Int J Clin Exp Pathol 2022;15(1):1-10
www.ijcep.com /ISSN:1936-2625/IJCEP0131439

Original Article

Analysis of Xinjiang HPV16 L1 gene polymorphisms: a newly developed, low-cost enzyme-linked immunosorbent assay

Zhe Wang[1], Yan Tang[1,4], Hongwei Dang[2], Xuxuan Zhang[1], Lijuan Pang[1], Pengyan Wang[3], Chuangfu Chen[3], Yan Ren[1]

[1]Department of Pathology and Key Laboratory of Xinjiang Endemic and Ethnic Diseases (Ministry of Education)/ Department of Pathology, The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi, China; [2]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Shihezi University, Shihezi 832000, Xinjiang, China; [3]College of Animal Science and Technology, Shihezi University, Shihezi 832000, Xinjiang, China; [4]Animal Science and Technology Branch, Xinjiang Agricultural Vocational Technical College, Changji 831100, Xinjiang, China

Received February 5, 2021; Accepted May 18, 2021; Epub January 15, 2022; Published January 30, 2022

Abstract: Background: Xinjiang, China shows the world's highest incidence and mortality rates of cervical cancer. Due to limited conditions available for medical examination, hybrid capture 2 (HC2) and other detection methods are used rarely, and early screening for human papillomavirus (HPV) cannot be carried out. Therefore, we established a double-antibody sandwich (DAS)-enzyme-linked immunosorbent assay (ELISA) based on a polymorphism of the Xinjiang HPV16 L1 strain (KU721788). Methods: According to the conserved sequence and specific epitope of Xinjiang strain HPV16 L1, we prepared two anti-HPV16 L1 monoclonal antibodies and combined them to construct a DAS-ELISA. Detection conditions for the DAS-ELISA were optimized, and HC2 was used as the control to verify the specificity, repeatability and coincidence detection of the DAS-ELISA. Results: The optimized conditions for the DAS-ELISA were: dilution of the capture antibody was 1:100; the enzyme-labelled antibody was 1:10; the sample reaction time was 45 min; the enzyme-labelled antibody was applied for 40 min, and the substrate color development time was 15 min. The quality of the DAS-ELISA for the detection of HPV 16 was very high, and there was no significant difference when compared with HC2. Conclusion: The DAS-ELISA developed on the basis of the Xinjiang strain (KU721788) polymorphism possesses the advantages of a detection rate similar to that for the HC2 assay currently used clinically, but it is more convenient operationally and at lower cost. DAS-ELISA is thus easier to implement for cervical cancer screening in economically depressed areas.

Keywords: Xinjiang HPV16 L1 gene polymorphisms, double antibody sandwich ELISA, hybrid capture 2, cervical cancer screening, monoclonal antibody.

Introduction

Each year, more than 85% of new worldwide cases of cervical cancer are concentrated in developing countries, and China accounts for approximately 12% [1]. Human papillomavirus (HPV)16 is the most dominant high-risk (HR)-HPV genotype, accounting for 31.39% of cervical intraepithelial neoplasia (CIN)1, 42.99% of CIN2, 75.18% of CIN3 and 71.17% of invasive cervical cancer (ICC) [2]. The HPV16 detection rate increases with the increase in the severity of the disease, and its dominant role in cervical lesions is beyond doubt. Epidemiologic studies have confirmed that HPV16 genomic polymorphisms can reflect the carcinogenic characteristics of the virus. Different HPV16 mutant strains show different carcinogenic potential and biologic activities, and show certain regional differences [3].

The HPV L1 protein can self-assemble into virus-like particles (VLPs) which induce high levels of neutralizing antibodies that are highly protective [4]. L1-VLPs are the components used to design specific prophylactic vaccines,

and they also constitute markers used in HPV detection. The expression levels of HR-HPV L1 protein in cervical tissues provide guidelines for the diagnosis of CC and CIN classification. Study of the HPV16 L1 epidemic strain in Xinjiang would be significant in disease detection and vaccine development, and although HPV16 L1 is significant overall, there are very few reports regarding the Xinjiang strain. We therefore investigated this strain and compared it with other vaccine strains (Reference sequencing: GenBank accession number DQ469930) [5].

