Universiteit Antwerpen

VACCINE-INDUCED HPV-SPECIFIC ANTIBODIES IN CERVICOVAGINAL SECRETIONS

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Centre for the Evaluation of Vaccination

Meeting HPV Prevention and Control Board

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Contents lists available at ScienceDirect

Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr

[redacted] vaccine-induced HPV-specific antibodies in cervicovaginal secretions. A review of the literature

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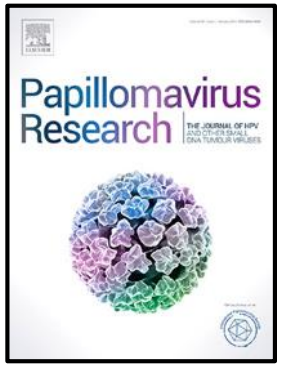


I/ WHY?

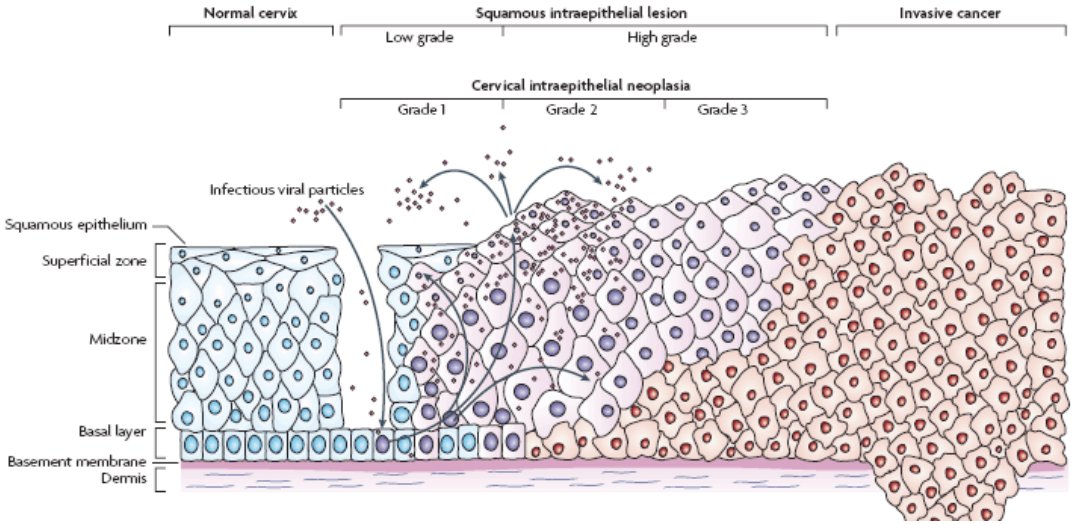
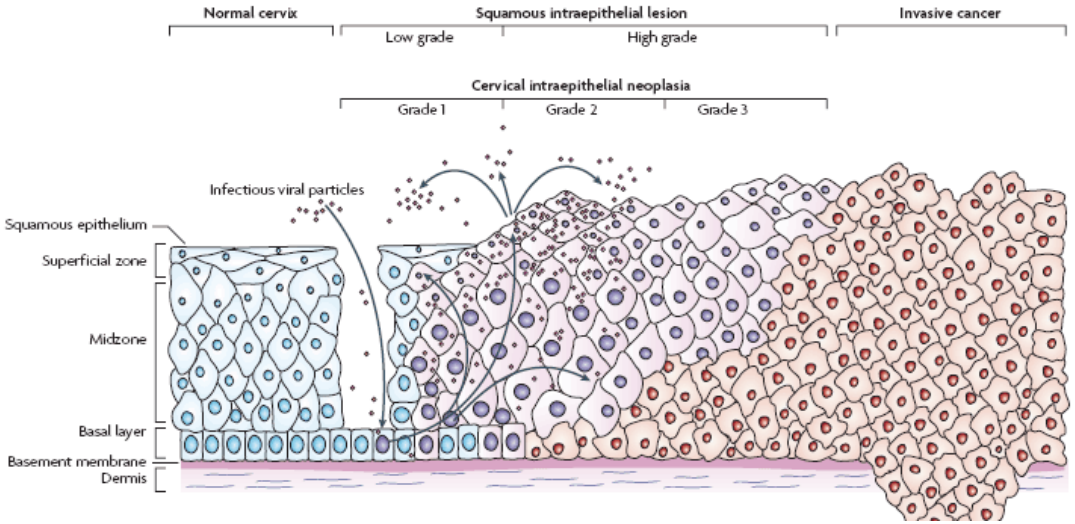


Figure derived from Kelloff & Sigman et al. 2007

- HPVs are specifically epitheliotropic
- Infect and propagate in the *female genital tract* (almost no viremic phase)
  - ! measure the induction of an effective immune response at disease-relevant sites

