Country Meeting

Prevention and control of HPV and HPV related cancers in France: the current landscape and way forward

The transition from Cytology to HPV-based screening

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Healthcare system / cervical cancer screening / HPV vaccination, Annecy, 2-3 décembre 2021 (visio Reims)

The transition from Cytology to HPV-based screening

HPV testing

HPV context and transition

Healthcare settings and controversies

(+ Co-testing)

1- Why HPV testing ?

HPV = necessary cause of cervix cancer (CC) → 99,7% of CC associated with a persistent hrHPV

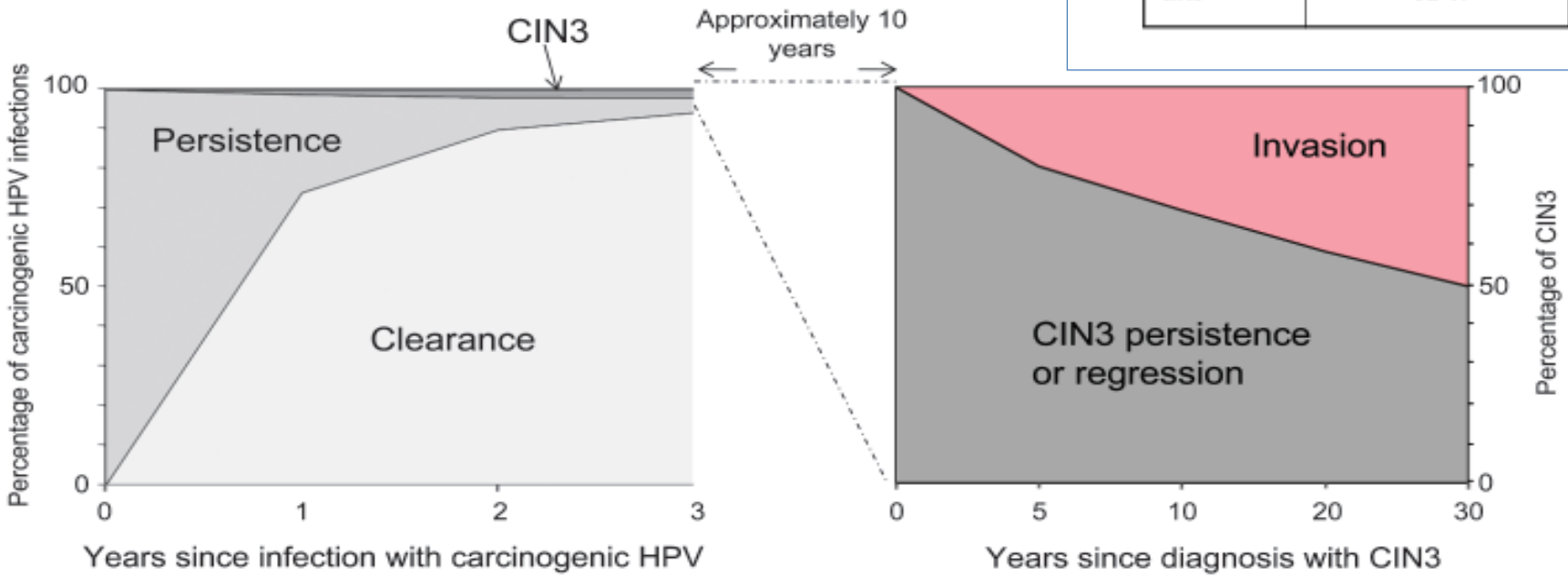
→ **efficacious primary prevention :** vaccination since 2007 ; Gardasil 9v since 2018 (France)

→ **secondary prevention**: HPV primary screening for women > 30 yrs

(France: HAS 2019, organisation pending)

Tableau 1 - Évolution des CIN (suivi de 2 à 7 ans) Östor, 1993

	Régression	Persistance	Progression vers une invasion
CIN2	43 %	35 %	5 %
CIN3	32 %	56 %	12 %



Screening = Sensitivity
Numerous Cohorts and randomized studies

- **Cytology:** lower sensitivity (CIN2+) → Se 40-70% → to repeat and PPV ↓ with vaccination
- **HPV testing**: higher sensitivity and higher NPV of HPV → extended HPV testing in time (5 yrs)