Effective screening can detect HSIL in time to administer appropriate treatment, which is an important means to reduce the incidence and mortality of cervical cancer. Currently, cytological testing by thin-prep cytological test (TCT) and Hybrid Capture 2 (HC2) are the two primary methods employed for the preventative screening for cervical cancer. These detection methods are especially important in developing countries, but due to their complexity and the need for certain molecular biology detection techniques, their wide application is limited.

It has been reported that anti-peptide antibodies induced by the surface oligopeptide of L1 18283 (^{55}PNNNKILVPKVSGLQYRVFR^{74}) and 18294 (^{284}LYIKGSGSTANLASSNYFPT^{300}) are conserved among different high-risk HPV types, can be used for extensive screening [6], and are believed to be highly sensitive for identifying patients with cervical cancer and precancerous lesions. Based on the aforementioned study, we developed a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using the conserved sequence of HPV L1 and polymorphisms of Xinjiang strain HPV16 L1. Our objective was to explore the feasibility of DAS-ELISA in the detection of HPV in exfoliated cervical cells.

Materials and methods

Ethical issues

Ethical approval was obtained from the Shihezi University School of Medicine Research Ethics Committee, and written informed consent was obtained from each patient.

Specimen collection

Exfoliated cervical cells were collected randomly from the First Affiliated Hospital of the Medical College of Shihezi University during a single year. Each exfoliated cell sample was diagnosed using HC2 performed according to the Digene Hybrid Capture(R) 2 (HC2) High-Risk HPV DNA Test kit instructions.

PCR amplification of the Xinjiang strain HPV16 L1 nucleotide sequence and bioinformatic analysis

Exfoliated cervical cells were collected from 198 patients aged 14 to 74 years who were positive for HR-HPV by HC2 testing, and placed in cervical preservation fluid. DNA from each sample was extracted for HPV L1 sequencing. Primers were synthesised, and PCR systems were used as previously described [7, 8].

PCR amplification products were visualized on 1.2% agarose gels, and fragments were retrieved, linked to the pMD19-T vector and transformed into *Escherichia coli* DH5a. A positive clone was selected for sequencing, and the sequence was BLAST-searched against the NCBI nucleotide database for sequence-alignment analysis. After splicing and proofreading gene sequences with DNAMAN (Ver. 7.0) software, the sorted sequences were submitted to the GenBank nucleic acid database using Sequin software.

Following DNAMAN software analyses of nucleotide and amino acid sequences of Xinjiang strain HPV16 L1, the B-cell epitope was predicted using ABCpred software (http://crdd.osdd.net/raghava/abcpred/ABC submission.html) [9] and the secondary structure was predicted using Predicprotein (https://www.predictprotein.org/home) [10]. The vaccine strain was used as a reference strain.

Establishment of the DAS-ELISA

According to the gene polymorphisms of Xinjiang HPV16 L1, the Yao Qiang Biological Technology Company (Shanghai, China) screened two monoclonal antibodies (MAbs) with excellent specificity and affinity using a conserved recombinant HPV16 L1 polypeptide and a B-cell-specific epitope polymorphism peptide (GenBank accession number KU721788). The company provided a capture antibody (anti-HPV16 L1-IgG1), an enzyme-labelled antibody fused to horseradish peroxidase (HRP; anti-HPV16 L1-IgG2b) and a recombinant HPV16 L1 protein (100 μg/mL).

For assays, 96-well microplates were coated with capture antibody (100 μL/well) in carbonate coating buffer (pH 9.6) for 1-3 h at 37°C. Plates were washed three times with PBST (1×PBS, 0.05% Tween-80) for 5 min, and 100 μL of skim milk (5%)/well was added and incubated for 3 h at 37°C in a humidified incubator. Plates were washed as described above, and 100 μL/well of sample (antigen) was added and incubated in a humidified incubator at 37°C for 1 h. Additionally, blank controls, negative controls (BSA), and positive controls (recombinant HPV16 L1 protein) were included. Plates were washed and HRP-labelled antibody (100 μL/well) was added and incubated for 1 h at 37°C in a humidified incubator. Plates were washed, 100 μL of TMB/well was added, and plates were incubated for 10-30 min at 37°C in the dark. The HRP reaction was halted by the addition of stop buffer (50 μL/well) and absorbance values were immediately measured at 450 nm.