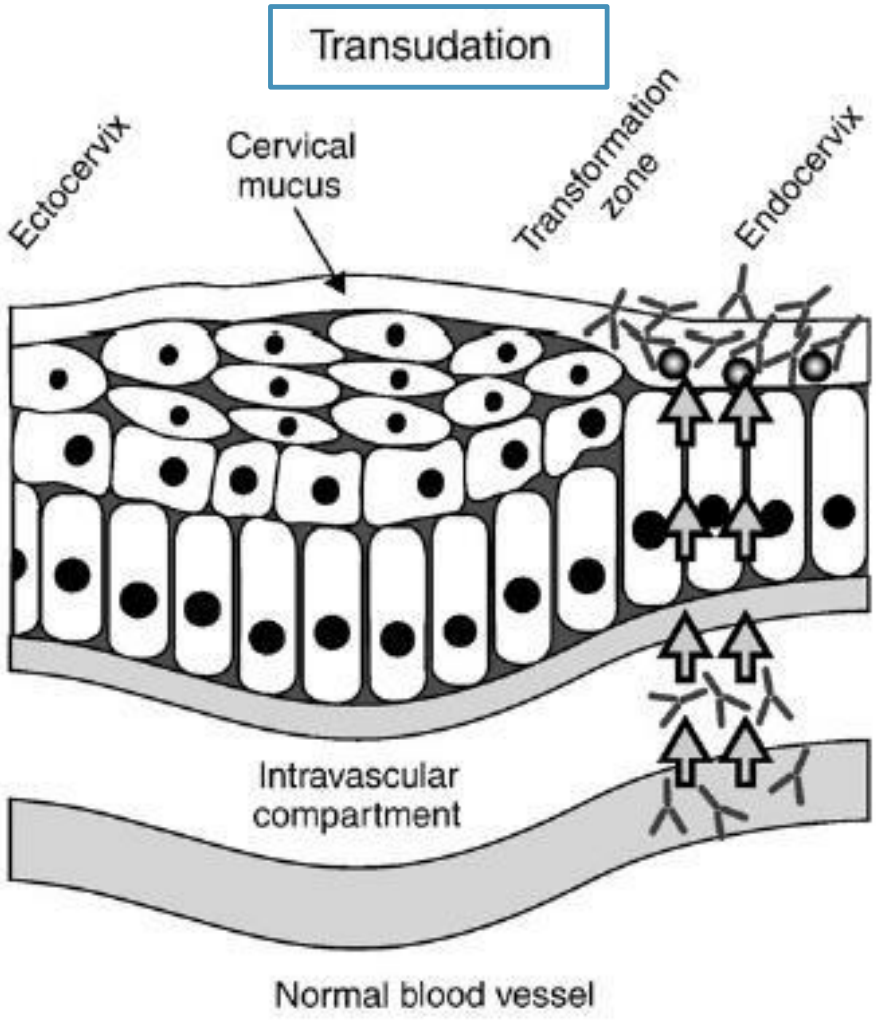
HPV-spec. antibodies in CVS

I / WHY?



- HPVs are specifically epitheliotropic
- Infect and propagate in the *female genital tract* (almost no viremic phase)
  - ! measure the induction of an effective immune response at disease-relevant sites

- To assess the current knowledge of specific antibody levels at the cervix
- To aid future studies investigating mucosal (HPV-specific) antibodies



