Dried blood spot samples for seroepidemiology of infections with Human Papillomaviruses, Helicobacter pylori, Hepatitis C virus and JC virus

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Running title
Dried blood spot seroepidemiology of HPV, H. pylori, HCV, JCV

Keywords
Dried blood spot, serology, antibody, epidemiology, multiplex

Abbreviations
CI, confidence interval; DBS, dried blood spot; GST, Glutathione S-Transferase; H. pylori, Helicobacter pylori; HCV, Hepatitis C virus; HPV, Human Papillomavirus; JCV, JC Polyomavirus; MFI, median fluorescence intensity; OR, odds ratio

Word count: 3111
Number of figures: 1
Number of tables: 2
Abstract

**Background:** To establish antibody analysis from dried blood spots (DBS) on filter paper for seroepidemiological infection and cancer association studies, we analyzed data from a population-based study in Mongolia.

**Methods:** Using multiplex serology, we analyzed 985 paired DBS and serum samples from the same donors for antibodies to 12 different proteins from 4 groups of infectious agents: Human Papillomaviruses (HPV), *Helicobacter pylori* (*H. pylori*), Hepatitis C virus (HCV), and JC Polyomavirus (JCV).

**Results:** Quantitative antibody reactivities in serum and DBS showed good correlation, with median correlation coefficients (Pearson’s R²) of 0.88 (range 0.80-0.90) for high-titre (*i.e.*, *H. pylori*, HCV, JCV) and 0.79 (range 0.72-0.85) for low-titre antibodies (*i.e.*, HPV). For high-titre antibodies, serum and DBS data were comparable (median slope of linear trend line 1.14, range 1.09-1.21) while for low-titre antibodies, DBS reactivities were lower than in serum (median slope 0.54, range 0.50-0.80). By extrapolating seropositivity cut-offs previously defined for serum to DBS, we found high agreement (>89% for all antigens) of dichotomized DBS and serum results and median kappa values for high- and low-titre antibodies of 0.86 and 0.78 (range 0.78-0.92 and 0.55-0.86), respectively. Epidemiological associations with known risk factors for HPV antibodies were as strong for DBS as for serum.

**Conclusions:** DBS provide a reliable alternative to serum or plasma for detection of antibodies against various pathogens by multiplex serology.

**Impact:** DBS do not require blood centrifugation and allow storage and shipment at ambient temperature thus facilitating field work for seroepidemiological studies especially in environments with limited technical infrastructure.
Introduction

Seroepidemiological studies of infectious diseases rely on blood-derived samples to detect specific antibodies against infectious agents. In contrast to tissue samples, full blood can be relatively easily obtained. However, centrifugation to separate blood cells from serum or plasma, and sample storage and shipment below 0°C require technical equipment sometimes not available in low-resource countries or remote field work locations. Therefore, we explored the feasibility of seroepidemiological studies based on dried blood spots (DBS) on filter paper cards which are commonly used for newborn screening. DBS cards have been employed in seroepidemiological studies of different viral or bacterial infections (1). To allow for a direct comparison, we analyzed paired DBS and serum samples from the same donors derived from intravenous blood of 985 women from a population-based study conducted in Mongolia (2).

Using multiplex serology, a fluorescent bead-based high-throughput method that allows the analysis of serum or plasma samples for antibodies to up to 100 different antigens simultaneously (3), these 1970 samples were tested for antibodies to 12 different proteins from 4 groups of infectious agents, most of which to our knowledge have not been analyzed by DBS serology: Human Papillomaviruses (HPV), *Helicobacter pylori* (*H. pylori*), Hepatitis C virus (HCV), and JC Polyomavirus (JCV).
Material & Methods

Study Population

The study base consisted of 1022 sexually active women (median age 36 years, range 16 to 63) from a population-based cross-sectional study (overall response rate 69%) conducted in Ulaanbataar, Mongolia (2). The aim of the study was to describe HPV prevalence and type distribution in Mongolia to help inform public health choices for cervical cancer prevention in Central Asia. The study was approved by the ethical review committees of the International Agency for Research on Cancer (IARC) and the Mongolian Health Ministry, and informed consent was obtained from all study participants. Paired serum and DBS samples from the same donors were available for 985 women (96%). For the remaining 37 women, paired analyses were not possible because either a valid serum or a valid DBS sample was missing.