Antibody concentration for optimal capture

Capture antibody was diluted 1:10, 1:50 and 1:100, and used to coat the ELISA plates. Standard samples at different dilutions (1:10, 1:50 and 1:100) were then added, and all of the other steps were carried out as described above.

Determination of the optimal enzyme-labelled antibody concentration

The optimal capture-antibody concentration was determined by coating plates using a serial dilution. Enzyme-labelled antibody was diluted 1:10, 1:50, and 1:100; all of the subsequent steps were performed as described above.

Optimal antigen (sample) reaction time

Using the optimal-capture antibody concentration, the standard (at a 1:10 dilution) was allowed to react for different times. All of the subsequent steps were performed as described above.

Determination of optimal reaction time for the enzyme-labelled antibody

After establishing the concentration and sample reaction time, the standard (1:10 dilution) was reacted for different times. All subsequent steps were performed as described above.

Optimal color development time

After determining the appropriate concentration, the sample reaction time and the enzyme-labelled antibody reaction time, color development was allowed to proceed for different time periods. All subsequent steps were performed as described above.

Determination of the cut-off value for the DAS-ELISA

We collected 20 HPV16-negative samples of exfoliated cervical cells that were determined to be HR-HPV-negative by HC2, and HPV16 L1-negative by PCR. Based on a literature report, the primers and reaction conditions for the HPV16 L1 PCR were determined and tested using the optimised DAS-ELISA [11]. The mean (X) and standard deviation (SD) of 20 samples were calculated to determine the cut-off value (X+/-2 SD) and the 450 nm OD value, where values ≥ X+/-2 SD were considered positive, and values < X+/-2 SD were considered negative.

Quality evaluation of DAS-ELISA detection of HPV 16

Specificity: A total of 50 samples of exfoliated cervical cells that were HR-HPV as determined by DAS-ELISA were tested for specificity, including 10 HPV16-positive, 10 HPV18-positive and 10 HPV45-positive samples; 10 samples of HR-HPV-positive strains other than 16, 18 and 45, and 10 samples of HR-HPV-negative cells. The detection process was as described above.

Repeatability: We selected 18 wells from three ELISA plates (different batches), with six wells per plate. These wells were assayed together to detect an identical HPV16 sample. By calculating the coefficient of variation (CV%), we analysed the inter-batch repeatability of the DAS-ELISA.

Coincidence detection: Exfoliated cervical cells were collected from 257 suspected cases of HPV infection at all ages for 1 year. Each sample was divided into two parts for the assay by HC2 and DAS-ELISA, respectively. Consistency between DAS-ELISA and HC2 was evaluated using McNemar's and Kappa-consistency tests [12]. The McNemar test analysis showed that the test level was 0.05. When $P < 0.05$, the difference between the two was statistically sig-

Table 1. HPV16 L1 nucleotide and amino acid differentiation sites

	Gene ID of HPV16 L1		Differential sites by N→C terminus
Nucleotide	KU721788 (1596 bp)	DQ469930 (1518 bp)	1-78 (ATGCAGGTGACTTTTATTTACATCCTAG TTATTACATGTTACGAAAACGACGTAAACGTTTACCATATTT TTTTTCAG-*) 402 (T-C); 465 (T-A); 945 (G-A); 1500 (A-G)
Amino acid	AMN09986 (531aa)	ABF06542 (505aa)	1-26 (MQVTFIYILVITCYENDVNVYHIFFQ-*)

Table 2. B-cell epitopes of KU721788 are more specific than DQ469930

Rank	Sequence	Start position	Score
8	YILVITCYENDVNVYH	7	0.86
11	QMSLWLPSEATVYLPP	26	0.82
32	CYENDVNVYHIFFQMS	13	0.57

nificant; P > 0.05 was considered no statistical difference. Kappa was used for the consistency test and analysis. The consistency was considered very poor (0-0.20), weak (0.21-0.40), moderate (0.41-0.60), highly consistent (0.61-0.80), or extremely consistent (0.81-1.00) [12].

