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Human Papillomavirus antibody reference reagents for post-vaccine surveillance serology

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Abstract

Suitably-controlled sero-surveillance surveys are essential for evaluating Human Papillomavirus (HPV) immunization programmes. A panel of plasma samples from 18 year old females was assembled, the majority being from bivalent vaccinees. Antibody specificities were evaluated by three independent laboratories and 3 pools were created that displayed either no antibodies to any HPV type tested, intermediate or high antibody levels to HPV16, HPV18, HPV31 and HPV45. These pools will be useful as control reagents for HPV serology.

Main text

The link between persistent infection with high-risk Human Papillomavirus (HPV) and the development of cervical cancer, the second most common cancer in women worldwide, is well established. The development of highly efficacious vaccines against the two most prevalent genotypes, HPV16 and HPV18 (14), represents one of the most significant advances in human vaccination for many years (13, 16, 20). Emerging data from efficacy trials also suggest some degree of cross-protection against non-vaccine types, including HPV31 and HPV45 (1, 16).

Serologic assays for the evaluation of HPV vaccine responses are currently limited to an ELISA (9), three multiplex assay systems (4, 6, 15), and a pseudovirus neutralization assay (2) and emerging data suggest that each system has some utility for characterizing HPV vaccine antibody specificity (3, 19). Protection against vaccine types is thought to be mediated by neutralizing antibodies (18) and while the mechanism of vaccine-induced cross-protection is uncertain, the measurement of antibodies against non-vaccine types (5, 12) may be useful as a potential correlate or surrogate of cross-protection (17). The only internationally available serologic standard is a WHO International Standard (IS) for HPV16 antibodies, derived from subjects with natural HPV16 infection (7) although a candidate IS for HPV18 antibodies, derived from subjects with HPV18 natural infection, is currently being characterized.

The aim of this study was to create serologic reference reagents for use as quality controls in post-immunization sero-surveillance surveys able to control for responses against vaccine (HPV16 and HPV18) and non-vaccine types (HPV31 and HPV45).

While IS are essential for assigning an international unitage of antibody levels, the daily quality control of serological tests needs to have access to secondary standards that are available in higher amounts than the IS itself. Such secondary standards should preferably be characterized by analysis of antibody level in parallel with the IS, to assign a traceable International Unitage (IU) to them (22). The reference reagents described in this paper have the high antibody levels that are typical of vaccinated subjects which makes them easier to use as reference standards for laboratories that perform serology mostly on vaccinated subjects and who have antibody levels substantially higher than found in the IS.

Twenty seven citrated plasma packs not required for transfusion were obtained from the NHS Blood and Transplant and tested negative for anti-HIV, anti-HCV and HBsAg. The plasma packs were selected from females aged 18 years old in September 2009 and for which a high proportion would have been vaccinated with the bivalent vaccine as part of the UK National HPV Immunization Programme 'Catch up' campaign (21). Serum is thought to be the ideal sample for HPV neutralization assays due to the potential for heparin to interfere with the assay (2); however, as these plasma samples were collected as citrated plasma packs, this is not expected to be an issue.

A plasma panel containing one aliquot of each coded sample was formally distributed to: (i) Laboratory A (Centre for Infections, Health Protection Agency, UK) for testing in a neutralization assay containing optiprep-purified pseudoviruses representing HPV16, HPV18, HPV31, HPV45 and the control Bovine Papillomavirus (BPV) made by transfection of 293TT cells with the appropriate bicistronic psheLL

L1L2 plasmid and the secreted alkaline phosphatase (SEAP) reporter vector (<http://home.ccr.cancer.gov/lco/plasmids.asp>) (2) with transduction of susceptible target cells resolved using the SEAP Reporter Gene Assay, Chemiluminescent (Roche) and Glomax Multi Detection System (Promega); (ii) Laboratory B (Global WHO HPV Reference Laboratory, Centers for Disease Control and Prevention, USA) for testing in the pseudovirus neutralization assay containing HPV16, HPV18 and the control BPV and detected using the SEAP Reporter Gene Assay (BD biosciences) and a Victor 2 Luminometer (Perkin Elmer); and (iii) Laboratory C (Global WHO HPV Reference Laboratory, Malmö University Hospital, Sweden) for testing in a multiplex serology assay with the following non-reporter containing HPV L1L2 pseudoviruses: α 1 (HPV32), α 2 (HPV3), α 7 (HPV18, 45, 68), α 9 (HPV16, 31, 33, 52, 58), α 10 (HPV6, 11), β 1 (HPV5), β 2 (HPV15, 38), and β 3 (HPV76) according to published methodology (6).

