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Short communication

Sourcing of the WHO human papillomavirus type 18 international standards for HPV antibody levels

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ABSTRACT

Background: HPV serology is important for studies of vaccine immunogenicity, but can not be performed in a comparable manner without international standardisation.
Objectives: To find suitable candidate sera from naturally infected persons for use as International Standards (IS) for antibodies to high-risk HPVs, with priority for HPV-18.
Study design: 946 healthy Thai women (median age 44, range 18–83) and 61 cervical cancer patients were screened using an HPV pseudovirion-Luminex assay to detect antibodies to genital (HPV-6,-11,-16,-18,-31,-33,-45,-52,-58,-68) and non-genital HPV types (HPV-5,-15,-32,-38 and -76). Suitable candidate sera should ideally be mono-specific (have reactivity against only one genital HPV) and have high antibody levels that are stable over time.
Results: Seroprevalences of HPV-16,-31,-52 and -58 were at least twice as high among cancer patients compared to healthy individuals. Thirteen healthy women who met the IS inclusion criteria in initial testing also consented to blood-bag donations. Donations from 2 women with high HPV-18 Ab titers were pooled to the HPV-18 candidate IS, later established as the WHO official IS for HPV antibodies. Sera that could potentially be used as candidate IS for other oncogenic HPVs have also been identified.
Conclusions: In the Thai population, seroepidemiology implicated HPV types HPV-16,-31,-52 and -58 as particularly associated with cervical cancer. A well characterized cohort study has allowed sourcing of materials for an IS for HPV-18 antibodies and could conceivably be used for IS for other HPV types as well.

1. Background

Persistent human papillomavirus (HPV) infection is established as a necessary cause for cervical, anogenital and oropharyngeal cancers [1,2]. Most genital HPV infections are transient and clear within 6–12 months [3]. Therefore, HPV DNA testing cannot measure cumulative HPV exposure. The antibody responses to the HPV capsid are known to be stable over time, providing a useful measure of cumulative HPV exposure [4].

Most HPV serology studies performed to date have been restricted to HPV-16 and -18 [5]. For Thailand, only HPV-16 and -18 seroprevalences have been described and not in relation to the cervical cancer [6]. According to GLOBOCAN, the rate of the cervical cancer in Thailand is very high with 23.2–32.4 cases per 100.000 [1]. Therefore, vaccination against HPV is urgent in this region and monitoring the HPV prevalence in population before and after vaccination is helpful for vaccination effectiveness evaluation.

We have previously developed and validated a high-throughput, multiplexed HPV serology method based on mammalian cell-produced HPV pseudovirions (PsVs) for 10HPV types (PsV-Luminex) [7]. We have continuously expanded this method with more HPV types for use in HPV seroepidemiology and vaccinology and it demonstrates a good correlation with natural infection [8].

A WHO collaborative study conducted in 2005 demonstrated that the availability of International Standards (IS) for antibodies to HPV serotypes would facilitate the standardization of HPV serology [9]. In the absence of such standards, individual laboratories had applied their own reference standards which were not harmonized with other laboratories and reproducibility between laboratories was therefore uncertain. The WHO's Expert Committee on Biological Standardization (ECBS) establishes international standards (IS) for biological substances used in the prevention, treatment or diagnosis of human disease. WHO ISs are recognized as the high-

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Table 1
Seroprevalences of studied HPV among 1007 Thai women.

HPV		Healthy women N = 946		Cervical cancer[a] patients N = 61		OR	95% CI	p value
		N	%	N	%			
HPV 6	Genital	82	8.7	8	13.1	1.59	0.67–3.62	0.238
HPV 11	HPV	43	4.5	6	9.8	2.29	0.84–5.92	0.063
HPV 16		53	5.6	19	31.1	**7.62**	3.97–14.58	<0.0001
HPV 18		41	4.3	4	6.6	1.55	0.45–4.73	0.415
HPV 31		35	3.7	8	13.1	**3.1**	1.27–7.28	0.004
HPV 33		54	5.7	6	9.8	1.8	0.67–4.6	0.187
HPV 45		15	1.6	1	1.6	1.03	0.13–7.97	0.974
HPV 52		53	5.6	16	26.2	**5.99**	3.02–11.77	<0.0001
HPV 58		59	6.2	10	16.4	**2.95**	1.33–6.38	0.002
HPV 68		47	5.0	1	1.6	0.32	0.02–2.2	0.237
HPV 5	Non	53	5.6	5	8.2	1.5	0.51–4.12	0.399
HPV 15	Genital	51	5.4	8	13.1	**2.65**[b]	1.1–6.16	0.013
HPV 32	HPV	37	3.9	4	6.6	1.72	0.5–5.29	0.31
HPV 38		59	6.2	9	14.8	**2.6**[c]	1.13–5.8	0.01
HPV 76		70	7.4	5	8.2	1.12	0.38–3.02	0.818
HPV any type		384	40.6	45	73.8	**4.12**	2.22–7.7	<0.0001
HPV single type		207	21.9	31	39.3	**3.69**	2.18–6.24	<0.0001
HPV any genital		285	30.1	40	65.6	**4.42**	2.48–7.91	<0.0001
HPV single genital		171	18.1	24	39.3	**2.94**	1.66–5.2	<0.0001
HPV any non genital		192	20.3	16	26.2	1.4	0.74–2.61	0.267
HPV single non genital		139	14.7	7	11.5	0.75	0.31–1.76	0.489
HPV 16 or 18		84	8.9	21	34.4	**5.39**	2.92–9.91	<0.0001
HPV 6 or 11		100	10.6	8	13.1	1.28	0.54–2.89	0.534
HR-HPV[d]		235	24.8	38	62.3	**5.00**	2.83–8.87	<0.0001