Results

PCR amplification of the Xinjiang strain HPV16 L1 nucleotide sequence

We submitted an HPV16 L1 fragment to the GenBank nucleic acid database and obtained GenBank accession number KU721788 and protein id AMN09986. A comparison of the Xinjiang strain KU721788 (protein id AMN-09986) with the vaccine strain DQ469930 (protein Id ABF06542) using DNAMAN provided an identity match of 94.86% for the nucleic acid sequence and 95.10% for the amino acid sequence (the differential sites are listed in **Table 1**). It is worth noting that the N-terminus of the Xinjiang strain includes an additional 26 amino acids that are absent in the vaccine strain, and that 505 amino acids coincide completely with the vaccine strain.

B-cell epitope prediction

Using ABCpred for B-cell epitope prediction, we selected a window length of 16 to utilize for prediction, with a threshold [0.1 to 1] of 0.51. The Xinjiang strain HPV16 L1 had 56 B cell epitopes for a mean score of 0.774 (scoring was between 0 and 1), with a minimum of 0.530 and a maximum of 0.950. The HPV16 L1 vaccine strain has 53 B-cell epitopes with an average score of 0.776, a minimum of 0.530 and a maximum of 0.950. The Xinjiang strain had three more epitopes than the vaccine strain (shown in **Table 2**), and the other epitopes (not shown) showed an identical sequence, although the scores were slightly different.

Secondary structure prediction

We used Predicprotein software to predict secondary structure and functional features. KU721786 had 11 protein-binding regions and 5 polynucelotide-binding regions, while DQ-469930 had only 14 protein-binding regions (shown in **Figure 1** as first line-binding sites). Through solvent accessibility analysis, we found that the distribution of amino acid residues was different from that of the exposed or buried protein, which may affect the hydrophobicity and antigenicity of the protein (shown in **Figure 1** as third line-solvent accessibility). In addition, the disordered regions were slightly different (shown in **Figure 1** as the fourth line-disordered region). Disordered regions are involved in many biologic processes, such as regulation, signal conduction, and cell cycle control, and they are related to protein or DNA molecular recognition and phosphorylation.

KU721786 and DQ469930 exhibited many loop structures (63.09% and 64.36%, respectively), suggesting the existence of multiple epitopes (shown in **Table 3**). The proportion of helices, strands and loops did not differ significantly, but the **Figure 1** second line-structure shows numerous slight changes in their positions, especially at the N-terminus.

Standardization of DAS-ELISA

We performed several tests to determine the optimal reaction conditions for the DAS-ELISA, and we determined the optimal conditions for the experiment by calculating the positive/negative ratio (P/N).

The following were our optimized conditions. The optimal dilution of the capture antibody was 1:100 (concentration 16.9 μg/mL, **Figure**

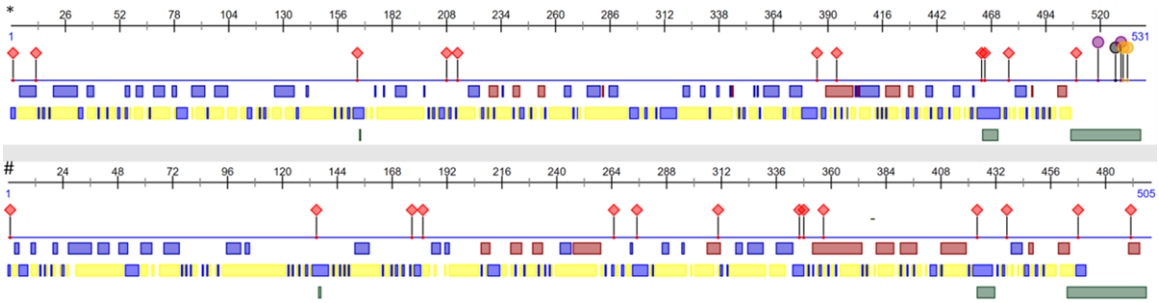

Figure 1. Secondary structures and functional features as predicted by Predicprotein. Note: *AMN09986 (531aa, coded by KU721788). #ABF06542 (505aa, coded by DQ469930). First line-binding sites: protein binding region; polynucelotide-binding region; second line-secondary structure: strand; helix; third line-solvent accessibility: exposed; buried; fourth line-disordered region: disordered.