	Sensibilité CIN2+	Spécificité CIN2+
HPV-ADN	96%	91%
CYTOLOGIE	53%	97%

Cuzick 2006

HPV tests Se / Sp

Evaluated assay	Study	Absolute sensitivity	Absolute specificity	Comparator assay Reference Assay	Absolute sensitivity	Absolute specificity	New/comparator assay Relative sensitivity	Relative specificity
GP5+/6+ PCR*	Meijer, 2009[9]	98.7%	96.0%	HC2*	98.7%	94.1%	1.00	1.02
PapilloCheck	Hesselink, 2010[18]	95.8%	96.7%	GP5+/6+ PCR	96.4%	97.7%	0.99	0.99
Abbott RT	Carozzi, 2011[19]	96.4%	92.3%	HC2	97.6%	92.6%	0.99	1.00
hrHPV test	Poljak, 2011[20]	100.0%	93.3%	HC2	97.4%	91.8%	1.03	1.02
	Hesselink, 2013[21]	95.6%	92.0%	GP5+/6+ PCR	98.5%	91.8%	0.97	1.00
cobas 4800	Heideman, 2011[22]	90.0%	94.6%	HC2	91.7%	94.4%	0.98	1.00
	Lloveras, 2013[23]	98.3%	86.2%	HC2	98.3%	85.3%	1.00	1.01
qPCR(*E6/E7*)	Depuydt, 2012[24]	93.5%	95.6%	HC2	83.9%	94.4%	1.11	1.01
APTIMA	Heideman, 2013[25]	95.5%	94.5%	GP5+/6+ PCR	100.0%	93.6%	0.96	1.01
Cervista	Boers, 2014[26]	89.0%	91.2%	HC2	93.4%	88.8%	0.95	1.03
	Alameda, 2015[27]	98.4%	85.2%	HC2	100.0%	86.4%	0.98	0.99
BD Onclarity	Ejegod, 2014[29]	92.9%	87.7%	HC2	94.2%	88.8%	0.99	0.99
HPV-Risk assay	Hesselink, 2014[28]	97.1%	94.3%	GP5+/6+ PCR	97.1%	94.1%	1.00	1.00

Validated in randomised trials demonstrating lower incidence of cervical cancer
Cross-sectional performance fully validated according to equivalency criteria
Cross-sectional performance partially validated according to equivalency criteria
No hrHPV DNA assay

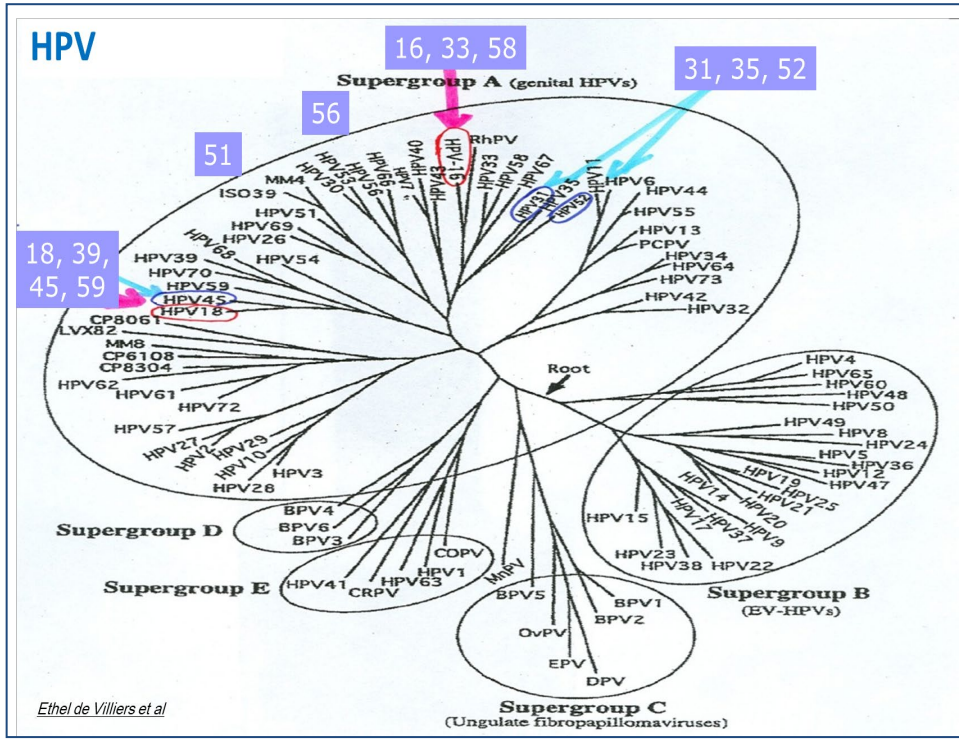
Arbyn 2015

2- HPV context and transition from cytology

Transition

- **cytology (co-testing)→ primary HPV screening (Se) + triage (Sp)**
- numerous HPV (13-14 hr HPV)
- **numerous HPV tests + new biomarkers**
- **quality of samples** (LBC, self-samples) +++
- one test not enough → **different algorithms** of HPV screening
- **screening and vaccination**: synergic
- **other HPV-induced cancers**

