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Serum antibodies to human papillomavirus (HPV) pseudovirions correlate with natural infection for 13 genital HPV types

Helena Faust[a,1], Mateja M. Jelen[b,1], Mario Poljak[b], Irena Klavs[c], Veronika Učakar[c], Joakim Dillner[a,d,e,*]

[a] Department of Medical Microbiology, Malmö University Hospital, Lund University, Jan Waldenströms gata 59, 20502 Malmö, Sweden
[b] Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia
[c] National Institute of Public Health, Trubarjeva 2, 1000 Ljubljana, Slovenia
[d] Department of Laboratory Medicine, Karolinska Institute and Karolinska Hospital, Stockholm, Sweden
[e] Department of Medical Epidemiology and Biostatistics, Karolinska Institute Nobels väg 12, 171 77 Stockholm, Sweden

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ABSTRACT

Background: Serology for human papillomaviruses (HPV) types -16 and -18 is established as an important tool for studies of HPV vaccinology and epidemiology. However, as there are a large number of oncogenic genital types of HPV there is a need for development of high-throughput, validated HPV serological assays that can be used for more comprehensive seroepidemiological studies and for research on multivalent HPV vaccines.
Objectives: To develop a multiplexed pseudovirion-based serological assay (PsV-Luminex) encompassing 21 HPV types and validate the method by correlating the serology with the presence of type specific HPV DNA in cervical samples.
Study design: Cervical swabs from 3,291 unvaccinated women attending organized cervical screening in Slovenia were tested with 3 different HPV DNA detection methods and presence of HPV DNA compared to presence of serum antibodies to pseudovirions from 15 genital HPV types (HPV-6,-11,-16,-18,-31,-33,-35,-39,-45,-52,-56,-58,-59,-68,-73).
Results: On average 51% of the HPV DNA positive women were seropositive for the same HPV type that was detected in the cervical specimen. We found a strong correlation with presence of HPV DNA and antibodies to the same HPV type for 13/15 genital HPV types (median OR = 5.7, CI 95% = 2.4–12.9). HPV-52 serology failed the validation and HPV-11 serology could not be validated because only a single woman was positive for HPV-11 DNA. The correlation between serology and HPV DNA status tended to be stronger among women infected with single HPV type (median OR = 10.5, CI 95% = 2.4–48.4) than among women with multiple HPV infections (median OR = 4.6, CI 95% = 1.8–11.7).
Conclusions: A multiplexed HPV PsV-Luminex assay has been developed and validated to correlate with natural HPV infection for 13 HPV types, thus enabling more comprehensive studies in HPV epidemiology and vaccine research.

1. Background

Persistent human papillomavirus (HPV) infection is established as a necessary cause for almost all cases of cervical cancer as well as for a proportion of vulvar, vaginal, penile, anal and oropharyngeal cancers.[1,2] Two highly effective vaccines are available against the two most oncogenic HPV types, HPV-16 and -18.[3,4] However, there is still intensive research on HPV prophylactic vaccines as the exact mechanism of vaccine protection is not known[5] and as second-generation, multivalent HPV vaccines are under development.[6]

Most genital HPV infections are transient and clear within 6–12 months.[7] Therefore, HPV DNA testing cannot measure cumulative HPV exposure. Although not all HPV infections lead to seroconversion,[8,9] the antibody responses to the HPV capsid are known to be stable over time (also after clearance of HPV DNA) providing a useful measure of cumulative HPV exposure.[10]

Most HPV serology studies performed to date have been restricted to HPV-16 and -18,[11] although some studies have also reported on seropositivity to other HPV types.[12,13] Multiplexed serology is a high-throughput method that could make it possible to study the seroepidemiology of multiple HPV types more routinely. So far, most multiplexed HPV serology studies have used GST-L1 fusion proteins as antigen.[11] The type-specific antibody

* Corresponding author at: Karolinska Institute, Department of Medical Epidemiology & Bioststistics, Nobels väg 12, 171 77 Stockholm, Sweden. Tel.: +46 768871126.
E-mail address: Joakim.dillner@ki.se (J. Dillner).
[1] Shared first authorship.

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Table 1
Seropositivity (%) for HPV types and polyomaviruses in two control groups (children ≤ 12 years and women with ≤1 lifetime sexual partner) and adult women participating in cervical screening.