Table 3. Secondary structure and binding sites by Predicprotein prediction

	Binding sites	Secondary structure strand		
		Helix	Strand	Loop
KU721786	11 Protein-binding regions 5 Polynucelotide-binding regions	18.08%	18.83%	63.09%
DQ469930	14 Protein-binding regions	18.02%	17.62%	64.36%

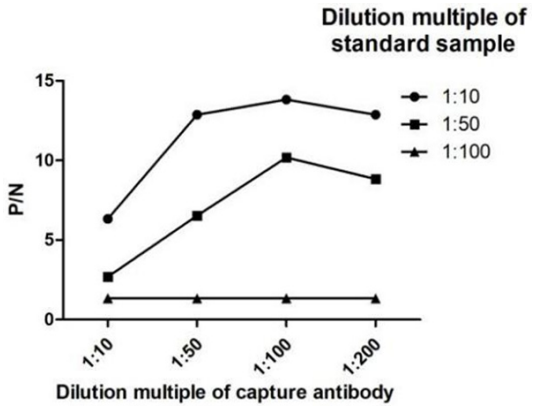

Figure 2. Optimal capture antibody concentration.

2), the optimal dilution of the enzyme-labelled antibody was 1:10 (**Figure 3**), the sample reaction time was 45 min (**Figure 4**), the enzyme-labelled antibody was applied for 40 min (**Figure 5**) and the substrate color-development time was 15 min (**Figure 6**). The specificity test results of our double-antibody sandwich ELISA showed that only HPV16 was positive (OD450 nm value ≥0.147), indicating that the specificity of the method was good (**Figure 7**).

The repeatability test showed that the intra-batch CV%s (2.60%, 2.40% and 5.03%) and inter-batch CV%s (3.00%, 2.99%, 2.56%, 3.40%, 1.90% and 3.00%) were < 5% (except for 5.03% at > 5%), indicating that the intra-batch/inter-batch reproducibility was acceptable (shown in **Table 4**).

Coincidence detection showed that for the same batch of 257 patient samples, 47 were HC2 positive, 210 were negative, 50 were DAS-ELISA positive, and 207 were negative. Of the 257 total samples, both methods showed positivity in 42 samples and 202 were negative. Of the 13 samples in dispute, 5 samples were positive by the HC2 method and negative by the DAS-ELISA and 8 samples were negative by HC2 and positive by DAS-ELISA. According to McNemar's test ($P = 0.0001 < 0.05$), the difference in diagnostic results was significant, while the kappa test [kappa = 0.835 > 0.75] indicated highly consistent diagnostic results (shown in **Table 5**).

Discussion

A general rule for macromolecules is that sequence determines structure, and structure determines function [13]. At the amino-acid level, the Xinjiang strain HPV16 L1 had only 26 more amino acids at the N-terminal than vaccine strain HPV16 L1, while the other 505 amino acids were identical. The two strains exhibited very high sequence identity, with nucleotides at 94.86% and amino acids at 95.10%, and the loop percentages in the secondary structure were 63.09% (KU721788) and 64.36% (DQ469930), respectively. Judging from these data alone, there should be little difference between the two proteins; however,

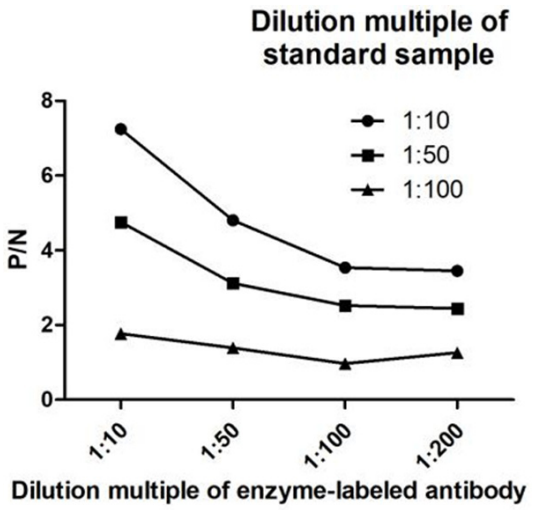

Figure 3. Optimal enzyme-labelled antibody concentration.

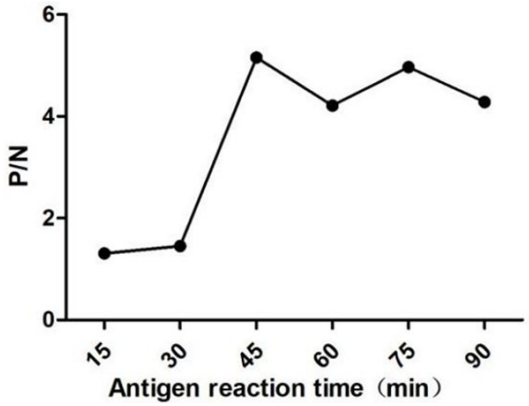

Figure 4. Optimal sample reaction time.

