

RESEARCH ARTICLE

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# Immune Response to Human Papillomavirus One Year after Prophylactic Vaccination with AS04-Adjuvanted HPV-16/18 Vaccine: HPV-Specific IgG and IgA Antibodies in the Circulation and the Cervix

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## Abstract

**Objective:** This study was designed to describe the course of IgG/IgA responses in cervical secretions and in serum one year after the first dose of intramuscular administration of the HPV16/18 AS04-adjuvant vaccine. **Methods:** Blood and cervical mucus samples were collected for immunologic assays, 7 months after the first doses and 1 year following the last boost vaccination (month 7) by enzyme linked immunosorbent assay (ELISA). The detection of IgG and IgA anti-HPV/VLP was developed for this purpose. **Result:** A total of 100% of serum samples were IgG antibody positive at a titer of 1:100 at both time periods and decreased according to the serum dilution. For serum IgA antibody, 95% were positive one month after vaccination and 79% were positive 1 year later. Similar results were observed with the cervical samples positive for both IgG and IgA antibodies at one month and decreasing 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 one month after vaccination when compared to 1 year post-vaccination ( $P < 0.0001$ ). **Conclusion:** Immune responses were significant one year after immunization, however it decreased in cervical and serum samples when compared to levels observed one month after the last dose. This suggests that a vaccine booster may be necessary to increase antibody titers.

**Keywords:** Immunoglobulin G- immunoglobulin A- HPV- vaccine

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## Introduction

Persistent infection with oncogenic human papillomavirus (HPV) types is a necessary cause of cervical cancer, a common cancer that accounts for approximately 12% of all female cancers. Of the 40 genital tract associated human papillomaviruses, approximately 15 have been classified as being 'high risk' for cervical oncogenesis. HPV types 16 and 18 are the most common oncogenic HPV types, responsible for about 70% of all cervical cancers (Schwarz et al., 2015; Huang et al., 2017).

Since 2007, HPV vaccination has been widely available in developed countries as well as in some developing countries. Two licensed vaccines have been used, the bivalent HPV16/18 (Cervarix®, GSK) and quadrivalent HPV6/11/16/18 (Gardasil®, Merck and Company)

vaccines. Both have contributed to a reduction in HPV prevalence. Studies have demonstrated continued decreases in the frequency of vaccine-targeted HPV types for up to 4 years after establishment of the vaccination program (Crowe et al., 2014; Gonçalves et al., 2014a; Meshier et al., 2016).

Markowitz et al., (2013) has shown that despite low vaccine coverage, HPV 16/18 prevalence was reduced by 56% among girls who had received the Gardasil® vaccine in regular and catch-up programs. Evidence is also emerging on the effectiveness of HPV vaccination in decreasing the frequency of low and high-grade precancerous cervical lesions (Rana et al., 2013; Pollock et al., 2014; Drolet et al., 2015). HPV vaccines are based on VLPs that allows the immune system to generate antibody titers that are 100 fold greater than occur upon natural infection (Kaufmann

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and Nitschmann, 2010; Lehtinen et al., 2012).

In October 2016, after Food and Drug Administration (FDA) approval a new dosing schedule for HPV vaccination, the Advisory Committee on Immunization Practices (ACIP) recommended a new 2-dose schedule for girls and boys who initiate the vaccination series at ages 9 through 14 years. Three doses remain recommended for those who begin the vaccination series at ages 15 through 26 years and for immunocompromised persons (Meites et al., 2016).

Earlier studies have shown that Cervarix® and Gardasil® vaccine induce persistently high levels of neutralizing antibodies against HPV 16, but antibody titers against HPV 18 decrease more rapidly. The antibody titers declined in the first months after completion of the vaccination schedule, and then reached a plateau (Dobson et al., 2013; Lazcano-Ponce et al., 2014; Dempsey et al., 2015). However, there is only limited data that indicates how quickly titers will fall back to natural infection antibody titer levels after induction of the amnesic response, indicating the potential need for booster immunizations

Despite there being a relatively extended period since the beginning of the clinical use of HPV vaccines no evidence-based data is available on the possible need for a booster vaccination. Thus, this study was designed to describe the course of IgG/IgA responses in cervical secretions and in serum one year after the first dose of intramuscular administration of the HPV16/18 AS04-adjuvant vaccine.