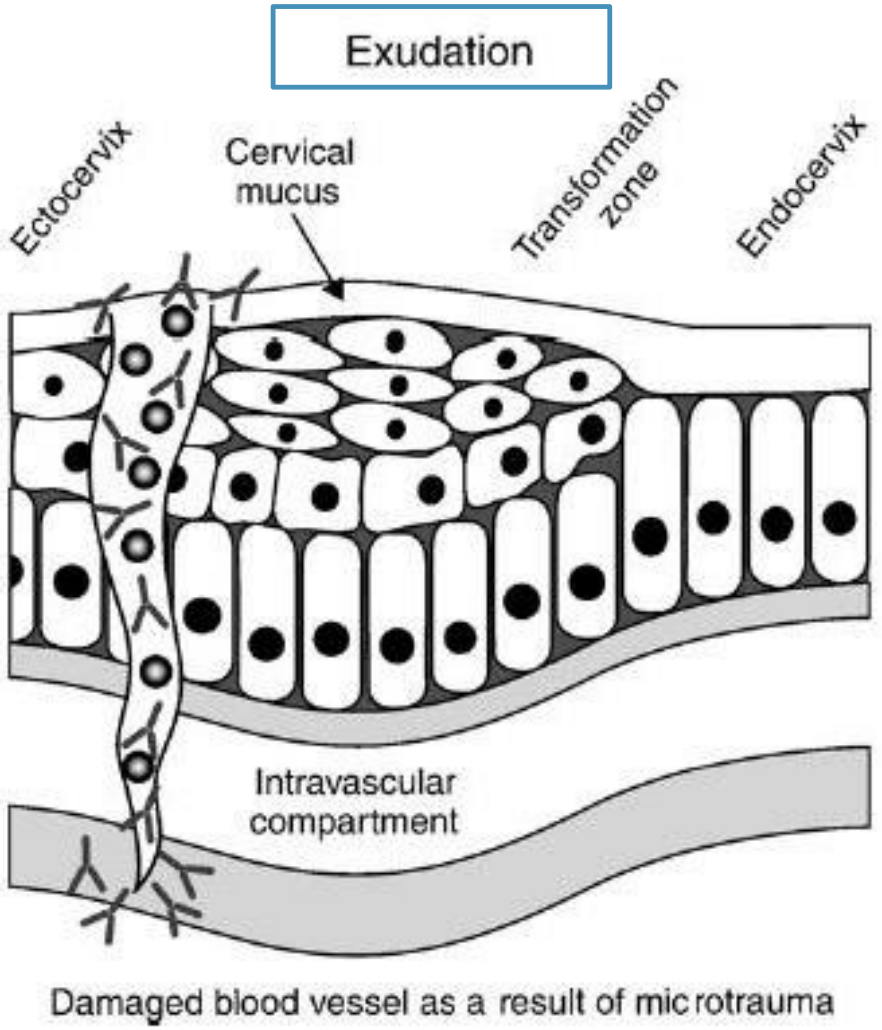


Figure derived from Schwarz et al. 2008

SEARCH RESULTS
EVALUATION OF VACCINE-INDUCED ANTIBODIES IN CVS

SEARCH RESULTS
2/ EVALUATION OF VACCINE-INDUCED HPV-SPECIFIC ANTIBODIES IN CVS

- 13 studies - 18 publications
- All papers reported paired CVS/serum data

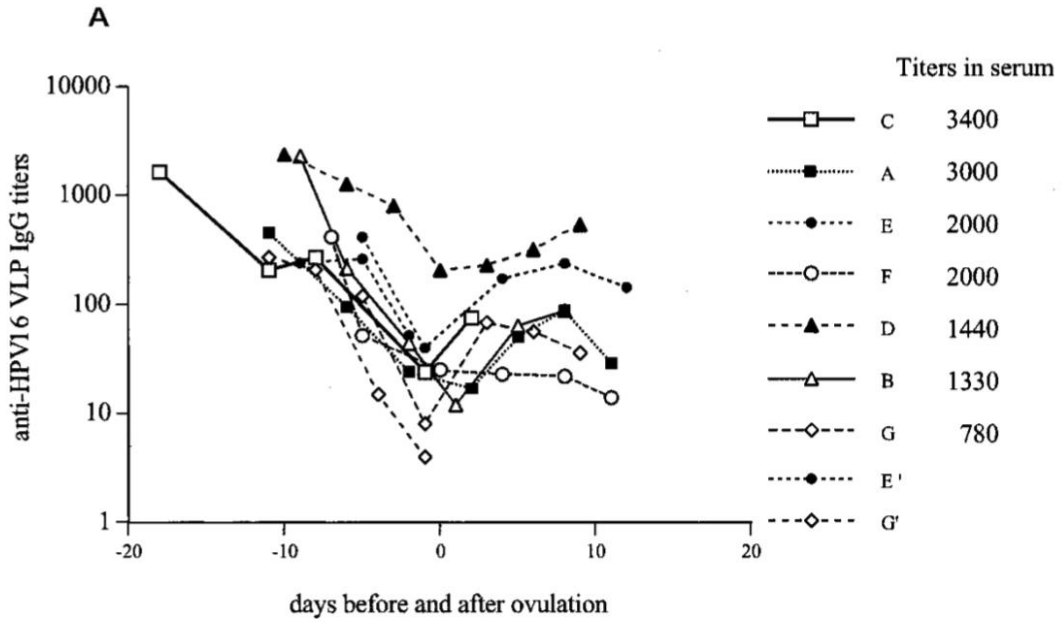
  - 3 studies => responses after **experimental vaccination** (HPV11/16 VLPs)
  - 1 study => responses to the **quadrivalent vaccine** (mucosal administration)
  - 7 studies => responses to the **bivalent vaccine**
  - 2 studies => **comparison** of bi- and quadrivalent vaccines
  - *Letter to the editor*

2/ VACCINE-INDUCED ANTIBODY ASSESSMENT IN CVS

2003

Specific Antibody Levels at the Cervix During the Menstrual Cycle of Women Vaccinated With Human Papillomavirus 16 Virus–Like Particles

Denise Nardelli-Haefliger, Daniel Wirthner, John T. Schiller, Douglas R. Lowy, Allan Hildesheim, Françoise Ponci, Pierre De Grandi



- Whether HPV immunization results in specific antibody levels in the female genital tract
- Whether these levels might vary during contraceptive or ovulatory cycles

- **Results were promising in terms of vaccine efficacy**
- **All participants developed a relatively high titer of HPV16 antibodies (IgG) in their cervical secretions after immunization**
- **CVS collection method: Weck-cell sponges**

2/ VACCINE-INDUCED ANTIBODY ASSESSMENT IN CVS

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2004

Vaccine. 2004 Jul 29;22(21-22):2943-52.

Dose-ranging studies of the safety and immunogenicity of human papillomavirus Type 11 and Type 16 virus-like particle candidate vaccines in young healthy women.

