advice
NILM	NA	Regular screening interval (in 3 calendar years)			
ASC-US	--> hrHPV negative --> hrHPV positive	<b>Result of reflex HPV test, with recommendation, will follow</b> Regular screening interval (in 3 calendar years) Repeat cytology in 12 months	--> NILM --> ASC-US	NA --> hrHPV negative --> hrHPV positive	Regular screening interval (in 3 calendar years) <b>Result of reflex HPV test, with recommendation, will follow</b> Regular screening interval (in 3 calendar years) <b>Immediate referral for colposcopic examination</b>
			--> ≥ LSIL		Immediate referral for colposcopic examination
LSIL	NA	Repeat cytology in 12 months	--> NILM --> ASC-US	NA --> hrHPV negative --> hrHPV positive	Regular screening interval (in 3 calendar years) <b>Result of reflex HPV test, with recommendation, will follow</b> Regular screening interval (in 3 calendar years) <b>Immediate referral for colposcopic examination</b>
			--> ≥ LSIL		Immediate referral for colposcopic examination
≥ ASC-H/AGC	NA	Immediate referral for colposcopic examination		NA	Immediate referral for colposcopic examination
INSU	NA	New sampling after 6 weeks at the earliest		NA	
Age range: 30-64 years					
Result hrHPV test	Result reflex cytology	(Integrated) advice	Result 2 <sup>nd</sup> triage (in 12 months)	(Result reflex cytology)	Advice
hrHPV negative	NA	Regular screening interval (in 5 calendar years)			
hrHPV non-16/18 positive	--> ≥ ASC-US --> NILM	<b>Result of reflex cytology, with recommendation, will follow</b> Immediate referral for colposcopic examination Repeat hrHPV testing in 12 months	--> hrHPV negative --> hrHPV positive	(cytology, not as triage)	Regular screening interval (in 5 calendar years) Immediate referral for colposcopic examination Result of reflex cytology will follow
HPV 16/18 positive	(cytology, not as triage)	Immediate referral for colposcopic examination Result of reflex cytology will follow			
HPVi		New sampling after 6 weeks at the earliest			

≥ASC-H/AGC = ASC-H or a more severe abnormality (HSIL, SCC, AIS, AC) or AGC

INSU: insufficient cytology, not representative sample (lack of epithelial cells/insufficient cellular material, cell lysis, abundant blood, ..)

HPVi: inconclusive HPV test result

NB: Immediate referral for colposcopic examination is understood within 3 months or faster, according to the severity of the screen-positive result (cfr. 5.3.1)

Cave: If on cytological examination **normal** endometrial cells are found in an entitled person > 45 years, additional advice is given: 'Correlation with clinic is indicated to exclude endometrial pathology in post-menopausal women'.

Cave: If on cytological examination **abnormal** endometrial cells are found at any age, additional advice is given: 'Exploration to exclude endometrial pathology'.

In bold: the actual integrated advice, after samples were send for reflex testing.

### 2.5. Advice for ≥65 year (exit or catch-up screening)

For the 65+ year-olds with catch-up or exit screening (being cotesting cytology and HPV), if no screening was reimbursed in the last 10 years, no integrated advice will be prepared by the laboratories. Each laboratory will provide a therapeutic advice based on the tests performed. The treating physician has to decide the necessary follow-up upon the separate reports and will find guidance in the table underneath (to be referenced by the lab together with the single therapeutic advice for this age group).

**ADVICE: ≥65y, in case of no reimbursed screening in the last 10 years (ANNEX 2, for a bigger format)**

Age range: ≥ 65 year, in case of no screening in the last 10 years			
Result hrHPV test and cytology within cotesting	Advice	Result repeat HPV testing (in 12 months)	Advice
HPV 16/18 positive, independent of cytology result	Immediate referral for colposcopic examination		
hrHPV non-16/18 positive	Result of co-test cytology with recommendation will follow		
--> cytology ≥ ASC-US	Immediate referral for colposcopic examination		
--> cytology NILM	Repeat hrHPV testing in 12 months	-> hrHPV negative	No further follow-up
		-> hrHPV positive	Immediate referral for colposcopic examination Result of reflex cytology will follow
hrHPV negative	Result of co-test cytology with recommendation will follow		
-> cytology ≥LSIL	Immediate referral for colposcopic examination		
-> cytology ASC-US	Repeat hrHPV testing in 12 months	-> hrHPV negative	No further follow-up
		-> hrHPV positive	Immediate referral for colposcopic examination Result of reflex cytology will follow
-> cytology NILM	No further follow-up		
HPVI	New sampling after 6 weeks at the earliest		

HPVI: inconclusive HPV test result