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Two L1-peptides are excellent tools for serological detection of HPV-associated cervical carcinoma lesions ☆

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Abstract

A persistent high risk human papillomavirus (HR-HPV) infection causes cervical intraepithelial lesions and cervical carcinoma. There is evidence that detecting anti-L1 antibodies could be successfully used for discriminating between cervical lesion patients and women having normal cytology. It was found that peptides 18283 (^{55}PNNNKILVPKVSGLQYRVFR^{74}) and 18294 (^{284}LYIKGSG STANLASSNYFPT^{300}) from the L1-surface exposed regions were specifically recognised by antibodies from the cervical lesion patient sera. These peptides were tested against 165 womens' normal cytology sera and 148 cervical lesion or cervical cancer patients' sera. Less than 3.6% of women's normal cytology sera recognised peptides 18283 or 18294; on the contrary, 91% to 96% of the cervical lesion (CIN I to CIN III) or cervical cancer patient sera recognised peptides 18283 and 18294. These data show that anti-peptide 18283 and 18294 antibodies in the patients' sera are strongly associated with the presence of HR-HPV associated cervical lesions, showing 92–97% sensitivity and 89–95% specificity in recognising precancerous and cervical cancer patients. These two peptides could be excellent tools for use in large-scale serological screening of women populations at risk of developing cervical carcinoma.

Keywords: HPV; HPV-patients; VLP; ELISA; HPV DNA; Cervical intraepithelial neoplasia; Cervical cancer

Cervical cancer is the second most common cancer amongst women worldwide; it has been estimated that 80% of new cases arise in the developing world [1]. Around 250,000 women die annually from this cancer and 500,000 new cases are diagnosed each year. There is strong evidence suggesting that this cancer is 100% attributable to infection with certain types of human papillomavirus (HPV); in fact, the World Health Organisation (WHO) has very recently recognised that this cancer is caused by HPV [2,3]. There are around 100 different HPV-types, of which around 40 types infecting the anogenital tract and causing cervical lesions and cervical cancer [4] have been called High Risk HPV (i.e., HR-HPV-16, -18, -31, -33, and -58). Early detection of cervical epithelial lesions or HR-HPV persistent infection, in symptomatic or asymptomatic women, using a simple reliable, specific, sensitive, cheap test that can be used in large-scale screening of female populations, represents an important tool for allowing rapid therapeutic action to be taken against cervical lesions and cervical cancer.

Most infections spontaneously disappear 2 years after genital HPV-incident infection; HPV DNA becomes

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undetectable in this transitory virus infection in about 90% of women [5]. However, a persistent HR-HPV infection in around 5% of them leads to the development of cervical lesions and cervical cancer. This is partly mediated by high viral load since there is evidence of a relationship between viral load and progression of cervical intraepithelial neoplasia (CIN), CIN II or CIN III diagnosis and the risk of developing cervical lesions and cervical cancer [5–7].

Most potentially oncogenic, persistent, long-term HPV infections induce an antibody response against virus proteins which can be detected by ELISA test and can be a good indicator of past as well as current infections and chronic active infection, associated with the presence of cervical lesions and the high risk of acquiring cervical cancer. In fact, it has been reported that women who are seropositive for HPV-16 present a higher risk of developing cervical carcinoma than seronegative women [8–11]. L1-viral capsid proteins are one of the targets for antibodies induced by persistent HPV-genital infection [12–16].

The L1 protein represents more than 90% of the total protein on the surface of the virus. This protein is able to assemble itself, forming virus-like particles (VLPs) (Fig. 3A). VLPs, mainly type 16, have been broadly used for studying the antibody response induced by genital HPV-infection. The VLP-antibody presence is stable in time [10], correlated with the number of sexual partners, and associated with persistent infection, viral load, and development of neoplasic lesions; this is rarely found in patients suffering from transitory infections. Seropositivity occurs more frequently in patients who have progressed to CIN III and invasive cancer than in those suffering from CIN I or CIN II and the antibody response is significantly higher in women having a higher viral load than those with lower viral load [10,11]. Prospective studies have shown that 70–90% of HPV-16 infected women are seroconverted between 6 and 18 months after HPV DNA has been detected and that this rarely occurs in patients detected as having transient HPV DNA [11,15–18].

However, other serological studies have shown that 20–50% of women suffering from HPV-associated lesions, with HPV DNA presence, do not present detectable levels of anti-VLP antibodies [19–22]; this is in part due to the lack of an optimised test and the fact that these antibodies' reactivity is type-specific for the virus. An optimised VLP-based ELISA test has been recently reported showing 93% sensitivity and 98.5% specificity for discriminating between positive and negative control sera [23].