## Materials and Methods

### Study population

In this study, we enrolled 35 healthy women who were received the three doses of HPV-16/18 AS04-adjuvanted vaccine (CERVARIX; GlaxoSmithKline Vaccines). Blood and cervical mucus sample were collected for immunologic assays, 7 month after the first doses and 1 year following the last boost vaccination (month 7). All participants provided written informed consent. The project protocol was reviewed and approved by the Ethical Committee (1034/2011 CEP-UNICAMP).

### IgA and IgG anti-HPV-VLP detection by ELISA

Initially details of the antigen preparation have been

described previously (Gonçalves et al., 2014b). Firstly, a plate of 96 wells was sensitized with 50 µL of antigen (HPV-16/18 vaccine) diluted in carbonate-bicarbonate buffer (Sigma-Aldrich) at a concentration of 10 µg/mL and incubated at 4°C for overnight. The plate was then washed three times with PBS-Tween 0.05% and blocked with 100µL of PBS with 10% of fetal bovine serum (FBS-Gibco) (PBS-FBS). Next step it was incubated for 2h at room temperature and washed three times with PBS-Tween 0.05%.

Cervical mucus and serum samples were diluted 1:100, 1:1,000, 1:10,000, 1:100,000, 1:1,000,000 and 1:10,000, 1:1,000, 1:10,000, 1:100,000, respectively in PBS-FBS, and 50µL of this dilution was added to each well. Following this, the samples were incubated for 2h at 37°C, and the plate was washed three times with PBS-Tween 0.05%. Next, secondary antibody (peroxidase-labeled anti-human IgG or IgA; Sigma-Aldrich) was diluted 1:10,000 in FBS 10%, and 50 µL was added to the wells and incubated for 1h at 37°C. After this, the plate was washed five times with PBS-Tween 0.05% and then 50 µL of substrate TMB (3,3',5,5'-Tetramethylbenzidine Liquid Substrate System; Sigma-Aldrich) was added to the wells before incubating for 30 min at room temperature. The reaction was stopped with 50 µL of 1N sulfuric acid, and the absorbance (optical density) of each well was read using an ELISA reader at 450 nm with a reference filter of 630 nm. The cutoff values for each antibody/sample were as follows: IgG/serum, 0.616; IgG/mucus, 0.611; IgA/serum, 0.173; and IgA/mucus, 0.294.

### Statistical Analysis

Data were entered into the GraphPad Prism software v6.0 (GraphPad Software Inc.), and submitted to one-way analysis of variance followed by Bonferroni's Multiple Comparison Test. All values were considered significantly at  $P < 0.05$ .

## Results

The ELISA assay for HPV-VLP (HPV-VLPs ELISA) was carried out using 35 samples of women who received HPV 16/18 vaccine. The samples were collected 7 month after the first dose and one year after the vaccination.

It was observed that approximately 100% of the IgG serum samples reacted when the antigen was present on

Table 1. Positivity for IgG and IgA Anti-HPV-VLP Detected by ELISA in Serum Sample after 7 Month and 1 Year Vaccination

Dilution	1/100	1/1.000	1/10.000	1/100.000	1/1.000.000
Sample					
IgG serum (7 months)	100%	97%	9%	0%	0%
IgG serum (1 year)	100%	23%	0%	0%	0%
IgA serum (7 months)	95%	12,5%	0%	0%	0%
IgA serum (1 year)	79%	4%	0%	0%	0%