Eight plasmas (29.6%) demonstrated no neutralization against any of the four HPV types tested, eighteen (66.7%) neutralized both HPV16 and HPV18 (twelve of these also neutralized both HPV31 and HPV45), while one sample (3.7%) was positive for HPV16 alone suggesting an HPV16 natural infection (**Figure 1**). No neutralization was seen against the control BPV pseudovirus (all titers <40). Based on sample positivity alone, there was 100% concordance (inter-rater agreement, $\kappa=1.000$; Stata 10.1, StataCorp, Texas, USA) between the neutralization datasets from Laboratories A and B. In addition, there was also a very good agreement between the magnitude of neutralizing antibody titers obtained by both laboratories for HPV16 (96% concordance, $\kappa=0.945$) and HPV18 (85% concordance, $\kappa=0.797$) when stratified by discrete titer intervals (<40, 40-160, 160-640, 640-2560, 2560-10240 and >10240).

A comparison of neutralization (Laboratory A) and multiplex serological (Laboratory C) assay datasets showed 100% concordance ($\kappa=1.000$) for HPV16 and HPV18, while for HPV31 and HPV45, concordance was lower at 96.3% ($\kappa=0.922$; McNemar test for discrepancies, $p=1.000$; Stata 10.1) and 85.2% ($\kappa=0.705$; $p=0.617$), respectively, although these discrepancies were not significant. Of the eight plasma samples for which no neutralization activity was detected, four of these had serological reactivity against one or more α or β HPV types not included in the neutralization panel: P07 (HPV6, HPV32), P16 (HPV6), P20 (HPV68, HPV76) and P27 (HPV38).

These data were used to assemble *HPV Negative*, *Intermediate HPV16/18 antibody* and *High HPV16/18 antibody* plasma pools whose specificity was confirmed by Laboratory A in the pseudovirus neutralization assay (**Table 1**). The WHO International Standard for HPV16 antibodies, IS16 (code: 05/134; 10 IU/mL) demonstrated type-specific neutralization of HPV16 at levels consistent with natural infection (**Table 1**) (7).

The HPV negative plasma pool (**Table 1**) comprised four plasmas (P03, P04, P12 and P19) that were negative for neutralizing antibodies against HPV16, HPV18, HPV31 and HPV45 (**Figure 1**) and negative for binding antibodies against all of the α and β HPV types by multiplex serology.

The intermediate HPV16/18 antibody plasma pool (**Table 1**) comprised two plasmas (P18 and P21) that displayed intermediate levels of neutralizing antibody against

HPV16 and HPV18; plasma P18 also displayed some neutralizing antibody activity against HPV31 (**Figure 1**). Both plasmas were positive for binding antibodies against HPV16 and HPV18 and, apart from P18 having some reactivity against HPV32 (α 1), these samples were negative for binding antibodies against other α and β HPV types tested by multiplex serology. The median HPV16 neutralizing antibody titer for this plasma pool when expressed as International Units (IU) was 187 IU/mL (Inter-Quartile Range, IQR, 126-211; n=3), consistent with being a low to intermediate vaccine response (7).

The high HPV16/18 antibody plasma pool (**Table 1**) comprised seven plasmas (P02, P06, P09, P14, P22, P24 and P25) that displayed high levels of neutralizing antibody against HPV16 and HPV18 and intermediate levels against HPV31 and HPV45 (**Figure 1**). The individual plasmas were positive for multiple α and β HPV types tested by multiplex serology including α 1 (HPV32), α 2 (HPV3), α 7 (HPV18, 45, 68), α 9 (HPV16, 31, 33, 52, 58), α 10 (HPV6), β 1 (HPV5), β 2 (HPV15, 38), β 3 (HPV76). The median HPV16 neutralizing antibody level was 6,668 IU/mL (IQR, 5,356 – 8,023; n=3), consistent with a high level vaccine antibody response (7).

Overall, the results obtained from testing the plasma pools were similar to those expected from the average of the responses for the individual plasma samples.

Antibody titers derived from natural infection are significantly lower than for vaccinees (8) and HPV16, HPV18, HPV31 and HPV45 co-infections, particularly in this age group, are rare (10, 11). Together these data suggest that the plasma samples with high titer antibodies against both HPV16 and HPV18 (including those

with reactivity against HPV31 and HPV45) are almost certainly a result of vaccination. Our panel is not perfectly representative of most vaccinees due to the age of vaccination in the catch-up cohort being rather older than the target age for routine immunization. However, the neutralization responses measured using these plasma pools are indistinguishable from the responses seen with sera taken from 13–14 year old vaccinees (5). Low levels of antibodies generated by vaccination and/or natural infection towards other α and β HPV types, as suggested from multiplex serology, cannot be ruled out.

Surveillance studies are important for estimating the prevalence of an infectious agent in, or for monitoring the impact of a vaccine on, a population or demographic group. With the recent introduction of the HPV vaccines, many countries are conducting post-vaccine surveillance, including sero-epidemiology surveys. These plasma pools will be useful as reference reagents. They are currently available as 250 μ L aliquots of liquid plasma archived at -80°C and can be obtained from the National Institute for Biological Standards and Control.