On the other hand, immunodominant regions have been identified within L1 protein specifically reacting with sera from patients suffering from cervical lesions and persistent HPV infection [24]. Some of these regions have been identified by using peptides; in fact, it has been reported that the ^{473}GLKAKPKFTLGKKA TPTTS^{491} peptide from the L1 protein is specifically recognised by sera from 91% of those patients whose biopsies contained HPV type 16 DNA, 24% of children, and 66% of other CIN VPH16 negative patients [8].

This article reports identifying two peptides from the HPV-16 L1 protein, solvent-exposed regions according to GETAREA software and the tridimensional structure reported for this protein [25], tested against 148 sera from patients with CIN I to III and patients suffering from cervical cancer compared to 165 sera from women having normal cytology. These peptides showed very high sensitivity (94.5–97.2%) and extremely high specificity (90.9–97.5%) for discriminating between HPV associated carcinoma and carcinoma lesion positive and negative women's sera, making these two peptides excellent tools for mass, rapid screening of large-scale female populations at risk of developing cervical carcinoma.

Materials and methods

Selecting peptide sequences to be synthesised. The L1-protein tridimensional structure reported in the protein data bank (Accession No. 1DZL) was analysed by GETAREA software to determine those regions surface-exposed and solvent accessible by using a 1.4 A radio probe [26]. Those L1-peptides presenting more than 30% of their sequence exposed to solvent according to the above-mentioned analysis, including all the loops exposed on the top of the pentamer structure, were tested against a positive or negative serum pool. One peptide presenting less than 30% of its exposed surface was included in this analysis as a negative control.

Peptide synthesis. 20-mer peptides, corresponding to HPV type 16 L1 [27], were synthesised by solid-phase multiple peptide system [28]. MBHA resin (0.7 mEq/g); t-Boc amino-acid, and low-high cleavage techniques were used. Peptides were analysed by MALDI-TOF mass spectrometry and reverse phase-high performance liquid chromatography (RP-HPLC) (data not shown).

HPV-16 VLP production and purification. HPV16 virus-like particles (VLP-16) were produced in insect cells and purified by ultra-centrifuging in CsCl gradients, according to a previously described procedure [29]. Recombinant baculoviruses encoding HPV16 L1 protein were used for infecting SF21 cells at a multiplicity of infection (MOI) of 20. Cells were harvested 4 days post-infection, and cytoplasmic and nuclear fractions were separated by Nonidet P-40 treatment (0.5%), followed by centrifuging (10,000*g*, 15 min). CsCl gradient fractions were collected and densities were determined by refractometry. Fractions having a density of around 1272 g/cm^3 were pooled in 1× PBS and ultra-centrifuged (4 °C, 1 h, 130,400*g*). VLP assembly was verified by electron microscopy.

Clinical specimens and patients. Cervical scrapes and blood samples were obtained from 313 women aged 18–55, attending the Instituto Nacional de Cancerología. 148 of these 313 women presented abnormal cytology, classified according to their diagnosis (underwent punch biopsy under t guidance of colposcopy for histological evaluation) in four groups: CIN I ($n = 21$), CIN II ($n = 14$), CIN III ($n = 41$), and invasive cervical cancer ($n = 70$). One hundred and sixty-five age-matched control women, who were cytologically normal, presenting no evidence of ongoing HPV infection, randomly selected from women attending the Gynaecological Outpatient Clinic of the Liga Colombiana de Lucha contra el Cancer in Bogota taking part in its cervical cancer surveillance programme were included in this study.

ELISA. 96-well ELISA-plates were coated with 1 μg of each L1-peptide (10 μg/ml) or with 0.5 μg VLP-16 (5 μg/ml) diluted in PBS, at

4 °C overnight. Plates were then washed four times with PBS containing 0.05% Tween 20 (PBS-T). Non-specific binding sites were blocked with 200 μl of 4% non-fat dried milk in PBS-T for 2 h at 37 °C. After washing as described above, 100 μl serum diluted 1:100 in blocking buffer was added to the plate and further incubated for 1 h at 37 °C. After washing, peroxidase-conjugated rabbit anti-human IgG (VECTOR) was diluted 1:500 in blocking buffer and 100 μl was added to each well. The plates were incubated for 1 h at 37 °C. After washing, 100 μl peroxidase substrate (TMBMR KPL) was added to the plates which were then read at 620 nm. The assay was performed by triplicate and considered valid only when the triplicates' coefficient of variation was lower than 10%.