Fife KH[1], Wheeler CM, Koutsky LA, Barr E, Brown DR, Schiff MA, Kiviat NB, Jansen KU, Barber H, Smith JF, Tadesse A, Giacoletti K, Smith PR, Suhr G, Johnson DA.

Author information

1 Department of Medicine, Indiana University School of Medicine, Indianapolis, USA.

+ 2 studies (Nardelli-Haefliger (2005); Huo et al. (2012))

Investigated CVS antibody titers after **mucosal vaccination**, as an ***alternative to intramuscular immunization***

The majority of identified vaccine studies explored the **presence of HPV-specific vaccine-induced antibodies at the cervix after bivalent HPV16/18 vaccination**

Vaccine group	Sampling time (month)	Kemp et al. 2008	Schwarz et al. 2010	Petäjä et al. 2011	Scherpenisse et al. 2013	Gonçalves et al. 2016	Ferreira Costa et al. 2018	Schwarz et al. 2009-2015-2017
Bivalent vaccine	0	x			x	x		
	1					x		
	6					x		
	7		x			x	x	
	12	x	x		x			
	18		x				x	x
	24		x	x	x			x
	36		x	x				
	48			x				
	60							x
	72							x
	120							x

2/ VACCINE-INDUCED ANTIBODY ASSESSMENT IN CVS

- HPV16/18 antibodies were detected in CVS of (young) women

  => 5-45% of cases HPV16 Abs were not detected although the antibody titers in serum were high

-

				HPV-16		HPV-18	
Sampling time (month)	**Study***	**Age† (years)**	**No. with evaluable samples**	**Serum positivity rates % (95% CI)**	**CVS positivity rates % (95% CI)**	**Serum positivity rates % (95% CI)**	**CVS positivity rates % (95% CI)**
7	HPV-010	18–45	65	100 (94.5, 100)	95.4 (87.1, 98.8)	100 (94.5, 100)	92.3 (83.0, 97.3)
12	HPV-028	26–65	12	100 (73.5, 100)	100 (73.5, 100)	100 (73.5, 100)	100 (73.5, 100)
18	HPV-014/ HPV-028	15–65	152	100 (97.6, 100)	71.1 (63.2, 83.1)	100 (97.6, 100)	55.3 (47.0, 75.1)
24	HPV-014/ HPV-012	10–55	216	100 (98.3, 100)	77.8 (71.6, 86.2)	100 (98.3, 100)	63.4 (56.6, 78.2)
36	HPV-012	10–25	108	100 (96.0, 100)	87.0 (79.2, 93.4)	100 (96.6, 100)	73.1 (63.8, 85.3)

2/ DIFFERENCES IN CVS ANTIBODY LEVELS INDUCED BY DIFFERENT HPV VACCINES

- 4 papers were identified that reported on 2 head-to-head studies
- Proportion of women with CVS antibodies: bivalent vaccine > quadrivalent vaccine
- Further long-term studies are needed to understand the clinical importance

Vaccine group	Sampling time (month)	Draper et al. 2013	Einstein et al. 2009-2011-2014
Bivalent vaccine vs. Quadrivalent vaccine	0		x
	7	x	x
	12		x
	18		x
	24		x
	36		x
	48		x

2/ VACCINE-INDUCED HPV-SPECIFIC ANTIBODY CVS LEVELS SHOW CORRELATION WITH SERUM LEVELS

- In general studies showed a moderate - strong correlation between HPV16/18 levels in serum and CVS

  => vaccine-induced HPV antibodies transudate from systemic to cervical mucosa

- To avoid any possible bias introduced by the presence of blood in the samples, in most studies the samples containing blood were excluded from the statistical analysis

2/ DETECTABLE VACCINE-INDUCED ANTIBODY LEVELS IN CVS ARE LOWER THAN SERUM LEVELS

- Nardelli-Haefliger et al. (2003) => **0.5 – 50%** of systemic levels
- Kemp et al. (2008) => **2-3 logs lower** than systemic levels
- Scherpenisse et al. (2013) => **2%** of systemic levels

? sufficient to offer protection

(immune correlate of protection remains to be determined)

2/ CROSS-REACTIVE ANTIBODIES DETECTABLE IN CVS

Studies	Non—HPV types
Draper et al. 2013	HPV31/45
Scherpenisse et al. 2013	HPV31/33/45/52/58

- Majority of genital samples were positive and a strong relationship was demonstrated with serum

METHODS USED

3/ METHODS

- **Collection methods used:**
  - Difficulty in the reliable collection of genital female secretions
  - Absence of an absolute method of sampling (most lavage/wicks)

- **Immuno-assays**

Characteristics	Cervical wick	Cervicovaginal lavages	Cytobrush/swab
Minimal trauma	+/-	+	☹
Known dilution of secretions	+/-	☹	+/-
Minimal dilution of secretions	+	☹	+
Sufficient material collected	☹	+	☹
Ease of collection	+	+/-	+
Self-insertion/self-collection possible	+	☹	+

+, good; +/- moderate

OTHER OPTIONS

Softcup

✓ Higher yield of total human IgG

N.N Mkhize et al. (2016), S. Abraham et al. (2019)

