Immunogenicity of HPV prophylactic vaccines: Serology assays and their use in HPV vaccine evaluation and development. Importance of international units for reporting immune response.

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HPV Serology and Vaccines

- HPV serology used as endpoint for:
  - ”Bridging studies” showing that vaccines are equally immunogenic for children (girls and boys) as for adolescents.
  - Validating different vaccine batches by immunogenicity
  - Studies on mode of administration, dosing, phase IV studies et c
  - Earliest endpoint for evaluating new vaccines
  - Studies on whether antibody levels wane (Requires other immunization strategy? Booster?)
  - Protective antibody levels
- Internationally standardised, relevant and proficient HPV immunogenicity testing important for progress in HPV vaccinology.

Basic principles

- An internationally accepted definition of an HPV antibody level must exist.
  → International Standard (IS) defining an International Unit (IU) is known to be required.
- Reproducible methods for analysing readouts should be used.
  → Parallell line method (PLL) known to increase reproducibility

- If no IS exists or non-reliable methods for analysing readouts are used, improvements in assays et c will not help.

International Standards

- Collaborative study by NIBSC established International standard sera with assigned IU for HPV 16 and HPV 18
- Ongoing project to establish International standard sera with assigned Units for HPV 6, 11, 31, 33, 45, 52 and 58
- The standard serum should be monospecific, antibodies to one HPV type.
- Assays based on antibody binding to target antigen and Neutralization assays are used for the validation.

Reference:
- Morag Ferguson, Dianna E. Wilkinson, Alan Heath, and Paul Matejtschuk, 'The First International Standard for Antibodies to HPV 16', Vaccine, 29 (2011), 6520-26.
- Fast H et al, Sourcing of the WHO Human papillomavirus type 18 international standards for HPV antibody levels, J Clin Virology 2016

Range of titres in HPV 16 immunoassays (Self-reported data from the labs)

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		Range of titres
NIB-01	HPV 16 natural infection	100-640
NIB-03	Pool HPV 6+11+16+18 #	<100-640
NIB-04	Vaccine	100-2560
NIB-05	Vaccine	384-2560
NIB-07	Vaccine	400-2560
NIB-08	HPV 16 natural infection #	<20-160
NIB-09	Natural HPV 6+11+16+18	<20-80
NIB-10	Vaccine	1600-10240
NIB-12	Negative	-
NIB-13	HPV 18 natural infection #	-
NIB-14	Vaccine	5381-40960
NIB-16	HPV 6+11 natural infection #	-

HPV 16 assays – potency of vaccinee sera: Raw data submitted to NIBSC- Amounts estimated using PLL

	Sample				
Lab	04	05	07	10	14
1	4.93	2.05	4.14	16.44	24.68
2	6.70	4.03	3.88	18.10	53.85
3	4.04	3.91	5.06	16.28	74.21
4	5.13	3.32	4.79	16.54	40.07
5	4.45	2.35	5.12	12.76	26.14
6	5.21	3.11	4.15	10.20	55.02
7	6.71	3.67	6.19	29.99	91.51
8 *	9.95	1.79	6.65	17.40	25.03
9	8.24	4.63	7.24	24.23	59.30
10 *	3.26	2.20	2.92	3.03	3.13
Mean	5.91	3.06	5.13	17.2	45.10
% GCV	35	39	25	37	63
Neutralisation data	*16*	*4*	*54*	*102*	*870*

NIB-01 - assigned unitage = 1.0 units

NIBSC

Take-home message from the first international collaborative study:

- In spite of **no attempt at harmonization of the assays themselves** (a wide variety of assays in use: Direct ELISA, Indirect ELISA, RIA, cLIA, neutralization assays) there was **approximately the same result,** on 2 conditions:
- 1. The same standard serum was used to define antibody unitage.
- 2. The same analysis method (Parallell line method) for calculating unitage from raw data was used.

- These conclusions already well known from serology in general – but formally shown to be true for HPV as well.

- Open Shareware for calculating PLL (Excel script):

Grabowska, K., Wang, X., Jacobsson, A., Dillner, J. Evaluation of cost-precision rations of different strategies for ELISA measurement of serum antibody levels. *J. Immunol. Meth*. 271. 1-15. 2002.