HPV DNA detection and typing. Cervical scrape samples from the transformation zone of the cervix were collected for detecting HR-HPV DNA. Cervical cells were suspended in PBS and stored at 20 °C until DNA-testing. HPV detection was performed according to previously described techniques [30]; each sample was tested with an HPV generic primer-mediated PCR with GP5+ and GP6+ consensus primers. Forty amplification cycles were carried out using a Perkin-Elmer 9600 thermocycler. Each cycle included a denaturing step at 95 °C for 1 min, one annealing step at 40 °C for 1 min, and a chain elongation step at 72 °C for 1.5 min. The first step was preceded by a denaturing step of 4 min and the last step was followed by a 10 min elongation step. SiHa cell line dilutions containing 1–10 copies of HPV16 (100 pg, 1 ng, and 10 ng) were used as positive control; distilled water was used as negative PCR control.

PCR-amplified products were further tested in an HPV type-specific hybridisation assay of GP5+/GP6+ PCR products with HPV-16, -18, -31, -33, -52, and -58 DNA-fragments [30–32]. HPV-X DNA included all samples which had tested positive with the consensus primers but were negative in HPV type-specific hybridisation assays.

Structural localisation of 18283 and 18294 peptides. Based on the previously determined HPV 16 L1 tridimensional structure by X-ray crystallography [25,33] (Protein Data Bank Accession No. 1DZL), 18283 and 18294 localisation on these molecules was established using Molecular Simulation (San Diego CA) Insight II software.

Results

L1-peptides recognised by IgG antibodies from cervical lesion patients' sera

Ten sera from CIN II/III patients (positive) and ten sera from women's normal cytology (negative) were