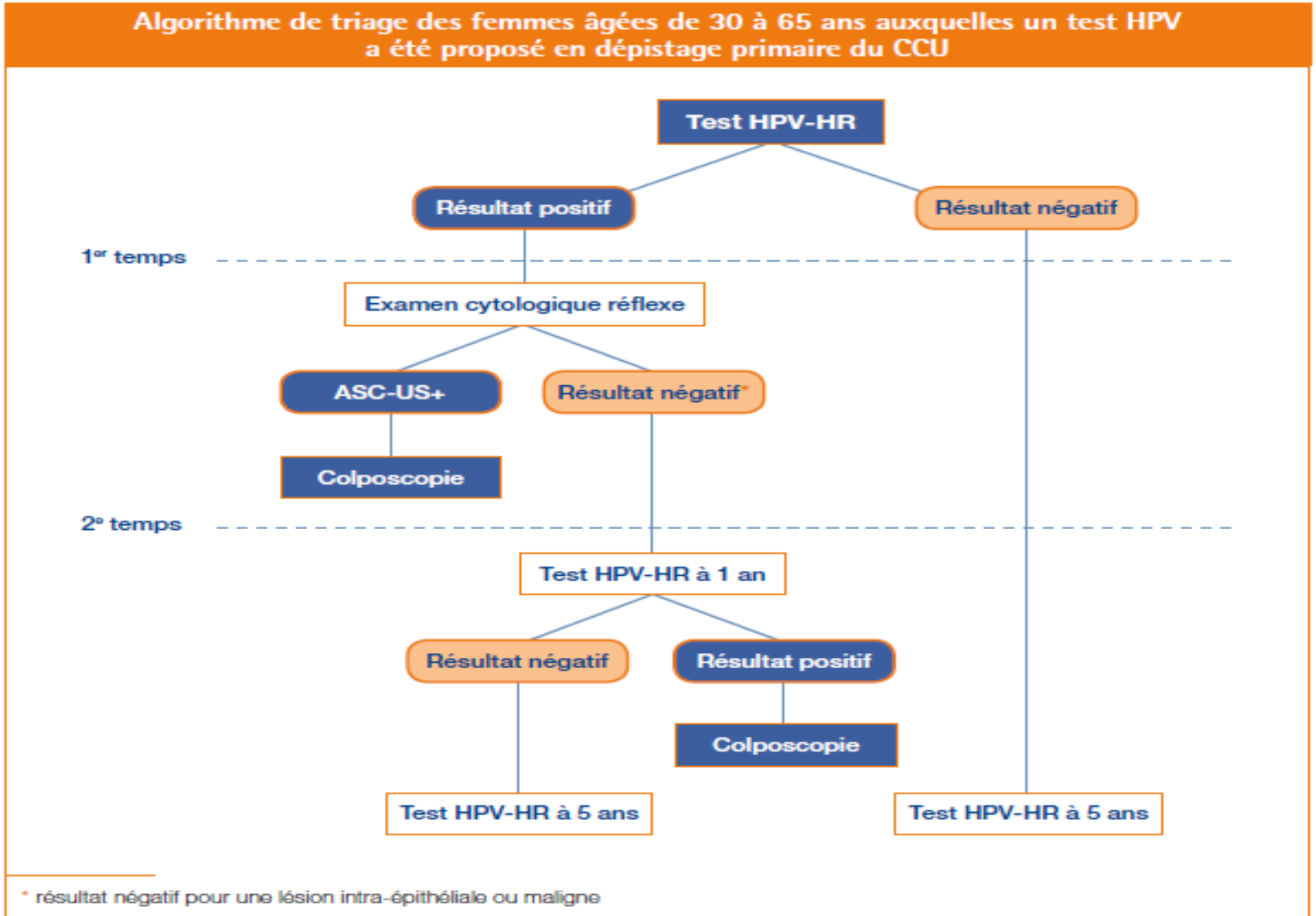
  → anal canal, vulvar…oropharyngal cancers



→ ALWAYS A NEED TO INCREASE, TO SIMPLIFY AND TO STANDARDIZE HPV SCREENING PROCESSES !