Data and sample collection

A standard questionnaire was administered to all study participants by trained interviewers, including questions on age, marital status, and sexual behaviour.

Blood samples were collected between September and November 2005. Venous blood samples for serologic testing (5 mL) were drawn into vacuum containers without anticoagulant. Seventy µL of full blood each were immediately applied to five spots on dried blood spot (DBS) filter paper cards (Whatman 903® Protein Saver Blood Collection Cards, Schleicher & Schuell, Dassel, Germany), and cards were dried at room temperature. After clotting of the remaining full blood and centrifugation at 2000 rpm for 5 min, 1 mL serum was transferred into a pre-labelled 2 mL vial (Safe-lock, Eppendorf, Hamburg, Germany). Serum and dried blood spot samples were stored at room temperature for up to 8 h and then at -20°C for up to one month. All samples were shipped at ambient temperature to the German Cancer Research Center (DKFZ) in Heidelberg, Germany and stored at -20°C until analysis.

Antigens

Serum and DBS samples from study participants were analyzed for antibodies to i) the major capsid (L1) proteins of the mucosal high-risk HPV types 16 (HPV-16), 18, 31, 33, 45, 52, and 58 (2, 4) ii) the JCV major capsid protein VP1 (5, 6), iii) the H. pylori proteins NapA (HP0243,
strain 26695) and GroEL (HP0010, strain G27) (7), and iv) the HCV (strain H77, subtype 1a) Core and NS3 proteins (Dondog et al., submitted).

**DBS processing**

We assessed suitability of DBS as source for antibodies first by determining the DBS elution volume corresponding to a 1:100 serum dilution. Antibodies against HPV-16 L1, HPV-18 L1, and JCV VP1 in 32 serum/DBS pairs were used for calibration. The best correlation was found for 4 punches with 2.85 mm diameter (corresponding to 15.4% of the spot’s area and applied blood volume, respectively, i.e. 10.8 µL of full blood) eluted in one well of a 96-well plate (Greiner Bio-One polystyrene flat bottom microplate) overnight at 4°C on a rocker (10 agitations per minute) in 100 µl phosphate-buffered saline (data not shown). Sixteen µL of this eluate correspond to 1.7 µL of full blood and, assuming a red cell content of 45%, 1 µL of serum.

**Multiplex serology**

The antibody analysis of serum samples was conducted as previously described in detail for HPV (3). Briefly, recombinant viral and bacterial proteins were bacterially expressed as double fusion proteins with N-terminal glutathione S-transferase (GST) and a C-terminal peptide (tag) consisting of the last 11 amino acids of the large T antigen of simian virus 40 (GST-X-tag) (8).

GST-X-tag double fusion proteins from cleared bacterial lysates were affinity-purified in situ through binding to glutathione casein-coated fluorescence-labelled polystyrene beads. Each fusion protein was bound to a spectrally distinct bead set (SeroMAP™ Microspheres, Luminex Corp., Austin, TX). Afterwards, fusion protein-loaded bead sets were mixed.

Sera were pre-incubated at 1:50 dilution in serum pre-incubation buffer consisting of phosphate-buffered saline, containing 1 mg/mL casein, 2 mg/mL lysate from bacteria expressing GST-tag without intervening viral or bacterial protein to block antibodies directed against residual bacterial proteins and the GST and tag fusion domains, 0.5% polyvinylalcohol, 0.8% polyvinylpyrrolidone and 2.5% Superchemiblock (Millipore, Billerica, MA) to suppress unspecific binding of antibodies to the beads themselves (9). Fifty µL of serum dilutions were incubated with the same volume of mixed bead sets, resulting in a final serum dilution of 1:100.
Sixteen µL of DBS eluates were mixed with 80 µL DBS pre-incubation buffer which was identical to the serum pre-incubation buffer but contained increased concentrations of the relevant background reducing components (i.e., 2.4 mg/mL GST-tag lysate and 3% Superchemiblock) to account for the changed ratio of sample and buffer volume.

Bound antibodies were detected with biotinylated goat-anti-human IgG (H+L) secondary antibody and streptavidin-R-phycoerythrin. A Luminex 100 analyzer (Luminex Corp., Austin, TX) was used to identify the internal colour of the individual beads and to quantify their reporter fluorescence (expressed as median fluorescence intensity (MFI) of at least 100 beads per set per serum).