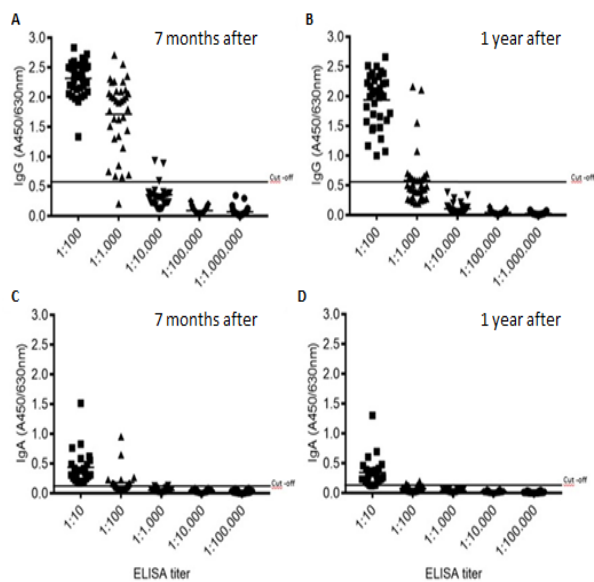


Figure 1. IgG/IgA Dilutions Regarding HPV-16/18 VLP in Serum. A, The Median of Absorbance Detected in Serum Sample for IgG anti-HPV-VLP at 1 Month after Vaccination ( $P < 0.0001$ ). B, The median of absorbance detected in serum sample for IgG anti-HPV-VLP at 1 year after vaccination ( $P < 0.0001$ ). C, The median of absorbance detected in serum sample for IgA anti-HPV-VLP at 1 month after vaccination ( $P < 0.0001$ ). D, The median of absorbance detected in serum sample for IgA anti-HPV-VLP at 1 year after vaccination ( $P < 0.0001$ ).

the first dilution of the both collections. The positivity, however, decreases, according to the dilutions. Regarding IgA reactivity in serum, initial conversion was observed in 95% on 7 month of vaccination and 79% after one year (Table 1).

Similar results can be show at the mucus samples with higher positivity on 7 month and decreasing after one year, lower levels of IgG and IgA antibody were detected in the cervical mucus (33%) and 29%, respectively, after one year of vaccination (Table 2).

The median absorbance detected in serum samples for IgG and IgA anti-HPV-VLP antibodies was significantly higher at 7 months after vaccination, on the dilution 1:100, 1:1,000, 1:10,000 and 1:10, 1:100, respectively, when compared with one year after vaccination 1:100,

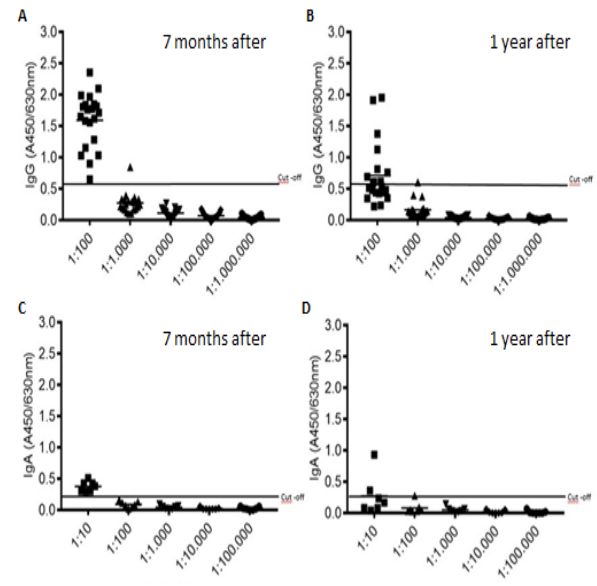


Figure 2. IgG/IgA Dilutions Regarding HPV-16/18 VLP in Cervix. A, The Median of Absorbance Detected in Cervical Mucus for IgG Anti-VLP at 1 Month after Vaccination ( $P < 0.0001$ ). B, The median of absorbance detected in cervical mucus for IgG anti-VLP at 1 year after vaccination ( $P < 0.0001$ ). C, The median of absorbance detected in cervical mucus for IgA anti-VLP at 1 month after vaccination ( $P < 0.0001$ ). D, The median of absorbance detected in cervical mucus for IgA anti-VLP at 1 year after vaccination ( $P < 0.01$ ).

1:1,000 and 1:10, 1:100, for IgG and IgA respectively ( $P < 0.0001$ ) (Figure. 1A, B, C and D).