First-void urine

✓ Young participants
✓ Increase participation
✓ Avoid microtrauma

Van Keer et al. (2019)

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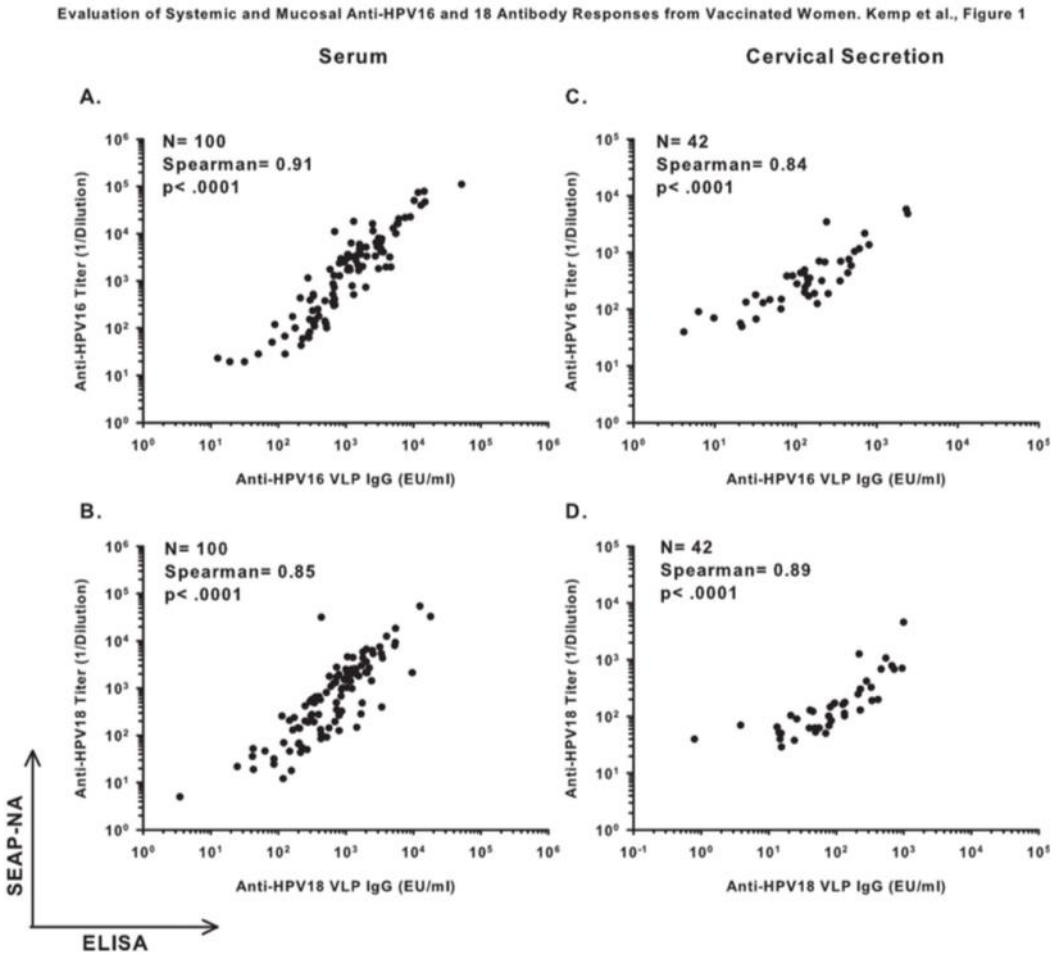
3/ METHODS

- Collection methods used
- Immuno-assays
  - Several assays have been used
  - In most studies; neutralizing antibody titers were not determined because a good correlation between ELISA titers and neutralizing titers *(Kemp et al. 2008)*.
  - Cut-off (HPV naïve person)
  - International initiative for standardizing and harmonizing assays for HPV antibody detection



INFLUENCING FACTORS

1/ Menstrual cycle dependent variation

2/ Blood contamination

3/ Influence of Age

- **NORMALIZATION**
  - Present levels in normalized form to correct:
    - for subtle changes due to hormonal/environmental influences
    - for differences in the techniques used to obtain the samples
    - e.g. diving by total IgG, amount of total protein, differences in weight/volume

CONCLUSION

- The extracted data were heterogeneous
  - meta-analysis was not feasible
- Vaccine-induced HPV antibody detection in CVS is feasible (although lower titers than in serum)
  - Wide variety of collection methods (washings/wicks)
  - Immuno-assays
- Need for specific methods to improve sensitivity and standardize the detection of HPV-specific antibodies in CVS