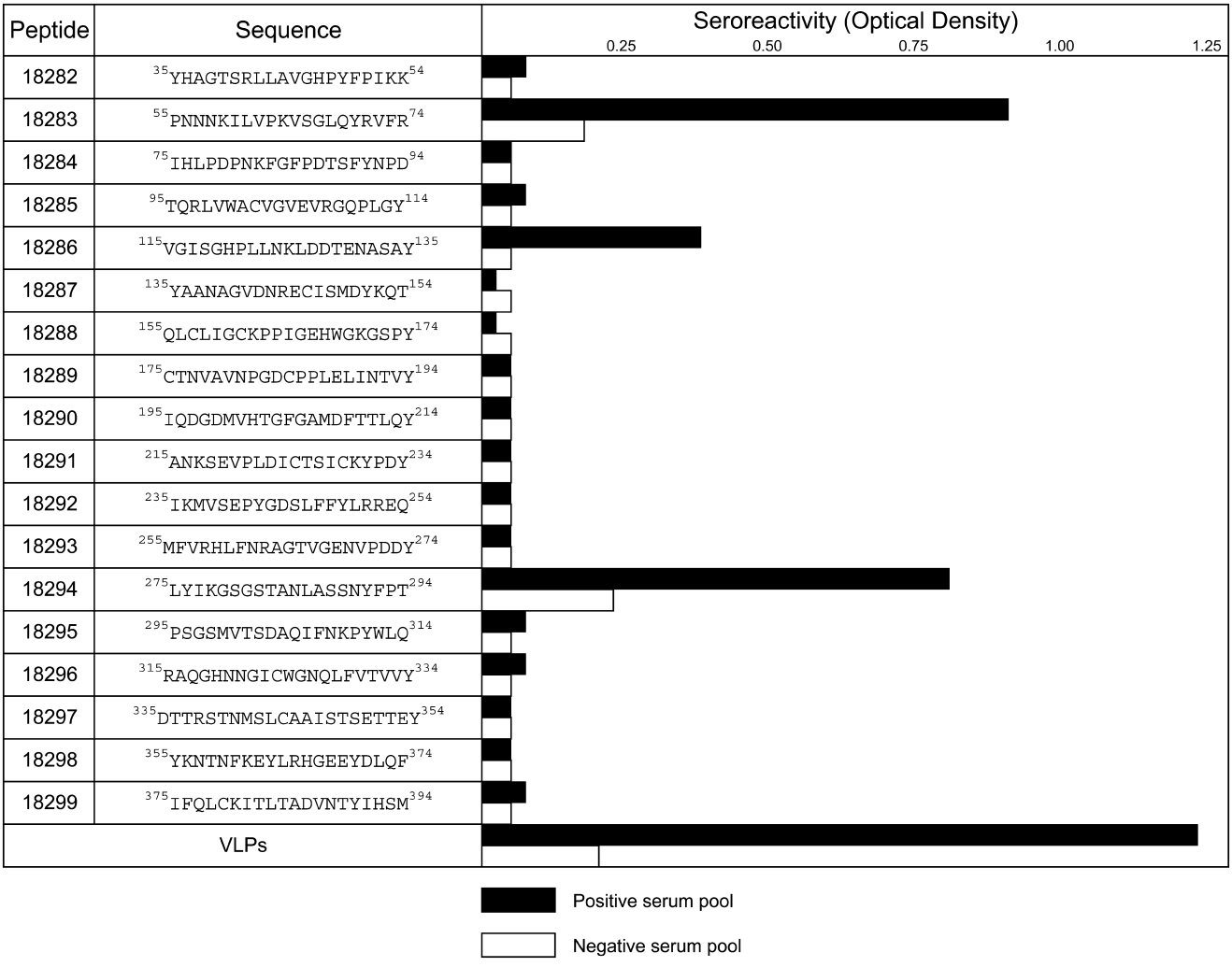

Fig. 1. L1-peptides screening ELISA of cervical lesion patients' sera. L1-peptides were tested in ELISA against a pool of ten sera from CIN II/III patients (black bars, positive) and a pool of 10 sera from normal cytology patients (white bars, negative). Optical densities (ODs) were obtained by using a serum pool at 1:100 dilution. VLP-16 was used as positive antigen control. Each peptide's sequence appears in the left-hand column.

used for preparing two serum pools. These serum pools were tested against sequential synthetic peptides spanning the L1 protein from residues 35 to 394 by ELISA. Peptides 18283 (^{55}PNNNKILVPKVSGLQYRVFR^{74}), 18286 (^{115}VGISGHPLLNKLDDTENASAY^{135}), and 18294 (^{275}LYIKGSGSTANLASSNYFPT^{294}) were specifically recognised by antibodies from the positive serum pool but not antibodies from the negative serum pool. Peptide 18283 and 18294 reactivity to these serum pools was similar to VLPs (Fig. 1), thus being chosen to be tested against 313 individual sera from both HPV-associated cervical lesion patients and women's normal cytology sera (control sera) using VLP-16 antigen as positive control and peptide 18295 (^{295}PSGSMVTSDAQ IFNKPYWLQ^{314}) as negative control (Fig. 1).

The increasingly ordered optical densities (ODs) obtained in ELISA for each serum tested against peptides 18283, 18294, 18295 and against VLP-16 showed that the ODs obtained by peptides 18283 and 18294 against HPV-associated cervical lesion patient sera were significantly higher than those against women's normal cytology sera and comparable to ODs obtained by using VLP-16. On the contrary, the ODs obtained by using peptide 18295 sera were lower than 0.2 (Fig. 2).

Peptides 18283 and 18294 presented similar mean ODs with values of around 0.4 against HPV-associated cervical lesion patients' sera and 0.16 against control sera; these values were slightly lower than those obtained with VLP-16 (mean ODs 0.6 and 0.19, respectively) but higher than those obtained with peptide 18295 (mean ODs 0.10 and 0.06, respectively). There were no differences in mean ODs obtained by peptides 18283, 18294 or VLP-16 in the sera obtained from patients having different disease status (CIN I, II, and III and cervical cancer) (Table 1).

Patient sera presenting ODs higher than or lower than the mean ODs obtained with control sera plus two standard deviations (2 SD) were considered to be seropositive or seronegative, respectively, for peptides 18283 and 18294 or VLP-16. This analysis showed that

Table 1
Peptide 10283 and 18294 seropositivity regarding status disease

Diagnosis	No. of positive responder (%)		
	Peptide 18283	Peptide 18294	VLP-16
Normal ($n = 165$)	4(2.4)	6(3.6)	3(1.8)
CIN I ($n = 23$)	22(96)	21(91)	23(100)
CIN II ($n = 14$)	14(100)	10(71)	14(100)
CIN III ($n = 41$)	37(90)	34(83)	40(97)
ICC ($n = 70$)	69(99)	69(99)	70(100)

Patients were diagnosed by biopsy under guidance of colposcopy; women classified as normal presented no cylogical alterations and more than 85% of them were not HR-HPV infected. The percentage of seropositive patients for 18283, 18294 or VLP-16 antigens appears in brackets. Seropositive patients were defined by using mean ODs plus two standard deviations for women having normal cytology.