CHU REIMS

HPV screening France - 2019



Various CC screening policies in Europe -2019 (Mavel 2020)

→ positive experience with national or regional HPV-based screening implemented

Table 1
Summary of current status of implementation of HPV-based cervical cancer screening in selected European countries and main characteristics of the screening programmes

Country	Implementation phase	Screening programme organisation	Year of implementation	Age range of women screened within the programme	Screening interval	Primary test used in the screening programme	Triage test used in the screening programme
The Netherlands	Implemented	National	2017	30–60 (65 if HPV-positive at the last screening)	5 years until age 40 10 years after age 40	HPV test	Cytology
Turkey	Implemented	National	2014	30–65	5 years	HPV test	Reflex HPV 16/18 genotyping and cytology
Italy	Implementation ongoing	Regional	2014–2018	30–64	5 years	HPV test	Cytology or HPV 16/18 genotyping
Sweden	Implementation ongoing	Regional	2017	23–64	3 years until age 49 7 years after age 49	HPV test in women after age 30; cytology in women age 23–29	Cytology
Finland	Implementation ongoing	Regional	2016	30–60 (some municipalities 25–65)	5 years	HPV test or cytology	Cytology
Spain	Implementation ongoing	Regional	2014	25–65	3 years for cytology 5 years for HPV or co-testing	Three options in women after age 31: cytology, HPV test or co-testing; cytology in women age 25–30	Cytology or HPV test or co-testing (depending on regional recommendations)
Norway	Implementation planned	National	2019–2021	25–69	3 years for cytology 5 years for HPV	HPV test in women after age 34, cytology in women age 25–33	Cytology
Denmark	Implementation planned	National	2020	23–65	3 years for cytology 5 years for HPV	HPV test replacing cytology in at least 50% in women age 30–59; cytology in women age 23–29; HPV test in women age 60–65	Cytology
United Kingdom	Implementation planned (ongoing in Wales)	National	Wales: 2018; England, Scotland, and Northern Ireland: 2019/2020	25–65	3 years until age 50 5 years after age 50	HPV test	Cytology
Belgium	Implementation planned	National	2020/2021	25–64	5 years	HPV test in women after age 30; cytology in women age 25–29	Cytology
Germany	Implementation planned	National	2020	20–60	Yearly for cytology 3 years for co-testing	Co-testing in women after age 35; cytology in women age 20–34	Cytology in women age 20–29, co-testing in women after age 30
Malta	Implementation ongoing	National	NA	>25	3 years for cytology 5 years for VIA or HPV	HPV test in women after age 30 or cytology in women age 25–50; visual inspection with acetic acid (VIA) in women after age 50	Cytology or HPV test

France (2019, pending)

3- Healthcare settings and controversies

Wentzensen Arbyn 2017

Ronco Lancet 2014, Cuzick J Clin Virol 2014, Schiffman 2015, Wentzensen 2016, Polman 2017, Olgivie JAMA 2018, Arbyn BMJ 2018, *Clarke 2018, Adcock CEBP 2019, Salazar 2019, Bhatla 2020, Maver 2020…*

Cancer Genome Atlas Reseach Work Nature 2017

How to switch to HPV-based screening ? To define :

- ❑ **HPV screening assays**
- ❑ **Triage tests**
- ❑ **Screening interval for HPV negative women**
- ❑ **Integrated HPV screening**
- ❑ **Link with vaccination**

1-HPV screening assays

- **HPV testing: early detection and diagnosis of CIN3 and long term protection**

→ more effective to detect precancerous lesion @ the first round than cytology-based screening
→ cancer risk @ 3 yrs after a negative HPV tests : 70% lower compared to a negative cytology

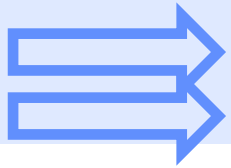
- better when cytology offers a lower performance

- no discrimination between transient and persistent HPV infection
  (easy HPV detection but clinical signification less easy)

- **no difference of efficacy with age** ; higher protection of women of 30-35 yrs old

→ for transition, evidence of higher program efficiency of HPV-based screening
→ better follow-up with same HPV test

→ FDA approved assays
→ International criteria and guidelines (Meijer 2009…)



5 tests FDA approved

Table 1 Comparison of the 5 FDA-approved testing platforms.