The median absorbance detected in cervical mucus samples was significantly higher at 7 months after vaccination, on the dilution 1:100, 1:1,000 and 1:10 for anti-HPV-VLP IgG and IgA, respectively, when compared with one year after, like 1:100 and 1:10, for IgG and IgA respectively ( $P < 0.0001$ ; Figure 2A, B, C and D).

In serum and cervical mucus samples, the median absorbance was significantly higher 7 months after vaccination and it is possible to see that decrease at one year after, according to dilutions (Figure 1 and 2).

## Discussion

Vaccination against oncogenic human papillomavirus

Table 2. Positivity for IgG and IgA Anti-HPV-VLP Detected by ELISA in Cervical Mucus Sample after 7 Month and 1 Year Vaccination

Dilution	1/10	1/100	1/1.000	1/10.000	1/100.000
Sample					
IgG mucus (7 months)	100%	5%	0%	0%	0%
IgG mucus (1 year)	33%	0%	0%	0%	0%
IgA mucus (7 months)	100%	0%	0%	0%	0%
IgA mucus (1 year)	29%	0%	0%	0%	0%

types is an intervention for cervical cancer prevention. Despite there being a relatively long period of time since the beginning of clinical use of HPV vaccines there is no information on the duration of vaccine efficacy and in addition, no evidence-base data is available on the need of a boost vaccination.

In this study, we investigated one month and one year post-vaccination antibody responses against HPV 16/18 by detection of IgG and IgA HPV-specific antibodies in cervical and serum samples. The reactivity of the IgG in serum samples was similar (100%) at one month and at one year after immunization at the lowest dilution tested (1:100). However, the percent positive women decreased according to the dilutions. Regarding IgA reactivity in serum, the initial conversion was observed in 95% of women one month after completion of vaccination and in 79% one year later. Similar results were seen in the cervical samples with a higher number of IgG and IgA antibody-positive women at month 1 and markedly decreasing after one year.

In a previous study with the same population, we described the course of IgA/IgG responses in serum and cervical samples after intramuscular administration of the HPV16/18 AS04-adjuvant vaccine, using the same methodology. We observed that the higher titers of IgG/IgA in both serum and the cervix were detected in month six and decreasing slightly in month 7 (Gonçalves et al., 2016).

Likewise, previous studies (Naz, 2012; Khatun et al., 2012; Gonçalves et al., 2016) also detected IgA/IgG in the cervix after administration of the HPV-16/18 AS04-adjuvanted vaccine. The levels of antibodies detected in the cervix were lower when compared to the serum. The latter could be explained if most or all of the antibodies detected in the cervix were the result of transudation or exudation from the circulation and not due to local antibody production in the cervix (Naz, 2012).

In this study, the median optical density detected in serum and cervical samples for IgG and IgA anti-HPV-VLP antibodies were significantly higher at one month, when compared to one year after vaccination.

Some trials have already suggested that two doses of the HPV vaccine are sufficient for HPV immunization. However, in this study even with the 3-dose regimen, after one year of vaccination IgG and IgA antibody levels decreased significantly in both cervical and serum samples. Usually, antibody titers decline in the first months after completion of the vaccination schedule, and then reached a plateau (Dobson et al., 2013; Dempsey et al., 2015).

It remains a challenge to determine the minimal neutralizing titer associated with protection. Until now, antibody titers do not define a surrogate level of protection against cancer or its precursors. However, protection against infection is linked to antibody persistence, which is directly related to the peak production of anti-HPV after vaccination. Conversely, it is recognized that antibody titers are highly dependent on the number and timing of vaccine doses (Olsson et al., 2009).

HPV immunization is the process or the act of making individuals immune, which is usually accomplished during childhood, adolescence and young adulthood.

Assuming that vaccine efficacy will wane over time, it will be interesting in the future to determine whether immune response patterns seen after a single or all three vaccine doses are the most predictive of long-term vaccine success or failure. If immunity wanes, then a booster may be required. The sufficiency of only two doses instead of 3 (to increase cost-effectiveness), or the need for an additional booster (4th immunization) to achieve lifetime immunity remain open questions.

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