

## Prevalence of Human Papillomavirus 16 and 18 Neutralizing Antibodies in Prenatal Women in British Columbia<sup>∇†</sup>

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**Human papillomavirus (HPV) type 16 and 18 neutralizing antibody (NAb) titers were measured in 1,020 prenatal women in British Columbia aged 15 to 39. HPV 16 and 18 NAbS were detected in 183/1,020 (17.9%) and 97/1,020 (9.5%), respectively, and 39 (3.8%) had NAbS to both types. Titers were similar across age strata.**

Measurement of type-specific antibody responses to human papillomavirus (HPV) is important for seroprevalence estimates and assessment of vaccine efficacy. Vaccine manufacturers have developed enzyme immunoassays (EIAs) targeting neutralizing epitopes (3, 10), but neutralizing antibody (NAb) tests could be a suitable alternative because they confirm blocking of infection of susceptible cells and they potentially measure antibodies to more epitopes than existing manufacturers' assays (13). Pseudovirus (PsV)-based NAb assays utilizing in vitro-generated HPV capsids containing a reporter plasmid have been described (1, 11). NAbS inhibit PsVs from infecting cells and from expressing the reporter plasmid product. For this study, we developed and validated a PsV NAb assay for HPV 16 and 18 and determined the seroprevalence among prenatal women in British Columbia (BC).

HPV 16 and 18 PsVs were prepared as previously described (1), except that the reporter plasmid encoded red fluorescent protein (RFP) (11). Electron microscopic examination of the PsV preparations showed typical papillomavirus morphology. Bands at 55 kDa (capsid protein L1) and 70 kDa (capsid protein L2) were observed on Western blot analysis with rabbit antisera. Cesium chloride density gradient ultracentrifugation showed that over half of the PsV fraction had a buoyant density of approximately 1.34 g/ml, consistent with capsids containing DNA. PsVs were titrated in 293TT cells by monitoring the cultures for red fluorescent cells, with each fluorescent cell representing one infectious unit.

NAb tests were performed as follows: sera were heated at 56°C for 30 min, and duplicate serial dilutions were prepared. Each serum dilution was mixed with 100 infectious units of the respective PsV and incubated for 1 h at 37°C, followed by transfer to 293TT cells on microtiter plates. Plates were incubated at 37°C and read after 4 to 6 days. The endpoint (100% neutralizing titer [NT<sub>100</sub>]) was the highest dilution of serum

which completely blocked cells displaying red fluorescence. Back-titrations of the PsV and serially diluted positive and negative serum controls were included in each run.

For initial NAb test validation, five anti-HPV positive control sera (two against HPV 16, one against HPV 18, one against HPV 6, 11, 16, and 18, and one against HPV 6 and 11) and one anti-HPV negative control obtained from the National Institute for Biological Standards and Control (NIBSC), United Kingdom, were titrated. NAb titers corresponded with known antibody status (Table 1), although some were near the assay cutoff (1:40). Control sera for routine use were obtained from a volunteer 1 month after receiving a full course of Gardasil vaccine and from an HPV 16- and 18-seronegative volunteer.

The prevalence of NAbS to HPV 16 and 18 was determined in 1,020 age-stratified anonymous sera from BC women undergoing prenatal testing. A sample size of 300 from each age stratum (15 to 19, 20 to 29, and 30 to 39 years) was estimated to provide a 95% confidence interval of 5% based on prevalence estimates of 7.7%, 19.4%, and 26%, respectively (8). Sera were selected between March 2007 and April 2008. Only age and city of residence were recorded for each subject. Sera were tested in duplicate for HPV 16 and 18 NAbS, and the geometric mean titer (GMT) was calculated. All sera demonstrating NAbS were retested to confirm the titer. Overall and age-specific prevalence rates of HPV 16 and 18 NAbS were determined. The chi-square test was used to compare HPV seropositivity rates by age group, one-way analysis of variance was used to test for mean GMT differences among age groups, and mean GMTs for those seropositive to one versus both HPV types were compared by using the *t* test. The study was approved by the University of British Columbia Clinical Research Ethics Board. Additional details regarding the methods for our study are available in the supplemental material.