**Assay design and data processing**

To optimize comparability, serum and DBS eluates of each person were analyzed in parallel on the same 96-well plate and the complete set of samples was tested within 3 consecutive assay days using one batch of antigen-loaded beads. Every day, binding of GST-X-tag double fusion proteins to glutathione casein-coated beads was quantified by monoclonal anti-tag antibody (8). The median inter-day coefficient of variation (CV) for the anti-tag MFI values for the various antigens was 5.1% (range 0.1% to 19.6%), indicating stable antigen binding to the beads throughout the three assay days.

Auto-fluorescence of each bead set and background reactions resulting from binding of secondary reagents to the antigen-loaded beads were determined in one well per plate without human serum (bead background). The sample background was determined from the reaction of the serum or DBS eluate with beads loaded with GST-tag. Bead background and sample background were subtracted from the raw MFI values obtained with the specific fusion proteins to obtain the antigen-specific reactivity of the sample (net MFI).

**Statistical analysis**

Serum MFI values for all antigens were categorized as antibody positive or negative by applying previously defined antigen-specific cut-offs (4, 6, 7) (Dondog et al., submitted). To extrapolate this definition to DBS, cut-offs were calculated to give the same seroprevalence for the DBS as present in the corresponding sera. The cut-off values for sera and DBS are indicated in Table 1.
All statistical analyses were performed with SAS Version 9.1.3. Correlation of continuous serum and DBS data was assessed by Pearson ($R^2$) correlation coefficients. For dichotomized data, overall agreement (sum of concordantly positive and concordantly negative samples divided by the total number of samples) and kappa values with their corresponding 95% confidence intervals (CI) were computed to estimate concordance of serum and DBS results. Categorization of socio-demographic characteristics, computation of Odds Ratios (OR) and their corresponding 95% CI, and tests for linear trends were performed as described previously (2). All tests were two-sided, and p-values below 0.05 were considered statistically significant.
Results

Antigens were classified into two groups based on their antibodies’ maximum median fluorescence intensity (MFI) reactivities. The HPV L1 proteins yielded maximum antibody signals below 10,000 MFI (hereafter referred to as “low-titre” antibodies), while the *H. pylori*, HCV and JCV antigens gave maximum antibody signals of up to 30,000 MFI (hereafter referred to as “high-titre” antibodies). The MFI values obtained for 985 DBS/serum pairs for all antigens are illustrated in Figure 1.

The relationship between the two serological markers followed a slightly J-curved pattern, most evident for the high-titre antibodies (Figure 1). Overall, the correlation coefficients ($R^2$) between DBS and serum MFI were high, ranging from 0.72 for HPV-58 L1 to 0.90 for HCV NS3. However, correlation coefficients were better for the high-titre antibodies (median 0.88, range 0.80-0.90) than for the low-titre antibodies (median 0.79, range 0.72-0.85) (Figure 1). For most antigens, DBS showed increased minimum reactivities between 30 and 80 MFI. For the low-titre antibodies, MFI in DBS were lower than in serum (median slope derived from linear regression 0.54, range 0.50-0.80), while for high-titre antibodies, direct comparability of serum and DBS MFI was better (median slope 1.14, range 1.09-1.21).

After extrapolation of the pre-defined serum cut-offs to the DBS, seroprevalences for all antigens in serum and DBS were equal by definition. Numbers of discordant reactions for each antigen were hence symmetrical (e.g., for HPV 16 L1, there were 725 samples concordantly negative, 180 samples concordantly positive, and 40 samples each DBS positive/serum negative and DBS negative/serum positive). Data on serum and DBS seropositivity cut-offs and concordance for all 12 antigens are presented in Table 1.

*H. pylori* NapA was the only antigen which required a slightly higher cut-off in DBS (124 MFI) than in serum (100 MFI) to give an equal seroprevalence (Table 1). For all other antigens, cut-offs for DBS were lower than for serum (median DBS cut-off 73% of the corresponding serum cut-off), with little variation (range 62-84%).