THANK YOU

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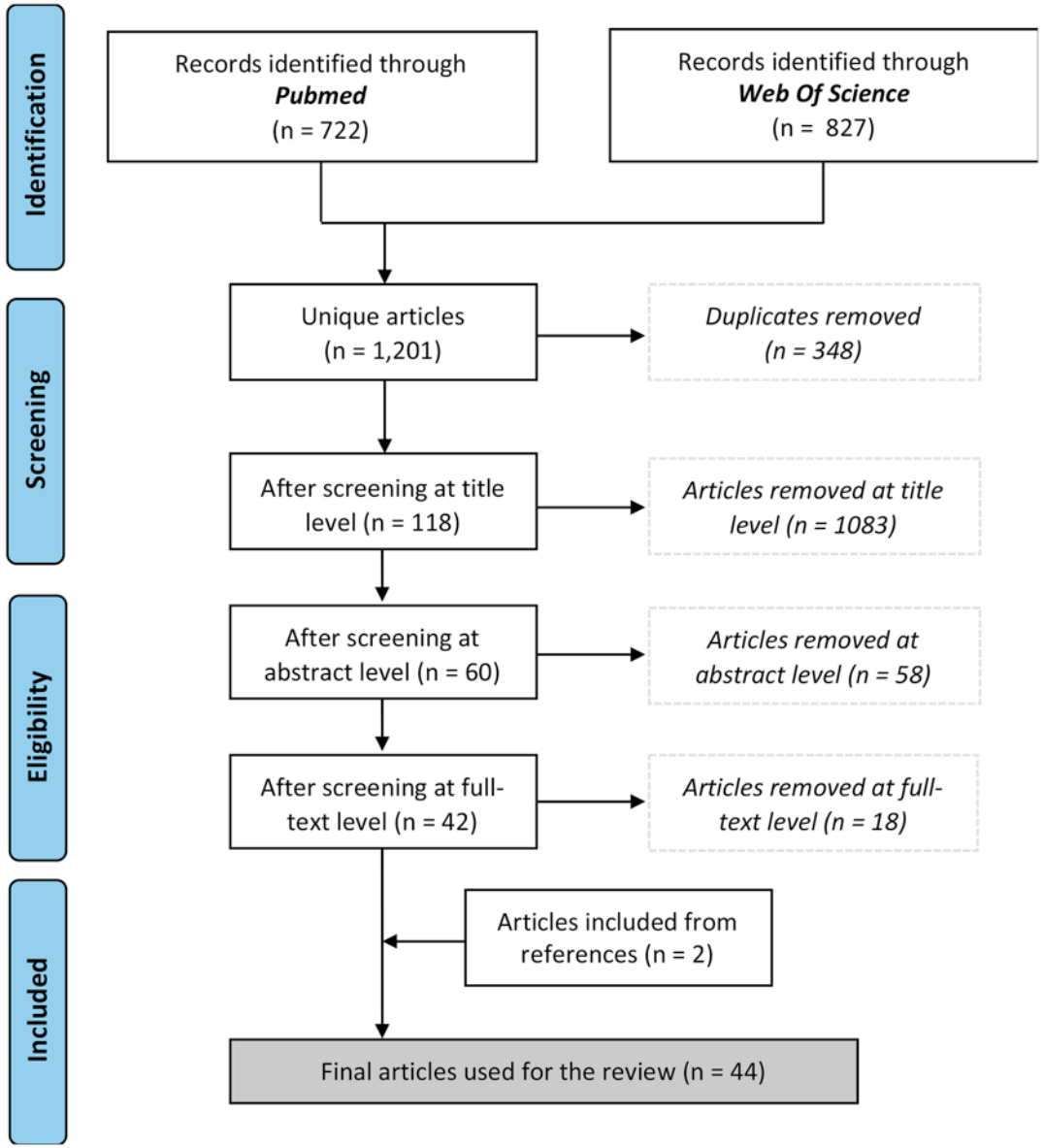
BACKUP SLIDES



Identification number: CRD42018104963

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

HPV-specific antibody levels in cervico-vaginal secretions (CVS) and its association with serum HPV-specific antibodies: a systematic review of the literature

Jade Pattyn, Severien Van Keer, Alex Vorsters

First author (year of publication)	Country of execution	Study Vaccine Vaccine schedule Vaccine administration	Number of participants	Mean age (total age range)	Total number of collected CVS samples (collection time (month (M)) from 1st vaccine dose) [e]	Methodology and antibodies analysed in CVS (class)	CVS collection method Storage	Main study outcome
I/ Experimental HPV immunization								
Nardelli-Haefliger et al. (2003)	Switzerland	HPV16 L1 VLP vaccine different immunization/dosing schedules no adjuvant i.m.	18; (n=7, taking oral contraceptives; n=11, ovulating)	Not reported 18 – 45 y	216 (M0-M2/M5, followed by twice weekly for 5 weeks)	VLP-based ELISA anti-HPV16 L1 (IgG)	Wick Samples were placed on ice, and PBS containing protease inhibitors were added. The liquid fraction (i.e., diluted cervical secretion) was frozen at –70 °C until analysis	The cervical titers among participants in the contraceptive group were relatively constant throughout the contraceptive cycle. In contrast, the cervical titers among participants in the ovulatory group varied during the menstrual cycle
Fife et al. (2004)	USA	HPV11 or 16 L1 VLP vaccine different immunization/dosing schedules i.m.	249	Median: 20 y 18 – 25 y	182 (M7)	VLP-based ELISA anti-HPV11/16 L1 (IgG/A)	CVL Not reported	Only about one half of the study participants who received the three highest experimental HPV11 vaccine doses had in their CVL detectable anti-HPV11 by month 7. The proportion was even lower in the HPV16 vaccine study
Nardelli-Haefliger et al. (2005)	Switzerland	HPV16 L1 VLP vaccine different immunization/dosing schedules no adjuvant nasal spray/bronchial aerosol or combination i.m./aerosol	32	Not reported 18 – 45 y	64 (M0-M2)	VLP-based ELISA anti-HPV16 L1 (sIgA, IgG/A)	Conducted as Nardelli-Haefliger et al. (2003)	Data suggest that aerosol administration of HPV VLPs may represent a potential alternative to parenteral injection. IgA was detected at the cervix in a subset of these vaccines