Of the 1,020 prenatal women, 183 (17.9%) were seropositive for HPV 16 (GMT mean, 1:118; median, 1:80; range, 1:40 to 1:640) and 97 (9.5%) were seropositive for HPV 18 (GMT mean, 1:143; median, 1:80; range, 1:40 to 1:640). Thirty-nine (3.8%) women, included in the respective totals, demonstrated NAbS to both HPV 16 and 18. While the proportion with HPV 16 NAb was highest in the 20- to 24-years age group (21.1%) and for HPV 18 in the 35- to 39-years age group (10.9%) (Fig. 1),

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TABLE 1. HPV 16 and HPV 18 neutralizing antibody titers for NIBSC standard sera

Sample ID	NIBSC serological status	Anti-HPV NT <sub>100</sub> for type:	
		16	18
HPV 01-02	HPV 16 seropositive	1:160	<1:40
HPV 08-02	HPV 16 seropositive	1:80	<1:40
HPV 13-02	HPV 18 seropositive	<1:40	<1:40 <sup>a</sup>
HPV 09-02	HPV 6, 11, 16, and 18 seropositive	<1:40 <sup>a</sup>	1:80
HPV 16-02	HPV 6 and 11 seropositive	<1:40	<1:40
HPV 12-02	HPV seronegative	<1:40	<1:40

<sup>a</sup> Partial (90%) neutralization was observed at this dilution for this sample.

the differences in proportions between the age groups were not statistically significant (HPV 16,  $P = 0.39$ ; HPV 18,  $P = 0.93$ ). Mean GMTs for HPV 16 ( $P = 0.74$ ) and 18 ( $P = 0.49$ ) were similar across all age strata (Fig. 2), with no statistically significant difference for those seropositive for one versus both HPV types (HPV 16,  $P = 0.65$ ; HPV 18,  $P = 0.94$ ). Retesting of seropositive samples confirmed no more than a twofold variation in titers between assay runs.

These data reflect the point prevalence rate of HPV NABs in a population of prenatal women in BC. For the age groups assessed, the prevalence of HPV 16 and 18 NABs was consistent with EIA-based results reported by others (6, 7, 9, 12, 16). Since it has been reported that 50% to 60% of naturally infected individuals do not have detectable antibodies (2), it is likely that the number of individuals exposed to HPV 16 and 18 infections within our prenatal population may be twice as high. Based on our age-stratified data, exposure to HPV 16 and 18 occurred at a young age and NAb titers were maintained across all age groups. This could reflect persistent HPV infection, reinfections, or long-term persistence of NABs (2, 14, 15, 17).

Limitations of this study included the following: (i) only prenatal women were tested, and results may not be generalizable to all females in BC. (ii) These women were by definition sexually active, and this may overestimate the population prevalence in BC females. (iii) Sample selection was sequential and, thus, not entirely random. (iv) Subject HPV immunization status was unknown, but individuals were unlikely to have been immunized, as the HPV vaccine had been licensed in Canada for less than 24 months, a publicly funded HPV immunization program had not been initiated, and most subjects were older than the HPV vaccine target group. (v) All testing in this study