The correlation of the dichotomized DBS and serum data yielded very good agreement (median 95.1%, range 89.2-98.0%) and median kappa values for the low- and high-titre antibodies of 0.77 and 0.90 (range 0.55-0.80 and 0.78-0.94), respectively, indicating very strong correlations beyond chance. The MFI reactivities of the discordant samples were mostly close to the cut-off (median MFI compared to cut-off 124%, range 108-159%).
The epidemiological association of risk-factors for seropositivity to any of the 7 high-risk HPV types is shown in Table 2 for both serum and DBS. Numbers of positively tested samples were similar for both antibody detection methods. For both serum and DBS, statistically significant associations with seroprevalence were observed for increasing age, increasing number of lifetime sexual partners and by marital status. These associations were equally strong for DBS as for serum. Similar observations were made for increasing age as main risk factor for HCV seropositivity in Mongolian women (Dondog et al., submitted) (data not shown).
Discussion

We directly compared antibody reactivities in 985 pairs of dried blood spots (DBS) on filter paper cards and serum samples derived from intravenous blood to explore the feasibility of DBS-based seroepidemiological studies. The main strengths of the present study are the large number of almost 1000 sample pairs tested in parallel, the use of state-of-the-art technique for detection of multiple antibodies, and the integration of 12 different antigens from 4 groups of infectious agents representing both low- and high-titre antibody responses: Human Papillomaviruses (HPV), *Helicobacter pylori* (*H. pylori*), Hepatitis C virus (HCV), and JC Polyomavirus (JCV).

The infectious agents involved in this study are known to cause different diseases. The mucosal high-risk HPV types 16, 18, 31, 33, 45, 52, and 58 are causally associated with several malignancies, particularly cervical cancer (10). *H. pylori* infection is related to severe gastrointestinal diseases including gastric cancer (11), and chronic HCV infection may lead to hepatocellular carcinoma and non-Hodgkin Lymphoma (10). Finally, JCV can cause progressive multifocal leukoencephalopathy in immunosuppressed patients (12). Despite this heterogeneity, antibody detection is valuable for each of these infectious agents, in order to assess their prevalence and to discover yet unknown disease associations in seroepidemiological studies (13). Our multiplex serology method was first developed for HPV serology (3) and the versatile bacterial protein expression system has facilitated the method's extension to antigens from other infectious agents. Multiplex serology-based polyomavirus (5, 6) and *H. pylori* serology (7) has also been reported. Furthermore, the application of multiplex serology to both HIV (Kranz et al., submitted) and HCV (Dondog et al., submitted), including validation against gold-standard serological tests, will soon be available in the published literature.

The seroprevalences of *H. pylori* (14) and JCV (5, 6) are quite high in adults of most populations, while antibodies to mucosal HPV (15) and HCV (16) are less abundant and variable by population. Mongolia is a high-risk country for infections with both mucosal HPV (2) and HCV (17) which makes our study population especially well suited for this comparison of serological methods. We included two exemplary *H. pylori* antigens with high (GroEL) and low (NapA) seroprevalence; these do however not allow assessing overall *H. pylori* seroprevalence. Our data on the overall prevalence of *H. pylori* in serum samples (81% as determined by *H. pylori* multiplex serology employing all 15 antigens (7), unpublished observation) and JCV (78%, this report) in Mongolian adults is in concordance with published data (18, 19).
DBS have been mainly used for viral or bacterial infections with high antibody titres like the HIV, Hepatitis B, and Measles viruses, or the bacterium *Mycobacterium leprae* (reviewed in (1)). HCV is the only of the four infectious agents analysed here for which DBS have been used before (e.g., (20)), but to our knowledge there is no published DBS data available for *H. pylori*, JCV or HPV. We considered the application of DBS for HPV serology the main challenge, because due to the absence of systemic infection, natural HPV antibody titres are low. However, HPV titres after vaccination are much higher than after natural infections (21), facilitating the use of DBS in HPV vaccination studies.

Our results indicate that overall quantitative DBS and serum results are well comparable. However, we observed deviation from the ideal (linear) DBS-serum correlation. For very low antibody reactivities (approx. 100 net MFI, i.e. after background subtraction), DBS yield higher baseline reactions, while for intermediate reactivities (approx. 1000 MFI), DBS generate lower MFI than serum. This drives the overall correlation of the low-titre antigens representing mostly weak and intermediate antibody reactivities to slopes below 1, i.e. overall lower DBS than serum values. Strong antibody reactivities (approx. 10,000 MFI) however yield similar MFI values in serum and DBS, resulting in a curved correlation as seen for the high-titre antigens. At present, we can only speculate about the causes underlying these phenomena. Baseline elevation in DBS could result from highly abundant, eluted full blood components not present in serum or plasma (e.g., haemoglobin) which mediate unspecific immunoglobulin binding to the viral or bacterial proteins used as antigens. The curved correlation could result from small concentrations of inhibitors or competitors competing with antibody binding to the antigens. With higher titres of antigen-specific antibodies in the DBS eluates, both phenomena are increasingly overcome.