Serology

- Key tool for studying cumulative exposure to infection and immunity and for vaccine research.
- Fitting of titration curves to International Standards and reporting in International Units is required for reproducibility and comparability.
- Models built on standardised serology give similar result to models built on PCR testing.

Head-to-head comparison of Bi- and Nonavalent HPV vaccine-induced antibody response

- Finnish women 25 year old at time of vaccination
- 3 doses of the vaccine according to protocol, sampling 6 months after third dose
- Gardasil 9; HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58
- Cervarix; HPV 16 and 18
- 184 women received Gardasil 9
- 188 women received Cervarix
- Randomised sera from Gardasil 9 and Cervarix in the binding assay including 17 different HPV types

Reference: JID, Arroyo et al, 10 May 2022

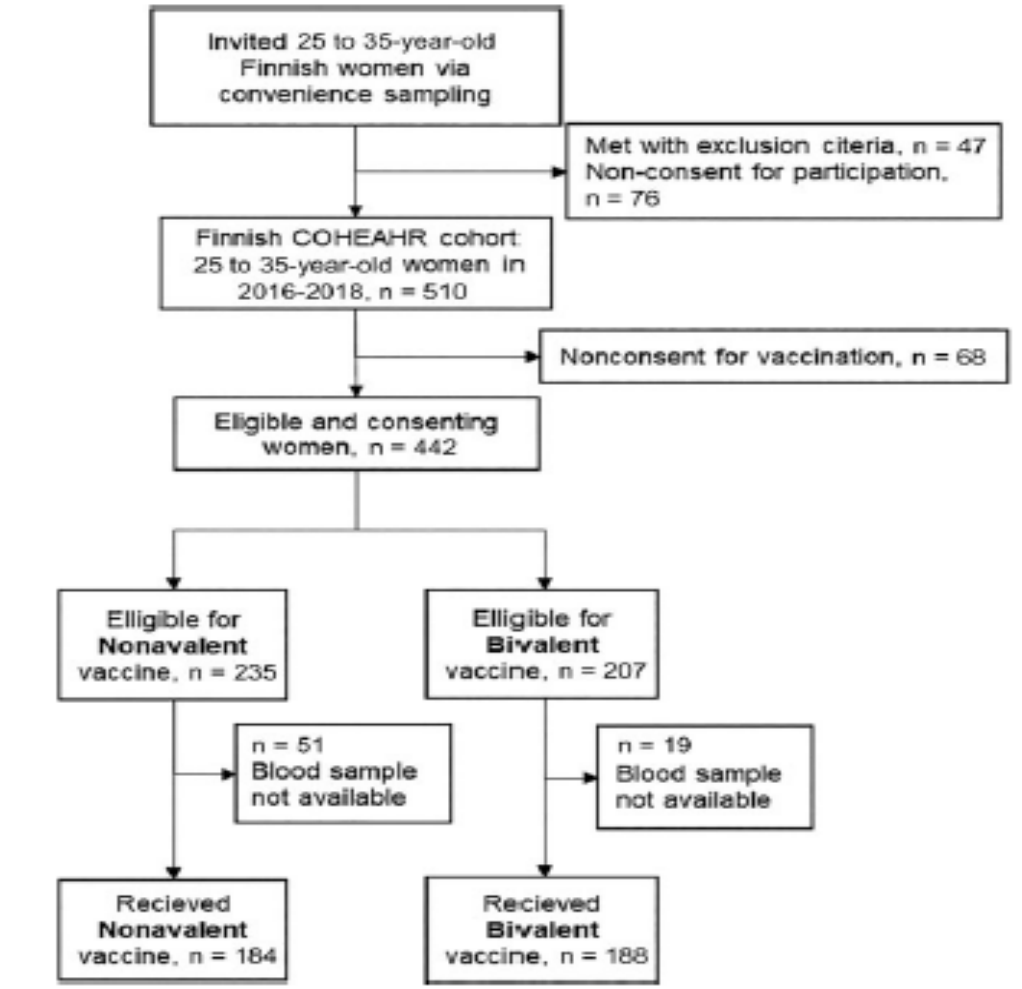

Figure 1. Flowchart of the study enrollment and participation.