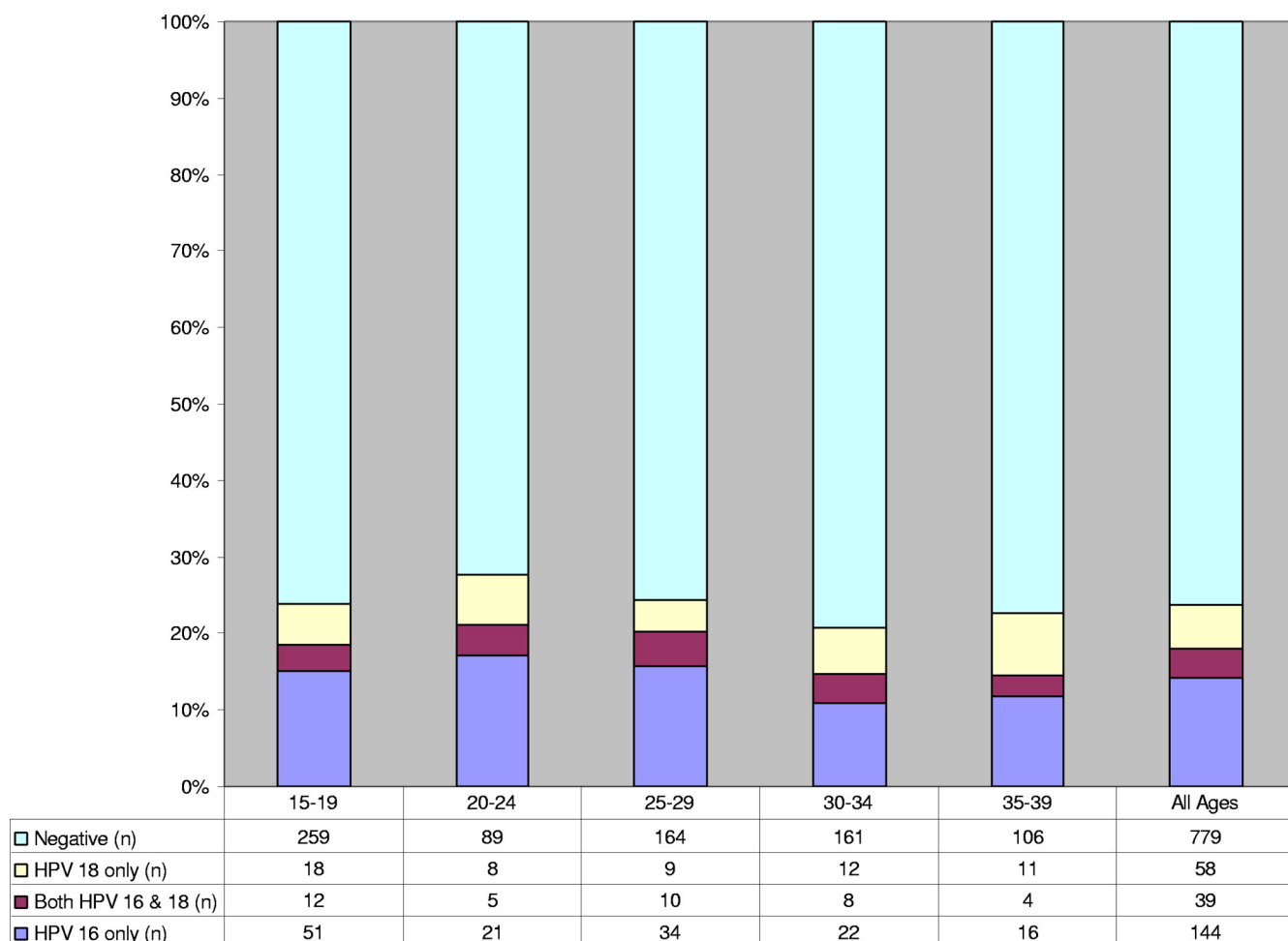


FIG. 1. Age distribution of HPV 16 and 18 neutralizing antibodies in prenatal women in BC ( $n = 1,020$ ).