First author (year of publication)	Country of execution	Study Vaccine Vaccine schedule Vaccine administration	Number of participants	Mean age (total age range)	Total number of collected CVS samples (collection time (month (M)) from 1st vaccine dose) [e]	Methodology and antibodies analysed in CVS (class)	CVS collection method Storage	Main study outcome
2/ HPV-6/11/16/18 (Gardasil®) immunization								
Huo et al. (2012)	England	HPV-6/11/16/18 vaccine 0,1,4 month schedule Sublingual (SL) drops/i.m.	18	24.2 – 26.3 y 19 – 31 y	81 (M0-M1-M2-M4-M5)	1/ VLP-based ELISA anti-HPV6/16/18 L1 (IgG/A) 2/ Neutralizing assay nAb-HPV16	Wick Samples were snipped into the top chamber of a Spin-X tube containing 300 mL sterile filtered extraction buffer and centrifuged at 4°C for 15 minutes at 13,000g. A repeat extraction was performed by adding additional extraction buffer to the top chamber, and then 8 mL heat inactivated FBS added to pooled secretions from each sample site, prior to separation into 200 mL aliquots and freezing at –80 °C until analysis	SL antigens induced 38-fold lower serum and 2-fold lower cervical/vaginal IgG than i.m. delivery, and induced or boosted serum virus neutralizing antibody in only 3/12 subjects. Neither route reproducibly induced HPV-specific mucosal IgA. The observation that SL immunization could boost pre-existing serum neutralizing activity points to the possible use of i.m. prime/SL boost schedules
3/ HPV-16/18 (Cervarix®) immunization								
Kemp et al. (2008b) [53]	Costa Rica	HPV-16/18 vaccine 0,1,6 month schedule i.m.	50	Not reported 18 – 25 y	47 (M0 (n=5) -M12)	1/ Neutralizing assay nAb-HPV16/18 2/ VLP-based ELISA anti-HPV16/18 L1 (IgG)	Wick Samples were placed into a 10 ml tube for storage in liquid nitrogen. After shipment the samples were stored at −70°C. Sponge were extracted in a buffer containing PBS, NaCl and Aprotinin. 300 µl of cervical extraction buffer was slowly added to the top of the sponge. 4 µl was added FBS. The extracts were aliquoted and frozen at −70°C until further testing	Strong correlations between SEAP-NA and ELISA were observed. Systemic and cervical antibody measures also correlated well except at mid-cycle. Correlations between antibody levels at one and twelve months following the start of vaccination were poor

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3/ HPV-16/18 (Cervarix®) immunization								
Schwarz et al. (2010)	Germany, The Netherlands, Finland, USA, Poland, Denmark	HPV-16/18 vaccine 0,1,6 month schedule i.m.	350	Not reported 10 – 65 y	553 (M7-M12-M18-M24-M36)	VLP-based ELISA anti-HPV16/18 L1 (IgG)	Wick Conducted as Kemp et al. (2008b) [53]. Samples were stored at -20°C or -70°C until antibody extraction	Good correlation was seen between HPV16/18 antibody levels at all time-points. The strong correlation between levels of HPV16/18 antibodies in serum and CVS up to 36 months post-vaccination supports transudation of serum antibodies as the mechanism by which antibodies are introduced into CVS
Petäjä et al. (2011)	Denmark, Estonia, Finland	HPV-16/18 vaccine 0,1,6 month schedule i.m.	321	24.2 y (Group 15 – 25y) 10 – 25 y	69 (only from the age 15-25 group; M24-M36-M48)	VLP-based ELISA anti-HPV16/18 L1 (IgG)	Wick Conducted as Schwarz et al. (2009) [61]	Anti-HPV16/18 antibodies in CVS were detectable for subjects aged 15–25 years (84% and 70%, respectively). There was a strong correlation between serum and CVS anti-HPV16/18 antibodies levels
Scherpenisse et al. (2013)	The Netherlands	HPV-16/18 vaccine 0,1,6 month schedule i.m.	1,151	15.1 y 14 – 16 y	649 (M0-M12-M24)	VLP-based multiplex immunoassay Anti-HPV16/18/31/33/45/52/58 L1 (IgG/A)	Wick Samples were collected from the tampons by addition of PBS containing complete protease inhibitor cocktail and subsequent centrifugation for 30 min, 3,200 g at 4°C. CVS samples were stored at -80°C until analysis	Post-vaccination, HPV16/18 IgG and IgA are detectable in CVS. The correlation of HPV16/18 IgG antibody levels between serum and CVS suggests that vaccine-induced HPV antibodies transudate and/or exudate from the systemic circulation to the cervical mucosa to provide protection against HPV infections