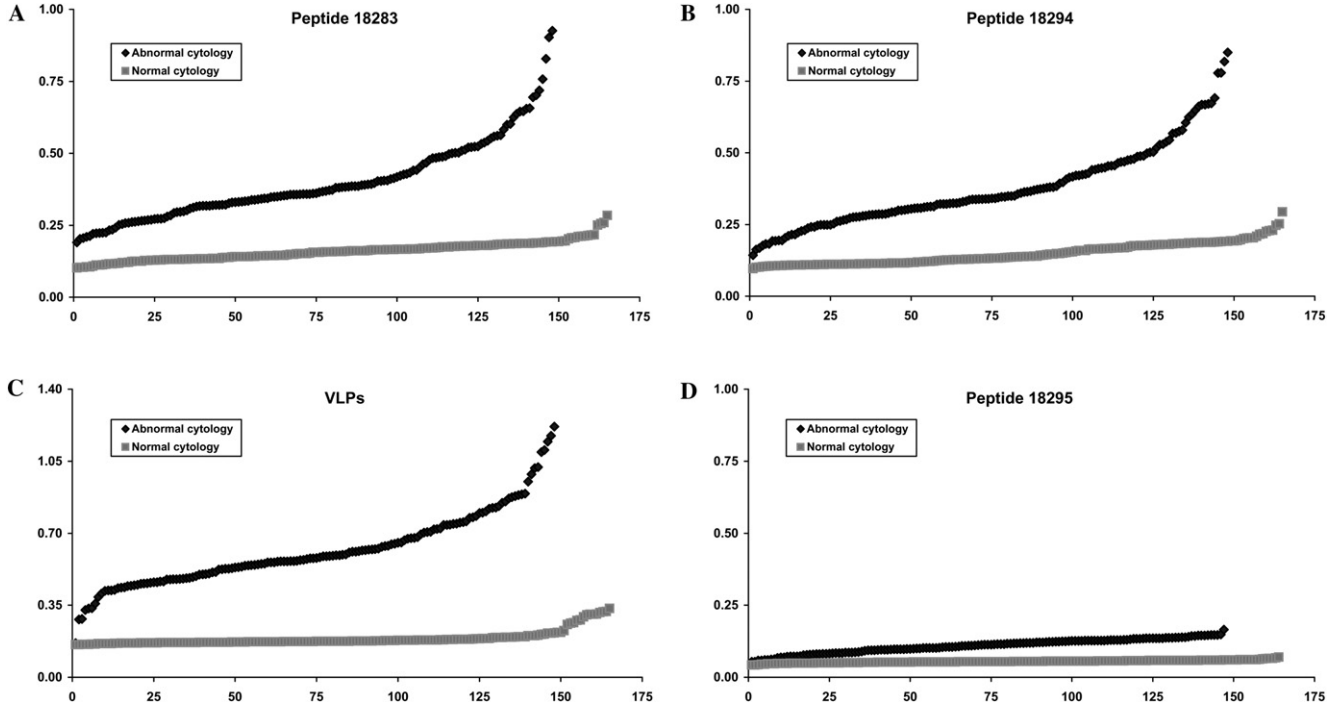

Fig. 2. ELISA results for peptides 18283 and 18294 against 313 patients' sera. Each patient's serum was tested in ELISA against peptides 18283 (A), and 18294 (B), using VLP-16 as positive antigen control (C) and peptide 18295 as negative antigen control (D). Black points and grey points represent the ODs obtained by using sera obtained from cervical lesion or cervical cancer patients and using normal cytology patient sera, respectively. Each serum was tested in triplicate and the mean value is represented in this figure; the standard deviation was always lower than 10% of the mean value.

2.4%, 3.6%, and 1.8% of the control sera was seropositive by using peptides 18283, 18294 or VLP-16 respectively. On the contrary, 96%, 91% or 99% of the HPV-associated cervical lesion patients' sera were seropositive by using peptides 18283, 18294 or VLP-16, respectively, the CIN III patients' sera presenting the lower seropositivity with the three antigens used here (18283, 18294 or VLP-16) (Table 1).

The sensitivity and the specificity were calculated by choosing different cut-off points, where the cut-off point was mean OD plus 2SD, sensitivity was 97–99% and 92–95% for 18283 and 18294, respectively, and specificity was 89–95% for both peptides when distinguishing between cervical lesion or cervical cancer patients and women with normal cytology (Table 2).

Comparing ELISA results and HPV DNA detected in these patients

Cervical samples from 104 out of the HPV-associated cervical lesion patients and 141 of the normal cytology women were tested for identifying HR-HPV DNA. HPV-16 DNA was detected in 23 patients; 19 belonging to patients diagnosed as having cervical lesions or cervical cancer and four belonging to women with normal cytology. The ELISA results showed that 19, 21, and 23 of the HPV-16 patients' sera were seropositive by using 18283, 18294, and VLP-16, respectively; two of the 18283 seropositive and one of the 18294 seropositive sera belonged to normal cytology women, suggesting that this last group of women are probably infected by HPV in spite of presenting normal cytology.

HPV-18 DNA was found in two patients, one from the patients diagnosed with invasive cervical cancer and seropositive for peptides 18283 and 18294; another belonging to the normal cytology women's group, being seronegative for peptides 18283 and 18294. HR-HPV-52 was found in one patient, belonging to normal cytology women and seronegative for peptides 18283, 18294, and VLP-16. HR-HPV DNA was found in 66 patients but they could not be specifically typed; these were named HPV-X. Forty-seven of these HPV-X patients were from the cervical lesion patient group, 46, 44, and 46 of them being seropositive for peptide 18283, 18294 and VLP-16, respectively. The other 19 women belonged to the normal cytology group; all of them being seronegative for peptides 18283 and 18294 or VLP-16. HR-HPV DNA was not detected in the 153 women with normal cytology (negative).