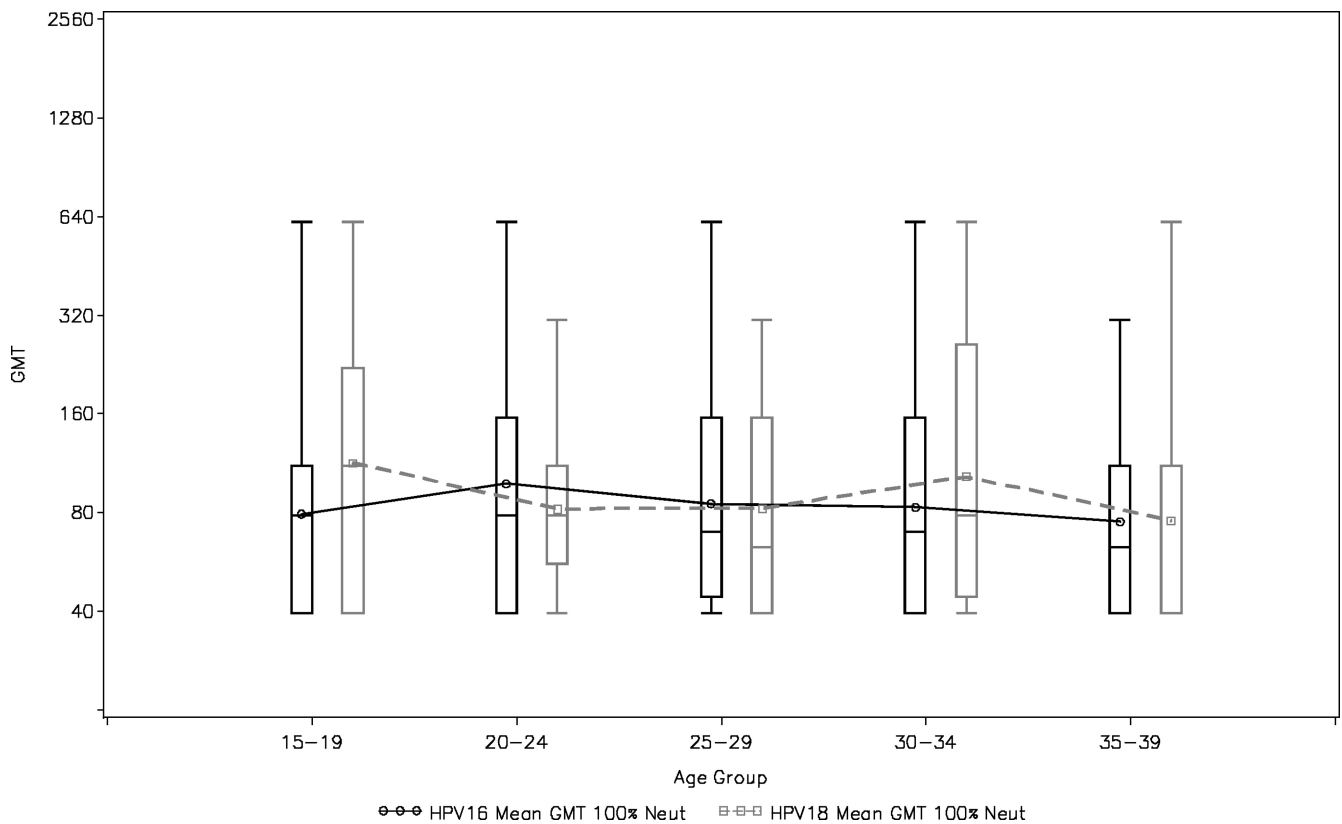


FIG. 2. Age-stratified HPV 16 and 18 neutralizing antibody GMTs in prenatal women in BC.

involved single lots of HPV 16 and 18 PsV. Reporter gene packaging efficiency may vary between lots and alter the NAb titers, but batch consistency can be monitored by using standardized sera (4, 5).

RFP was chosen as the reporter gene. Although secreted alkaline phosphatase-based assays are machine-read and run-to-run variation can be tracked objectively, they involve more handling steps. In contrast, RFP-PsV assays involve fewer steps and are visually read, which could potentially make them more subjective. However, duplicate testing of samples and retesting of seropositives on separate runs confirmed run-to-run reproducibility.

In conclusion, HPV 16 and 18 type-specific NAb from natural infection can be reliably measured by a PsV-based NAb assay. PsV NAb assays may provide a more reliable indicator of neutralizing activity than monoclonal antibody-based EIAs because they confirm the actual blocking of infection of susceptible cells. This NAb assay could provide an alternative to vaccine manufacturers' assays to understand prior population exposure to vaccine HPV types in order to optimize the use of the vaccine and to independently monitor vaccine antibody responses.

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