Test	Hybrid Capture II	Cervista	cobas	Aptima	BD Onclarity
Manufacturer	Qiagen	Hologic	Roche	Gen Probe (Hologic)	Becton Dickinson
Year FDA approved for reflex HPV testing and HPV/Papanicolaou co-testing	2001	2009	2011	2011	2018
Year approved for primary screening	N/A	N/A	2014 (ThinPrep only)	N/A	2018 (SurePath only)
Method	DNA (non-PCR based) Signal amplification: full genome probe	DNA (non-PCR based) Signal amplification: L1, E6, and E7 genes	DNA (PCR based); Target amplification: L1 gene target	mRNA (PCR based); Target amplification: *E6/E7* gene target	DNA (PCR based); Target amplification: *E6/E7* gene target
Genotypes detected	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 with genotyping of 16 and 18	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; genotyping as separate test (16, 18/45)	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68; simultaneous, discrete identification of 16, 18, and 45
Clinical trial	ASC-US/LSIL Triage Study (ALTS), 2006 CAP	Cervista HPV HR	ATHENA[12]	CLEAR trial	Onclarity trial (baseline phase)[13]
Clinical validation	Extensive	Limited	Limited	Limited	Limited
Sensitivity for CIN2/3	63.6%-100%[2,14-24]	92.8%-100%[25]	71.1%-99%[2,15-21,26]	55.3%-100%[2,14,17-20,22-24,26-30]	85.7%-100%[18,31-33]
Specificity for CIN2/3	6.2%-98.4%[2,14-24]	–	24%-86.2%[2,15-21,26]	28.8%-99.2%[2,14,17-20,22-24,26-30]	17%-98.8%[18,31-34]
Built-in internal control	No	Yes (HIST2H2BE)	Yes (ß-globin)	Yes, an internal control transcript (HPV16 E6/7 transcript) is added to each reaction at the target capture step	Yes (ß-globin)

Abbreviations: N/A, not applicable; PCR, polymerase chain reaction.

Salazar 2019

hrHPV

HPV testing

Classification αHPV :

- **Groupe 1 : carcinogenic**

HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

- **Groupe 2A : probably carcinogenic : HPV 68 (preuves limitées)**

- **Groupes 2B : possibly carcinogenic :**

HPV 26, 53, 64, 65, 66, 67, 69, 70, 73, 82 (preuves limitées)

- **Groupe 3 : non carcinogenic : HPV 6, 11**

→ pool of 14 hrHPV :
HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (Gardasil 9®)

HPV testing :

→ HPV testing without genotyping: cocktail test with 13-14 hrHPV (DNA / RNA)
→ HPV testing with complete genotyping: 20-50 hr HPV (vaccination controls)
→ HPV testing with partial genotyping: HPV 16/18 (+33?) and other HPV group(s)

→ assay to detect rare HPV, variants, integration, viral load… (epidemiology, next routine ?)
→ indirect markers (consequence of infection): p16, methylation…

HPV testing performance and gold standards

✓ **International consensus recommendations** (HPV test validation, Se/Sp/reproductibility…QC)

✓ **Clinical validation** with numerous positive and negative samples (Meijer 2009, Stoler 2007, Arbyn 2015)

→ **VALGENT studies**:
VALidation of HPV **GEN**otyping **T**ests

VALGENT: A protocol for clinical validation of human papillomavirus assays

Marc Arbyn [a,*], Christophe Depuydt [b], Ina Benoy [b], Johannes Bogers [b], Kate Cuschieri [c], Markus Schmitt [d], Michael Pawlita [d], Daan Geraets [e], Isabelle Heard [f], Tarik Gheit [g], Massimo Tommasino [g], Mario Poljak [h], Jesper Bonde [i], Wim Quint [e]

✓ **Recommendations from HPV CNR** (*Centre National de Référence*) **France (Besançon) :**

https://cnr-hpv.fr/wp-content/uploads/2021/01/Liste-des-trousses-de-detection-et-de-genotypage-des-HPV-validees-par-les-fabricants-de-milieux-v8.pdf

✓ **List of HPV tests for primary CC screening**
(Arbyn, CMI 2021)

ELSEVIER

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Systematic review

2020 list of human papillomavirus assays suitable for primary cervical cancer screening