Structural localisation of peptides 18283 and 18294

Peptides 18283 and 18294 were determined by GETAREA software as being exposed on the HPV-16 L1 VLP surface and, according to the 3D structure reported by Chen et al. [25], they are forming part of the C β-strand and the B–C, F–G loops (Fig. 3B). The frontal view shows that they are neighbours in the L1 assembled pentamer (Figs. 3C and D).

Discussion

Persistent HR-HPV-infection is responsible for cervical intraepithelial lesions and cervical cancer; such persistent infection induces IgG antibodies, of interest in this work being the L1-capsid protein. There is evidence that the presence of these antibodies is an indicator of the risk of acquiring cervical lesions and cervical cancer. Although the majority of the epitopes recognised by these antibodies seemed to be conformational, several works using peptides have successfully allowed linear epitopes to be identified [8,9,24,34,35]. Even the nona-peptide (IHSMNSTIL) from L1-protein is capable of discriminating between HR-HPV and LR-HPV infected women diagnosed as having low grade squamous intra-epithelial lesions (LSIL) [25]. However, our peptide 18299 tested in this work, containing part of this nona-peptide sequence, did not react with the positive serum pool; it could be that the differences in sequences altered peptide-reactivity against serum antibody or that this peptide was split into peptides 18299 and 18300, and the resulting reactivity was conformational, more than sequential.

Peptides from L1-regions which were exposed or not to the solvent, according to the GETAREA algorithm and their X-ray structure, were tested in this work against a pool of CIN II/III patients' sera, using normal cytology patients' sera as control. Peptides 18283 (^{55}PN NNKILVPKVSGLQYRVFR^{74}), 18286 (^{115}VGISGH

Table 2
Serological test for cervical lesion determination

Cut off point	Sensitivity			Specificity		
	Peptide 18283	Peptide 18294	VLP-16	Peptide 18283	Peptide 18294	VLP-16
Mean + 2 SD	97.30	92.57	99.32	94.55	95.15	91.52
Percent 90	99.32	95.27	99.32	89.70	89.70	90.30
Percent 95	97.30	91.89	97.97	95.15	95.15	95.15

The sensitivity and the specificity of peptides 18283, 18294 or VLPs for discriminating between cervical lesion or cervical cancer and normal women were calculated, choosing different cut off points, including percentile 90% and 95% for determining the seropositive patients.