[a] Invasive cervical cancer or carcinoma *in situ*.
[b] 7 cervical cancer patients out of 8 were HPV15 and HR-HPV seropositive at same time.
[c] 7 cervical cancer patients out of 9 were HPV38 and HR-HPV seropositive at same time.
[d] HR-HPV: HPV 16, 18, 31, 33, 45, 52, 58, 68.

est order of reference for biological substances, and are arbitrarily assigned a potency in International Units (IU). Their primary purpose is to calibrate secondary reference standards used in routine laboratory assays in terms of the IU, thereby providing a uniform resultsí reporting system and traceability of measurements, independent of the method used. The International Standards are available through the National Institute for Biological Standards and Control in United Kingdom (www.nibsc.org).

An IS for antibodies to HPV-16 was established in 2009 by the WHO ECBS [10]. The IS for HPV-18 was established in 2012 and is described in this paper. We sought to source a candidate IS that contained high antibody levels against one HPV types, but not against related HPV types ("mono-specific"). We preferred an IS containing pooled serum from at least two individuals, to reduce the probability that an IS could contain a peculiar antibody response found only in a single individual.

We describe the seroprevalences of fifteen HPV types in relation to cervical cancer (invasive cancer or carcinoma *in situ*) in Thailand and describe the sourcing of the now established IS for HPV-18 and putative IS for other medically important genital HPVs.

2. Objectives

To find suitable candidate sera from naturally infected persons suitable as International Standard (IS) for antibodies to high-risk HPVs, with priority for HPV-18. To describe HPV seroprevalence among Thai women, in relation to cervical cancer.

3. Study design

The project was approved by the ethical and research committees of the National Cancer Institute (NCI), Thailand (EC 122/2009, decision taken 18.12.2009). The epidemiological study on HPV was conducted by the NCI of Thailand. Women attending the cervical cancer screening program at NCI were recruited to the study. The cytology samples of screened women were analysed with Pap-smear tests and classified as cytologically normal or as having cervical cancer (without separating cancer *in situ* or invasive squamous cell carcinoma). Participants who agreed to be part of the study gave informed consent to the use of their sera in the study and in the production and establishment of the candidate standard. The serum samples were collected during 2009 and kept at −80 °C until testing. One-thousand and seven serum samples arrived to WHO HPV Laboratory Network (HPV LabNet) Global Reference Laboratory (GRL) in Sweden for HPV antibody testing. The serum samples of 946 healthy Thai women (median age 44, range 18–83) and 61 cervical cancer patients were tested using a HPV pseudovirion-Luminex assay to detect antibodies to HPV types -5,-6,-11,-15,-16,-18,-31,-32,-33,-38,-45,-52,-58,-68 and -76 [11]. Pseudovirions were produced in-house by transfection of 293TT cells, as described [12] Activated Bio-rad COOH beads were kept at +4 °C and used within 2 weeks. To define seropositivity, a cut-off of 250 Mean Florescence intensity (MFI) units was applied to all HPV types [7]. Donated blood-bags were analysed with a somewhat improved methodology containing more HPV types (HPV types 3,-35,-39,-56,-59 and 73 were added). The improved protocol freezes the activated beads, which results in a higher sensitivity [8]. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using the Statcalc module of Epi-info version 3.5.1 (available at www.cdc.gov).

4. Results

Overall HPV seropositivity for any one of the 10 tested genital HPV types was 30% among the 946 healthy Thai women and 66% among the cervical cancer patients (Table 1). HPV-16,-31,-52 and -58 had at least twice as high seroprevalences in cervical cancer patients compared to the healthy individuals. Antibodies to HPV-16 and HPV-52 demonstrated very strong association with cervical cancer (p values < 0.0001 for both). Antibodies to HPV-31 and HPV-