Disclosure of conflicts of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Neutralization of HPV16 (filled circles), HPV18 (filled triangles), HPV31 (open circles), HPV45 (open triangles) and control BPV (asterisk) pseudoviruses using individual plasmas. HPV16, HPV18 and BPV neutralization titers were assembled using data generated by both Laboratories A and B while HPV31 and HPV45 titers were generated by Laboratory A only. Negative values (<40) were assigned a titer of 20 and a maximum level was assigned when titers were $\geq 10,000$. Plasma samples are arranged according to whether they were selected for inclusion into the High HPV16/18 (02, 06, 09, 14, 22, 24, 25), Intermediate HPV16/18 (18, 21), HPV Negative (03, 04, 12, 19) antibody pools.

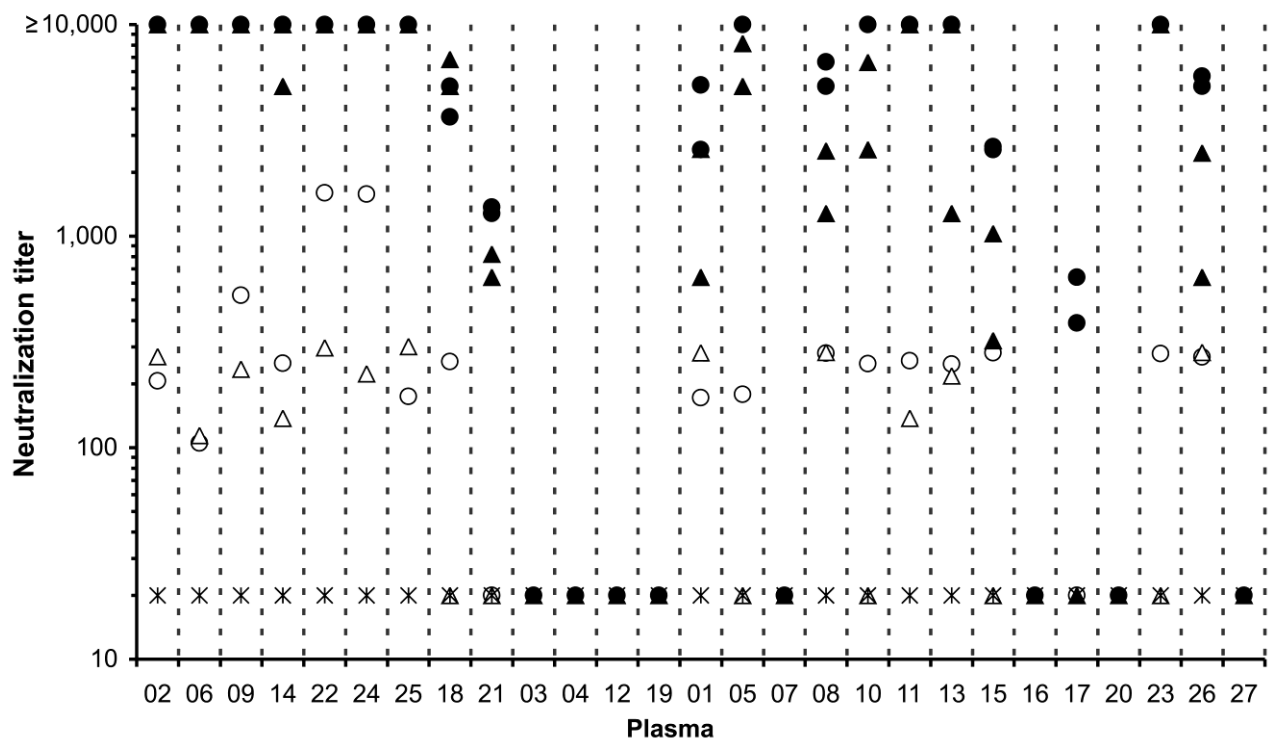


Table 1. Neutralization of vaccine and non-vaccine HPV pseudoviruses by plasma pools and IS16

HPV antibody reagent	Average neutralization titers (%CV) against indicated HPV pseudovirus ^a				
	HPV16	HPV18	HPV31	HPV45	BPV
HPV Negative	<40 (0%)	<40 (0%)	<40 (0%)	<40 (0%)	<40 (0%)
Intermediate HPV16/18	3,538 (2.1%)	5,655 (1.7%)	40 (13.9%)	<40 (0%)	<40 (0%)
High HPV16/18	161,838 (2.4%)	163,866 (4.8%)	1,271 (6.7%)	148 (3.2%)	<40 (0%)
IS16 ^b	295 (12.9%)	<40 (0%)	<40 (0%)	<40 (0%)	<40 (0%)

^a Average neutralization titers derived from three experiments with %CV of the Log₁₀ titers in parentheses

^b International Standard for HPV16 antibodies (IS16)