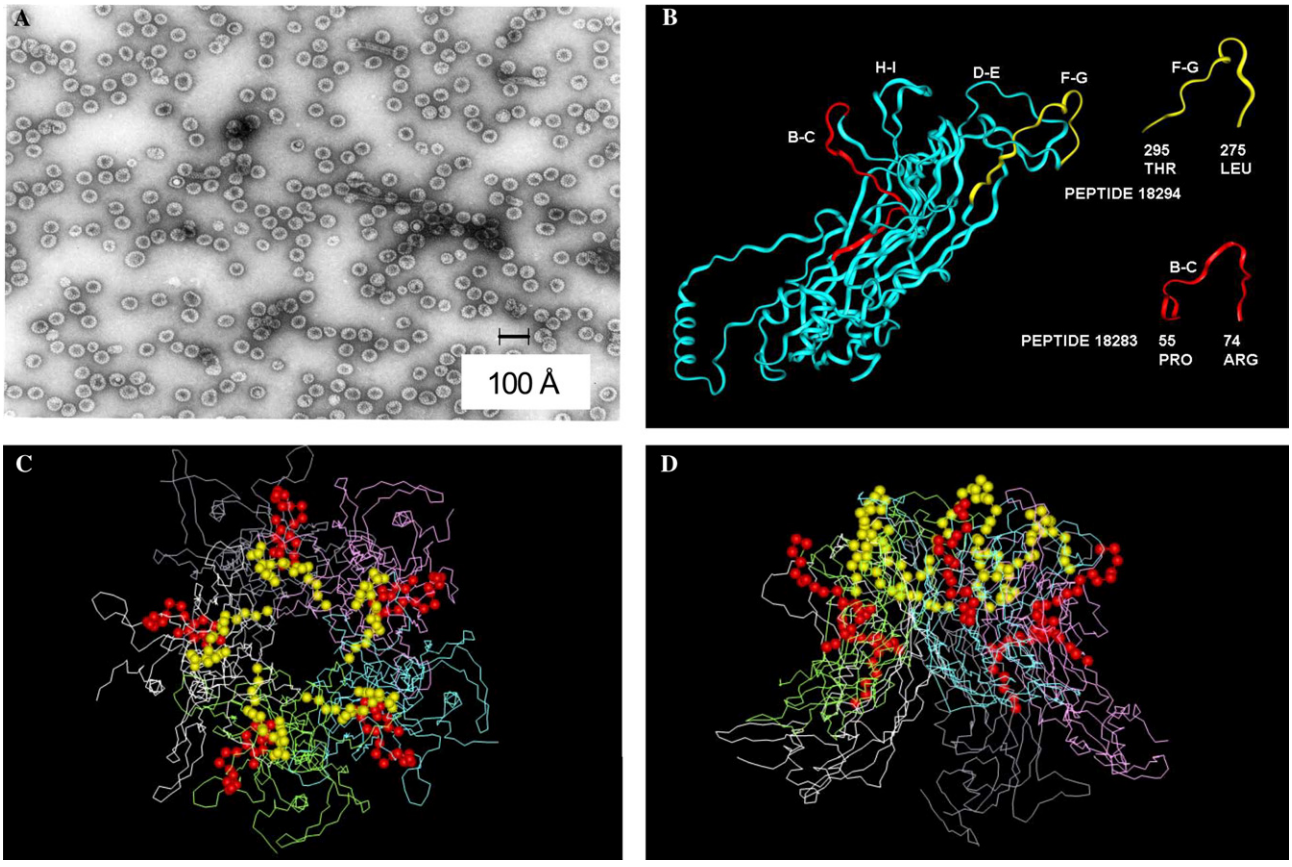

Fig. 3. L1-pentamer localisation of peptides 18283 and 18294. The pentamer structure based on previously reported pentamer model coordinates (Protein Data Bank Accession No. code 1l0t) was built using Insight II (2000) Biopolymer module software (Accelrys Software, USA) run on an Indigo 2 Station (Silicon Graphics). Peptide 18283 (in red) and 18294 (in yellow) sequences were located on this structure, showing that part of the peptide sequences were surface-exposed on the pentamer and accessible for interacting with antibodies. (A) The VLP electron microscopy at 39.000× magnification. (B) The localisation of peptides 18283 and 18294 on the L1 structure. The main loops are shown and named according to the L1 structure reported by Chen et al. [25]. (C) A top view of the interacting face of the pentamer and (D) a lateral view of the spike pentamer, clearly showing that both peptide sequences are solvent exposed.

PLLNKLDDTENASAY^{135}), and 18294 (^{275}LYIKGSG STANLASSNYFPT^{294}) were specifically recognised by antibodies present in a pool of CIN II/III patients' sera; these three peptides presented 35–50% of their surface exposed to the solvent in the L1-tridimensional structure (Figs. 3C and D). Taking into account that 18283 and 18294 present reactivity similar to VLP-16, they were chosen to be tested against the 313 humans' sera; using VLP-16 as positive antigen control and peptide 18295 (^{295}PSGSMVTSDAQIFNKPYWLQ^{314}) as negative antigen control (this peptide is not exposed in the L1-tridimensional structure). Parts of peptide 18283 and 18294 sequences were highly conserved even in LR-HPV, but other parts of these sequences were variable.

We have previously reported that peptide 18283 and 18294 sequences that bind with high affinity to HPV-infection susceptible cells (VERO and HeLa cells) are capable of blocking VLP-binding to these cells and are involved in HPV-attachment to host cells [36], contain epitopes recognised by antibodies from HPV-infected patient sera, and are the target for neutralising antibodies [37,38]. Most 18283 and 18294 peptide amino acids are surface-exposed not only in the L1-structure but also in the reported pentamer structure (Figs. 3C and D) [25,33]. The most prominent region of the spike pentamer is formed by peptide 18294 FG-loop and part of the 18283 sequence is exposed not only at the top of the spike (very close to peptide 18294) but also at the side of the pentamer spike. This indicates that part of the 18283 and 18294 sequences are accessible on the virus surface, very probably allowing antibodies to interact with these peptide regions (Figs. 3C and D). In fact, it has been reported that part of these peptide sequences or peptide analogue sequences are more frequently recognised by patients suffering from cervical lesions (CIN I to CIN III) and patients with cervical cancer [8,34,35]; part of these sequences are also recognised by neutralising antibodies and antibodies induced by immunisation with VLPs [37,38].

There were no differences in mean ODs and the number of positive responses regarding disease status suggested that most people exposed to HR-HPV

recognised the epitopes present in 18283 and 18294. The lower reactivity than with VLP-16 could have been due to there being more epitopes inducing antibodies in VLP-16 (perhaps peptide 18286), including conformational ones, or antibody affinity differences being higher for VLP-16 than for peptides 18283 and 18294.