In the presence of paired serum and DBS samples, we were able to extrapolate pre-defined serum cut-offs to the DBS data, by choosing DBS cut-offs that resulted in identical seroprevalence estimates, a key outcome in epidemiological studies. This method resulted, by definition, in symmetric discordance, and was more robust against individual outliers than regression-based methods (data not shown). After application of the extrapolated cut-offs, kappa values for DBS/serum concordance were high for the antigens with high-titre antibodies (with a median of 0.90), thus approaching values that are found for reproducibility of multiplex serology (typically ≥ 0.95). Overall concordance was still good for the HPV antigens, with the exception of HPV-58 with a kappa value of 0.55. However, kappa statistics are strongly influenced by prevalence, and HPV-58 was the type with the lowest seroprevalence (below 5%). For all antigens, antibody reactivities of the discordant samples
were mostly close to the respective cut-off. We therefore believe that discordance was largely a stochastic event. Indeed, epidemiological associations of established risk-factors for HPV (2) and HCV (Dondog et al., submitted) seropositivity in Mongolia were shown to be equally strong for DBS as for serum.

The availability of paired data from both methods is an exception. Purely DBS-based studies will rely on simultaneous collection of samples from appropriate control groups to define internally valid cut-offs (e.g., children and/or virgins when studying sexually transmitted diseases). Alternatively, in the absence of such controls, our data also suggest how existing cut-offs established in serum may be extrapolated to DBS. For 11 out of 12 antigens, we found the DBS cut-offs to be approx. 73% of the corresponding serum cut-offs, within a relatively narrow range of ±11%, probably owing to random variation. A limitation of the DBS method is that standardized reference sera cannot be used.

DBS facilitate field work in epidemiological studies especially in low resource countries, because blood does not need to undergo centrifugation, and the DBS cards can be stored and shipped at ambient temperature. Blood sampling procedures may also be facilitated, since sufficient amounts of blood for DBS preparation from adults and even children may be obtained from the finger tip, the earlobe or the heel as done routinely with diabetes patients and newborns, thereby strongly reducing costs of seroepidemiological studies. Of note, however, we used blood drops from intravenous puncture to prepare DBS, which facilitates high quality and reproducibility of DBS. Imprecise application of too little or too much blood per spot may reduce DBS quality.

In conclusion, our data indicate that DBS provide a reliable alternative to serum samples for seroepidemiological studies and allow antibody determinations for various pathogens inducing high- but also low-titre antibody responses.
Acknowledgment

We thank Ute Koch and Monika Oppenländer for excellent technical assistance.
Table 1  Cut-offs and dried blood spot/serum concordance

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cut-offs (MFI)</th>
<th>Sera¹</th>
<th>DBS²</th>
<th>Prevalence³ (%)</th>
<th>Agreement⁴ (%)</th>
<th>Kappa (95% CI⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV16 L1</td>
<td>422 263</td>
<td>22.4</td>
<td>91.9</td>
<td>0.77 (0.72-0.81)</td>
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<tr>
<td>HPV18 L1</td>
<td>394 298</td>
<td>19.3</td>
<td>93.9</td>
<td>0.80 (0.76-0.85)</td>
<td></td>
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<tr>
<td>HPV31 L1</td>
<td>712 523</td>
<td>13.1</td>
<td>95.1</td>
<td>0.79 (0.73-0.84)</td>
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<tr>
<td>HPV33 L1</td>
<td>515 374</td>
<td>9.2</td>
<td>95.1</td>
<td>0.71 (0.63-0.79)</td>
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<tr>
<td>HPV45 L1</td>
<td>368 232</td>
<td>16.9</td>
<td>94.5</td>
<td>0.80 (0.75-0.85)</td>
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<tr>
<td>HPV52 L1</td>
<td>547 406</td>
<td>10.7</td>
<td>93.9</td>
<td>0.68 (0.60-0.76)</td>
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<tr>
<td>HPV58 L1</td>
<td>592 448</td>
<td>4.8</td>
<td>95.9</td>
<td>0.55 (0.43-0.68)</td>
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<tr>
<td>JCV VP1</td>
<td>500 324</td>
<td>78.4</td>
<td>98.0</td>
<td>0.94 (0.91-0.97)</td>
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<tr>
<td>NapA</td>
<td>100 124</td>
<td>45.5</td>
<td>89.2</td>
<td>0.78 (0.74-0.82)</td>
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<tr>
<td>GroEL</td>
<td>100 80</td>
<td>86.0</td>
<td>96.6</td>
<td>0.86 (0.81-0.91)</td>
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<tr>
<td>Core</td>
<td>1492 967</td>
<td>19.4</td>
<td>98.0</td>
<td>0.94 (0.91-0.96)</td>
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<td>NS3</td>
<td>371 310</td>
<td>24.9</td>
<td>96.1</td>
<td>0.90 (0.86-0.93)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Serum cut-offs were pre-defined in Clifford et al. 2007 (HPV), Antonsson et al. 2010 (JCV), Michel et al. 2009 (H. pylori), and Dondog et al. submitted (HCV)
² DBS, dried blood spots
³ Cut-offs for DBS were chosen such that prevalences in serum and DBS were identical
⁴ (concordantly positives + concordantly negatives) / total study population
⁵ CI, confidence interval
⁶ PyV, Polyomavirus
Table 2 Association of socio-demographic characteristics with antibodies to any of the 7 high-risk HPV types as detected by serum and dried blood spots