Source of expression vectors	VIRUS		N=133 Children (≤ 12 years)	N= 71 Women (≤1 lifetime sexual partner)	N= 3291 Women attending cervical screening	Cut-off (MFI)
J. Schiller	HPV 6	Genital HPV types	2.3	9.9	19.2	591
J. Schiller	HPV 11		0.8	0.0	5.8	400
J. Schiller	HPV 16		2.3	14.1	25.2	400
J. Schiller	HPV 18		0.0	2.8	9.5	400
J. Schiller	HPV 31		0.0	1.4	17.3	400
J. Dillner	HPV 33		3.0	2.8	11.2	400
S. Beddows	HPV 35		2.3	12.7	12.5	400
S. Beddows	HPV 39		3.8	7.0	17.6	635
J. Schiller	HPV 45		0.8	1.4	5.7	400
J. Schiller	HPV 52		3.0	0.0	9.3	1400
R. Roden	HPV 56		0.8	4.2	10.4	657
J. Schiller	HPV 58		1.5	5.6	19.7	400
S. Beddows	HPV 59		0.8	7.0	12.5	400
J. Dillner	HPV 68		3.0	8.5	12.2	400
R. Roden	HPV 73		2.3	11.3	12.5	400
J. Dillner	HPV 3	Non genital HPV types	0.0	2.8	5.0	400
Schiller	HPV 5		0.8	9.9	20.1	400
J. Dillner	HPV 15		0.8	11.3	13.1	400
J. Dillner	HPV 32		2.3	11.3	6.5	589
J. Dillner	HPV 38		1.5	1.4	9.3	400
J. Dillner	HPV 76		3.8	9.9	14.7	1066
K. Sasnauskas	JCV	Polyoma viruses	12.0	49.3	65.1	400
C. Buck	MCV		31.6	49.3	68.2	400
	HPV any type		15.0	63.4	76.6	
	HPV single type		7.5	35.2	21.0	
	HPV any genital		11.3	45.1	65.8	
	HPV single genital		4.5	29.6	21.5	
	HPV any non genital		6.8	35.2	40.2	
	HPV single non gen.		6.0	26.8	23.1	
	HPV≥10 types		0.0	1.4	3.8	

response to HPV is predominantly against conformational epitopes that are exposed on native virions, but not on disrupted virions. We have previously developed and validated a high-throughput, multiplexed HPV serology method based on mammalian cell-produced HPV pseudovirions (PsVs) for 10 HPV types (PsV-Luminex).[14] In the present study, we have expanded this method to include pseudovirions from 21 HPV types and 2 polyomaviruses (merkel cell polyomavirus (MCV) and JC polyomavirus (JCV)). Validation of HPV serology for the less common HPV types is a challenge, as very large cohorts with both serum sampling and reliable HPV DNA testing are required in order to obtain sufficiently large validation panels. We used a population of 3291 women who had already been tested for presence of HPV DNA status for 15 genital HPV types[15,16] to evaluate the performance of our extended multiplexed serology assay.

2. Objectives

To develop an expanded multiplexed HPV serological assay based on HPV pseudovirions and to evaluate the performance of the assay by comparing serology with HPV DNA status.

3. Study design

3.1. Study populations

In the present study, 3321 women 20–64 years of age (average age 36 years) who participated in the national cervical cancer screening program (DP ZORA) in Slovenia were consecutively enrolled into the Slovenian HPV prevalence survey (SHPVPS).[15,16] From each consenting participant, both a cervical smear and a blood sample were collected. Collection of cervical smears is described in detail elsewhere.[16] Whole blood samples (5 mL) were stored at +4 °C for less than a week before centrifugation for 5 min at 3000 rpm. The serum was aliquoted and stored at −30 °C until further analysis. Thirty women who had been vaccinated against HPV were excluded.

Two negative control serum panels were included: serum samples from 133 Swedish children not older than 12 years of age (average age 6 years)[17] and 71 serum samples from adult (average age 43 years) Swedish women who reported up to 1 life-time sexual partner.[14,18]

3.2. HPV DNA testing

HPV DNA testing and genotyping is described in detail elsewhere.[15] Briefly, cervical samples were tested in parallel with hybrid capture 2 HPV DNA test (hc2) (high-risk probe cocktail B) (Qiagen, Hilden, Germany) and realtime high risk HPV test (RealTime; Abbott, Wiesbaden, Germany). All samples with concordant positive RealTime/hc2 results and all samples with discordant RealTime/hc2 results were further analyzed with the linear array HPV genotyping test (Linear Array; Roche Molecular Diagnostics, Branchburg, NJ), with an HPV-52 type-specific real-time PCR assay (INNO-LiPA HPV Genotyping *Extra* Test (Innogenetics, Ghent, Belgium)) and, if necessary an in-house GP5+/GP6+ PCR assay. In addition, 1000 randomly selected samples were also tested for the presence of 37 HPV genotypes with Linear Array HPV genotyping test (Učakar et al., manuscript in preparation). Since the hc2 and RealTime assays do not include detection of HPV types -6, -11 and -73, the comparison of DNA prevalences and seroprevalences of