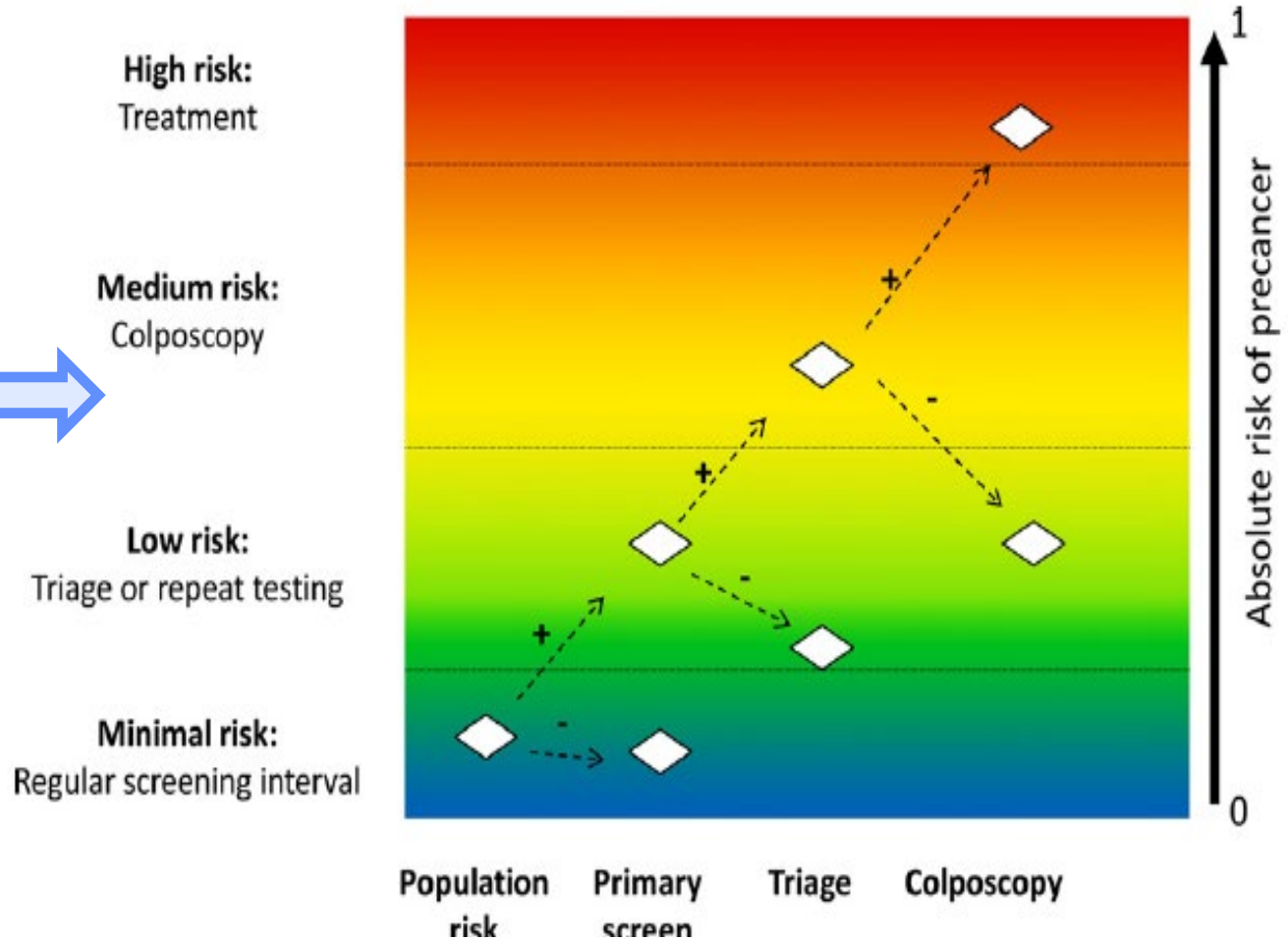
Marc Arbyn [1, 2, *], Marie Simon [3], Eliana Peeters [1], Lan Xu [1, 4], Chris J.L.M. Meijer [5], Johannes Berkhof [6], Kate Cuschieri [7], Jesper Bonde [8], Anja Ostrbenk Vanlencak [9], Fang-Hui Zhao [10], Remila Rezhake [1, 10, 11], Murat Gultekin [12], Joakim Dillner [13], Silvia de Sanjosé [14], Karen Canfell [15, 16], Peter Hillemanns [17], Maribel Almonte [18], Nicolas Wentzensen [19, †], Mario Poljak [9, †]