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Dried Blood Spots</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>pos (%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>204</td>
<td>66 (32.4)</td>
</tr>
<tr>
<td>25-34</td>
<td>250</td>
<td>79 (31.6)</td>
</tr>
<tr>
<td>35-44</td>
<td>252</td>
<td>96 (38.1)</td>
</tr>
<tr>
<td>45-54</td>
<td>212</td>
<td>100 (47.2)</td>
</tr>
<tr>
<td>55+</td>
<td>54</td>
<td>26 (48.2)</td>
</tr>
<tr>
<td><strong>ptrend</strong></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>691</td>
<td>269 (38.9)</td>
</tr>
<tr>
<td>Single</td>
<td>212</td>
<td>62 (29.3)</td>
</tr>
<tr>
<td>Widowed</td>
<td>35</td>
<td>19 (54.3)</td>
</tr>
<tr>
<td>Separated/Divorced</td>
<td>32</td>
<td>17 (53.1)</td>
</tr>
<tr>
<td><strong>Lifetime number of sexual partners</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>418</td>
<td>148 (35.4)</td>
</tr>
<tr>
<td>2</td>
<td>241</td>
<td>89 (36.9)</td>
</tr>
<tr>
<td>3</td>
<td>141</td>
<td>56 (39.7)</td>
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<tr>
<td>4</td>
<td>73</td>
<td>33 (45.2)</td>
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<tr>
<td>5+</td>
<td>81</td>
<td>35 (43.2)</td>
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<tr>
<td><strong>ptrend</strong></td>
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^1 OR, odds ratio; CI, confidence interval
Figure legends

Figure 1  Scatter plots of antigen-specific dried blood spot (DBS) median fluorescence intensities (MFI) versus serum MFI (985 pairs). Plots for all antigens representing low (A to G) and high (H to L) titre are shown: A, Human Papillomavirus type 16 (HPV-16) L1 (R² 0.85, slope 0.52); B, HPV-18 L1 (R² 0.80, slope 0.54); C, HPV-31 L1 (R² 0.79, slope 0.59); D, HPV-33 L1 (R² 0.74, slope 0.53); E, HPV-45 L1 (R² 0.84, slope 0.54); F, HPV-52 L1 (R² 0.74, slope 0.50); G, HPV-58 L1 (R² 0.72, slope 0.80); H, JC polyomavirus (JCV) VP1 (R² 0.80, slope 1.14); I, Helicobacter pylori (HP) NapA (R² 0.88, slope 1.09); J, HP GroEL (R² 0.86, slope 1.12); K, Hepatitis C virus (HCV) Core (R² 0.89, slope 1.17); L, HCV NS3 (R² 0.90, slope 1.21). Dashed lines indicate cut-offs as provided in Table 1. Insets in the upper left corner of each plot show the same data on linear axes. Please note the maximum values for the insets' axes (10,000 MFI for plots A to G; 30,000 MFI for plots H to L).
References


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Cancer Epidemiol Biomarkers Prev Published OnlineFirst December 6, 2011.

Updated version Access the most recent version of this article at: doi:10.1158/1055-9965.EPI-11-1001

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