	Bivalent HPV Vaccine (n = 188)				Nonavalent HPV Vaccine (n = 184)				Lowest Detectable Antibody Level Bivalent/ Nonavalent	Unit	*P* Value, Difference in Antibody Median Level	*P* Value, Difference in Proportion Seropositive
	Median	Minimum	Maximum	No. (%) positive	Median	Minimum	Maximum	No. (%) positive				
HPV6	0	0	815.5	85 (45.21)	32.28	0	11064.14	181 (98.37)	0.12/1.29	IHU	<.0001	<.0001
HPV11	0	0	216.21	26 (13.83)	14.18	0	376.58	182 (98.91)	0.09/0.36	IHU	<.0001	<.0001
HPV16	1140.11	6.86	109027.4	188 (100)	265.08	2.69	15163.39	184 (100)	6.86/2.69	IU	<.0001	NA
HPV18	170.54	0	29999.78	187 (99.47)	22.28	0	9418.97	183 (99.46)	1.06/0.31	IU	<.0001	1
HPV31	1.85	0	1791.49	178 (94.68)	21.66	0.11	1937.33	184 (100)	0.08/0.11	IHU	<.0001	.0044
HPV33	0	0	501.15	80 (42.55)	7.97	0	629.61	181 (98.37)	0.04/0.07	IHU	<.0001	<.0001
HPV35	0	0	2493.39	66 (35.11)	31.17	0	5288.8	107 (58.15)	7.38/10.76	IHU	<.0001	<.0001
HPV39	0	0	1550.5	7 (3.72)	0	0	540	7 (3.80)	252.00/275.00	MFI	.98	1
HPV45	2.24	0	6929.66	152 (80.85)	45.8	0	1426.97	182 (98.91)	0.24/0.28	IHU	<.0001	<.0001
HPV51	0	0	1521	14 (7.45)	0	0	1099	17 (9.24)	281.00/279.00	MFI	.748	.6616
HPV52	0	0	52.15	30 (15.96)	31.75	0	6422.03	175 (95.11)	0.78/1.31	IHU	<.0001	<.0001
HPV56	0	0	340.5	3 (1.60)	0	0	328	1 (0.54)	278.5/328.00	MFI	.861	.6304
HPV58	0	0	34787.6	90 (47.87)	17.01	0	463.49	182 (98.91)	0.02/0.09	IHU	<.0001	<.0001
HPV59	0	0	684	7 (3.72)	0	0	324	2 (1.09)	261.50/300.50	MFI	.658	.1878
HPV66	0	0	272	1 (0.53)	0	0	0	0 (0.00)	272.00/NA	MFI	.929	1
HPV68	0	0	672288	35 (18.62)	0	0	7514.07	41 (22.28)	37.36/49.14	IHU	.497	.4544
HPV73	0	0	2359.5	34 (18.09)	0	0	1749	39 (21.20)	269.00/253.00	MFI	.597	.5322

Anti-HPV antibody levels are given in IU for the HPV types where an international standard has been established by the World Health Organization (for HPV16 and 18) or in IHU for the other types. For some HPV types, the seroreactivity was so low that antibody levels in units could not be calculated and the results for these types are therefore presented as the crude MFI obtained when testing sera in a 1:50 dilution. Differences in antibody levels across the vaccines were assessed with nonparametric Wilcoxon rank-sum test and relative proportions using 2 proportion Z test and its associated *P* value.

Abbreviation: HPV, human papillomavirus; IHU, in-house units; IU, international units; MFI, mean fluorescence intensity; NA, not applicable.

Head to head summary

- Bivalent HPV vaccine (Cervarix) higher antibody titres to HPV 16 and HPV 18 compared to the nona-valent vaccine
- The bivalent HPV vaccine commonly induced antibodies against the nonvaccine HPV types 31/33/35/45 and 58
- The nonavalent vaccine induced hither antibodies against HPV 6/11/31/33/45/52/58 and 35

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