2- Triage is required

❑ **pre-cancer end-points**
→ to define women at risk (alert treshold):
CIN2+, 2 HPVhr+ tests, HPV16/18+
→ **risk stratification using triage program (colposcopy)**

❑ various options of triage
❑ but optimal strategies limited

✓ to not mis a HG lesion
✓ to check HPV persistence
✓ to not increase colposcopy
✓ to not overtreat



Wentzensen 2016

❑ **Triage tests (specificity) with growing number of differents options + new molecular tests…**

- ✓ cytology (in many countries / France)
- ✓ partial HPV genotyping
- ✓ viral load
- ✓ p16/Ki67 (more longitudinal studies needed)
- ✓ methylation signatures (viral genes, human genes)
- ✓ HPV-E6 proteins detection…
- ✓ tumoral biomarkers… (*Cancer Genome Atlas Reseach Work Nature 2017*)

→ **large comparison and longitudinal studies are still missing**
→ **new markers pending**
→ **to simplify testing in next future** (global testing using one NGS DNA seq test ?)

3- Screening intervals for HPV negative women

❑ **5 yrs:**
→ important point
→ better safety than CYTO analysis every 3 yrs (reduced frequent screening)

❑ **5 yrs is very safe:** extended intervals with repeated negative screens
→ participation at longer intervals: good compliance
→ education of women

→ physicians (MP, gyneco) may accept recommandation against annual pelvic examination
→ with formation
→ with ease of computer to check screening of women

4- Integrated HPV screening

❑ **Integrated HPV screening:**

→ different countries, cultures, infrastructures, settings

→ costs with different healthcare funds

→ to switch countries with low CC rates and established cytology-based screening

❑ **To evaluate the performance of an entire screening program** (Wentzensen Arbyn 2017):

- organized management HPV+ women: follow-up at longer intervals (compliance), treatments
- organized administration: adherence to screening policy, invitations and reminders (majority of CC = women with no participation in regular screening)
- + self sampling kits (mailing, pharmacies) to increase coverage,
  → using sensitive HPV PCR assays (Arbyn BMJ 2018)

❑ **Challenge +++**: **synergic integration of screening and vaccination programs**

→ organized settings with vaccination & screening registries programs

- with a continuous decrease of CC risk
- now, vaccinated women reach the age of screening

→ future: organized vaccination for young girls and boys ? (school ?)

→ in the long term with vaccination: ↓PPV of cytology ; ↓carcinogenic HPV types, with lower CC risk

HPV vaccination coverages in Europe

Nguyen-Huu, Vaccine 2020

Various VC
Different populations
Different settings

High % of HPV VC :
→ efficient infrastructures (schools)
→ free programs with efficient invitations and reminders
→ easy access to vaccines