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3/ HPV-16/18 (Cervarix®) immunization								
Gonçalves et al. (2016) [b]	Brazil	HPV-16/18 vaccine 0,1,6 month schedule i.m.	60	27.2 y 19 – 43 y	60 (M0-M1-M6-M7)	VLP-based ELISA anti-HPV 16/18 L1 (IgG/A)	CVL Samples were obtained by PBS (No storage temperature reported)	After the third vaccination, there is a strong agreement between cervical and systemic IgG antibody responses and a weak agreement between cervical and systemic IgA antibody responses. The induction of IgA antibodies seems to be secondary to that of IgG antibodies in response to HPV i.m. vaccination
Ferreira Costa et al. (2018) [b]	Brazil	HPV-16/18 vaccine 0,1,6 month schedule i.m.	35	Same population as Gonçalves et al. (2016); age not specified	70 (M7-M18)	VLP-based ELISA anti-HPV/VLP (IgA/G); HPV type not specified	CVL Not reported	Cervical samples were positive for both IgG and IgA antibodies at 7 months and decreased after 1 year to 33% and 29%. The median absorbance in serum and the cervix for IgG and IgA anti-HPV-VLP antibodies was significantly higher at month 7 after vaccination when compared to 1 year post-vaccination
Schwarz et al. (2009) [c]	Germany, Poland	HPV-16/18 vaccine 0,1,6 month schedule i.m.	531	35 y 15 – 55 y	149 (M18-M24)	VLP-based ELISA anti-HPV16/18 L1 (IgG)	Wick Samples were collected per woman and stored at −20°C until antibody extraction. The extraction protocol was conducted as Castle et al. (2004)	There was a high correlation between HPV16 and HPV18 antibody levels (IgG) in CVS and sera, regardless of age
Schwarz et al. (2015) [c]			488	Not reported 15 – 55 y	190 (M60-M72)			A strong correlation between anti-HPV16/18 levels in serum and CVS samples 6 years after vaccination indicates a long-lasting transudation of serum antibodies across the cervical epithelium
Schwarz et al. (2017) [c]			470	Not reported 15 – 55 y	107 (M120)			Correlation coefficients for antibody titers in serum and CVS were 0.64 (anti-HPV16) and 0.38 (anti-HPV18)

First author (year of publication)	Country of execution	Study Vaccine Vaccine schedule Vaccine administration	Number of participants	Mean age (total age range)	Total number of collected CVS samples (collection time (month (M)) from 1st vaccine dose) [e]	Methodology and antibodies analysed in CVS (class)	CVS collection method Storage	Main study outcome
4/ HPV-16/18 (Cervarix®) vs HPV-6/11/16/18 (Gardasil®) immunization								
Draper et al. (2013)	UK	HPV-16/18 vaccine HPV-6/11/16/18 vaccine Both 0,1,6 month schedule i.m.	198	Not reported 12 – 15 y	50 (M7)	VLP-based ELISA anti- HPV16/18/31/ 45 L1 (IgG)	Cytobrush Samples were rehydrated with PBS and subjected to centrifugation within a Amicon tube for 5 min at 2,500 g. Two such extractions were performed and the eluted material pooled and subjected to centrifugation at 13,000 g to remove cellular debris. The clarified supernatant was aliquoted and stored at -80°C	Levels of neutralizing and binding antibodies in genital secretions were closely associated with those found in the serum, with Cervarix® having a median 2.5 fold higher level of HPV-specific IgG ratio in serum and genital samples than Gardasil®
Einstein et al. (2009) [d]	USA	HPV-16/18 vaccine 0,1,6 month schedule i.m. HPV-6/11/16/18 vaccine 0,2,6 month schedule i.m.	920	30.2-30.7 y 18 – 45 y	165 (M0-M7)	1/ Neutralizing assay nAb-HPV16/18 2/ VLP-based ELISA anti-HPV16/18 L1 (IgG)	Wick Conducted as Schwarz (2009)	Positivity rates for anti-HPV16/18 nAb in CVS and circulating HPV16/18-specific memory B-cell frequencies were higher after vaccination with Cervarix® compared with Gardasil®
Einstein et al. (2011) [d]			799	31 y 18 – 45 y	222 (M12-M18-M24)	VLP-based ELISA anti-HPV16/18 L1 (IgG)		Positivity rates and levels of antigen-specific IgG antibodies in CVS were not significantly different between vaccines
Einstein et al. (2014) [d]			524	31 y 18 – 45 y	170 (M36-M48)			Limited CVS samples were available. Positivity rates of anti-HPV16/18 IgG antibodies in CVS appeared higher in the Cervarix® group compared with the Gardasil® group, while CVS antibody levels were of similar level. Antibody levels in serum and CVS are poorly correlated, especially for HPV18