18283 and 18294 reactivity with IgG from cervical lesion or cervical cancer patients' sera was higher than with IgG from control sera. There was 97–99% and 92–95% sensitivity for 18283 and 18294, respectively, by choosing a different cut-off point; specificity was 89–95% for both peptides when distinguishing between cervical lesion or cervical cancer patients and normal cytology women; results were very similar to VLP-16 (Table 3).

HR-HPV DNA could not be identified in 32 out of the 143 patients suffering from cervical lesions or cervical cancer (22%); 26 of the 165 control patients were detected as having HR-HPV DNA (16%). As a consequence of the HPV DNA detection method's sensitivity and specificity, some HR-HPV DNA negative control patients who could have recently acquired HPV-infection did not present visible epithelial lesions or simply the cervical lesions were not detected by cytology. On the other hand, more than 90% of the HR-HPV infected patients, suffering from CIN I, CIN II, CIN III or cervical cancer were seropositive for peptides 18283 and 18294. On the contrary, only 11% and 15% HR-HPV infected control patients were seropositive; this indicates that HR-HPV-infection induced antibody recognised peptide 18283 and 18284, and that the majority (around 85%) of the HR-HPV infected control women did not present detectable anti-18283 or 18294 antibodies. This could probably have been because the infection was very recently acquired or in part because these control patients were infected with HPV-types very different to HPV-16 (18283 and 18294 sequences belong to HPV type 16).

Peptides have been useful for discriminating between HR-HPV infected patients suffering from cervical lesions and LR-HPV infected patients or normal cytology patients; however, peptides 18283 and 18294 presented a higher sensitivity and specificity than the previously reported peptides, perhaps as a consequence of the sequences of both peptides being exposed on the top not only in the L1-structure but also in the pentamer structure and these two peptides are longer than most previously reported peptides (Figs. 3C and D), allowing higher and wider area for interacting with the antibodies.

Peptides 18283 and 18294 thus represent powerful tools for discriminating between patients suffering from cervical lesions and cervical carcinoma associated with HPV and women having normal cytology. All these data also suggest that detecting anti-peptide 18283 and 18294 antibodies in all women's sera was associated with the presence of past or present HR-HPV infection and the presence of HR-HPV-associated cervical lesions.

The sensitivity of these peptides was higher than that reported by cytology (50–70%) and it was comparable to DNA-detection techniques; moreover, the specificity was comparable to the cytology and DNA-detection techniques, allowing the use of this peptide-based ELISA in low resource settings to be considered for mass HPV-infection screening. The study was carried out on a large population of women, adding considerable statistical power to the test.

The fact that both synthetic peptides showed very similar reactivity by ELISA with purified VLP-16 offers a greater advantage for HPV-associated cervical carcinoma lesions than VLP-16, due to the inherent difficulties in producing large amounts of these purified virus-like particles, their biological instability, the need for a cold chain to preserve them properly, batch-to-batch variability, etc. These problems are not present in synthetic peptides.

Peptides 18283 and 18294 can be synthesised in kilogram amounts (as previously done with our SPf-66 synthetic malaria vaccine), their synthesis and production can be monitored step-by-step following good manufacturing procedures (GMP), their reproductibility from batch to batch can be assessed very accurately by methodologies such as HPLC, mass spectroscopy (MS),

Table 3
Comparing HPV DNA detection, and peptide 18283 and 18294 serological results

DNA-type detected (n)	No. of seropositive women					
	Cervical lesion patients			Normal cytology women		
	Peptide 18283	Peptide 18294	VLP-16	Peptide 18283	Peptide 18294	VLP-16
HPV-16 ($n = 23$)	17	20	19	2	1	4
HPV-18 ($n = 2$)	1	1	1	0	0	1
HPV-52 ($n = 1$)	0	0	0	0	0	0
HPV-X ($n = 66$)	46	44	46	0	0	0
Negative ($n = 153$)	31	28	36	0	1	4

A HPV DNA fragment was amplified by using GP5+/GP6+ primers as described in Materials and methods. The amplified fragment was hybridised with HPV-16, -18, -52, -54, -56, -59, -67, -81, and -90 DNA-fragments. HPV-X patients were those whose samples amplified with the generic primers but did not hybrdise with specific primers. Seropositive are those women showing reactivity with the corresponding peptides or VLPs as previously described.

circular dichroism (CD), amino acid sequencing, and even nuclear magnetic resonance (NMR). Freeze-dried synthetic peptides (or even in solution) are very stable over long periods of time (years and even decades) and therefore do not require a cold chain. Last, but not least, synthetic peptides are very cheap to produce.

Due to the simplicity of the ELISA test and its worldwide use, linked to the specificity and sensitivity of these two peptides in recognising precancerous and cervical cancer patients, these two peptides represent excellent tools for use in mass, rapid, cheap, reliable, large-scale screening of women populations at risk of developing cervical carcinoma.

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