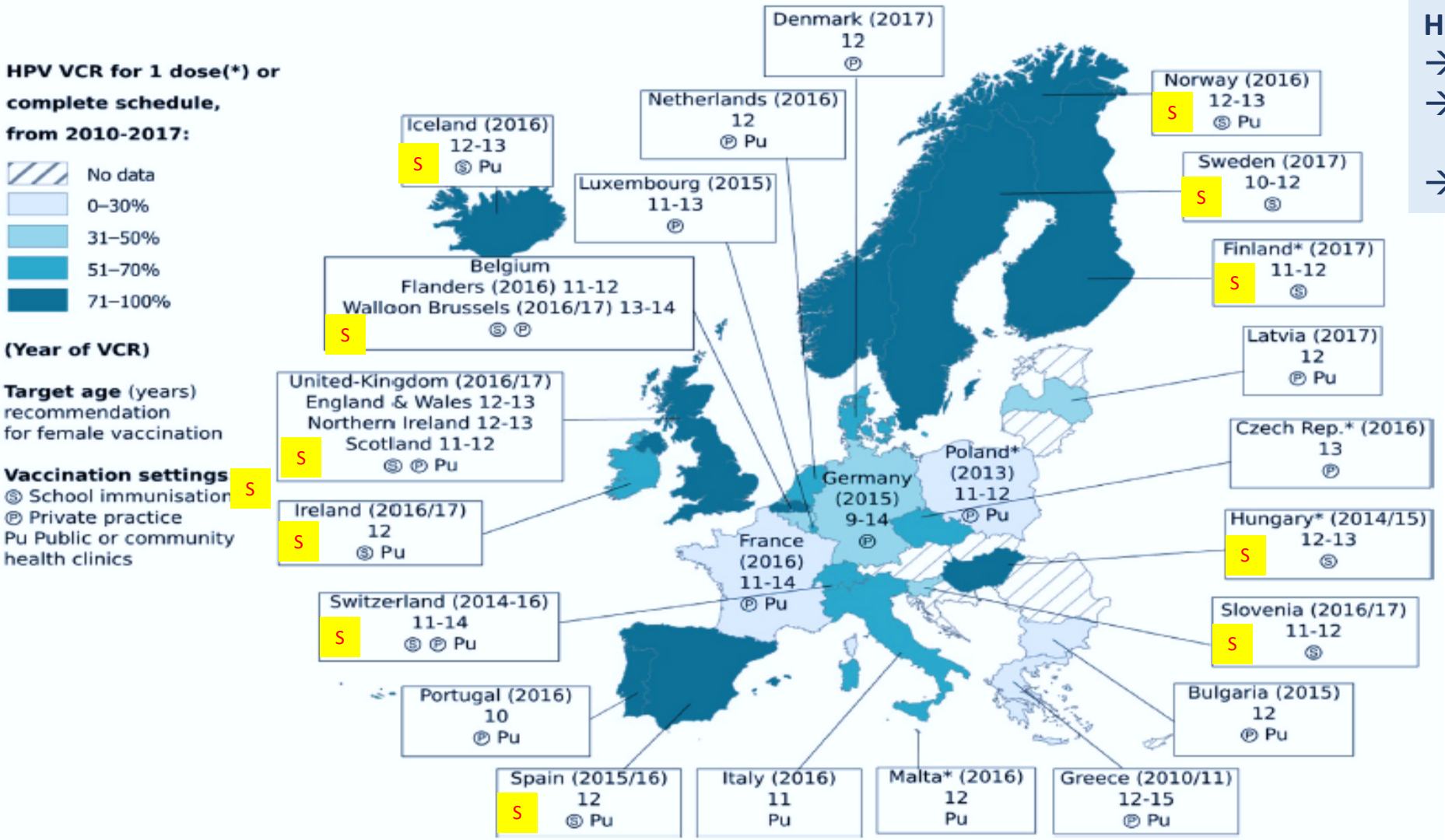


Fig. 3. Human Papillomavirus vaccination coverage rates (HPV VCR) in target agegroups in females and vaccination settings in European countries, 2010–2017.

Co-testing vs HPV testing alone

→ to not miss a subset of CC (HPV- et CYTO +)

❑ **explanation of negative HPV test in CC :**

1. very rare CC would be not caused by HPV
2. rare CC caused by rare hrHPV, not included in the panel of 13-14 hr types
3. during HPV integration: parts of HPV genome (L1) may be lost (numerous L1 HPV assays) → not enough evaluation
4. pb of quality of samples and necrotic materials

❑ for transition, **actually limited benefit of adding CYTO to HPV testing**

→ more expensive with many HPV negative and normal CYTO cases

→ after neg HPV test, an additional CYTO neg do not provide more reassurance

→ addition of CYTO to HPV test raised sensitivity (Bhatla 2020):

- ✓ by only 5% for CIN2+
- ✓ and 2% for CIN3+ compared to HPV test alone (with loss of Sp)
- ✓ the CYTO component contributed only 5 cases per million women per year to the sensitivity of the combined test

Conclusion

- ❑ **Efficiency of HPV screening**
- ❑ **Cytologic triage at present time**
- ✓ but may change

→ **impact of self sampling (molecular tests)**

→ **impact of vaccination** with ↓ PPV CYTO and HPV (HPV 16/18 (+31/33/45/52/58))

- ❑ **At present time 2 tests**: screening with partial genotyping (16/18) and CYTO for triage

- ❑ **<u>Next assays independant of HPV types</u> ?**
  - ✓ p16/Ki67 for triage ?? (cumulated risk CIN2+ @ 5 yrs > abnormal CYTO)
  - ✓ methylation testing (L1/L2HPV)
  - ✓ and/or methylation of human genes (EPB41L3…)

  - ✓ global testing combining HPV genotyping and methylation targets
  - ✓ to add molecular markers for targeted therapies
  - ✓ *for cancers: circulating tumor DNA (diagnosis and follow-up of treatments)*