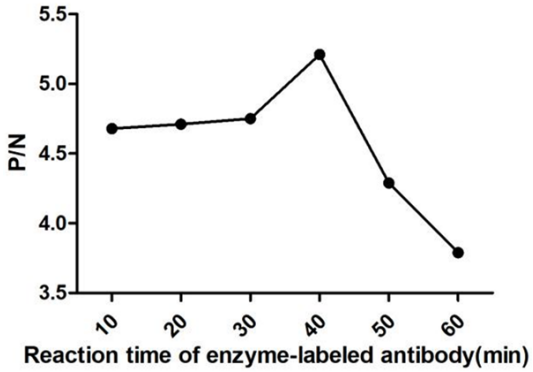

Figure 5. Optimal reaction time of enzyme-labelled antibody.

when we assessed the position of the secondary structure and the composition of antigen epitopes, we uncovered many small differenc-

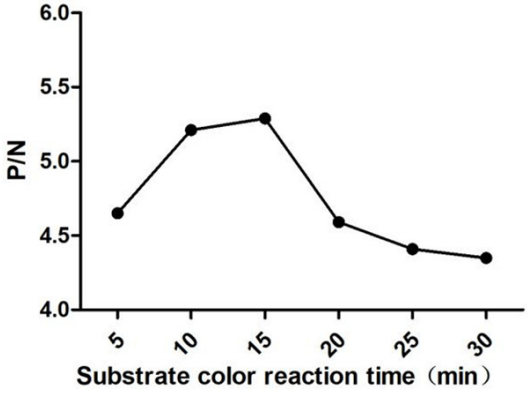

Figure 6. Optimal color developing time.

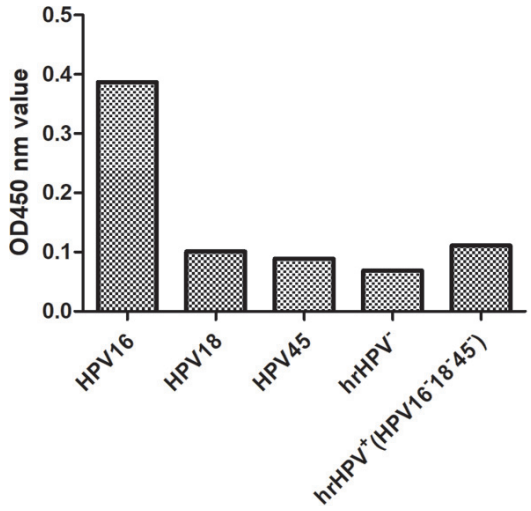

Figure 7. Specificity analysis of the DAS-ELISA.

es between them. These differences are expected to produce cumulative effects and affect the higher structure of the proteins, thus affecting some of their functions-including protein binding sites, hydrophilicity and the number, specificity, and affinity of antigen epitopes.

Antibodies bind to antigens at sites known as antigenic determinant regions, which are also called B-cell epitopes. B-cell epitopes are essential to several biomedical applications such as rational vaccine design, disease diagnostics, and immune-therapeutics [14].

From these results, we can infer that Xinjiang strain HPV16 L1 retains good immunogenicity and reactivity compared with the vaccine strain HPV16 L1, and this information may be used to develop vaccines or diagnose disease. Of course, there are also some notable differenc-

Table 4. Intra-batch assay/inter-batch assay coefficients of variation

		First batch	Second batch	Third batch	Inter-batch assay		
					SD	Means	C/V (%)
OD450 nm value		0.329	0.336	0.349	0.01015	0.338	3.00
		0.345	0.325	0.336	0.01002	0.335	2.99
		0.329	0.335	0.346	0.00862	0.337	2.56
		0.345	0.334	0.322	0.01150	0.334	3.40
		0.335	0.328	0.341	0.00651	0.335	1.90
		0.324	0.315	0.305	0.00950	0.315	3.00
Intra-batch assay	SD	0.00885	0.00804	0.1675			
	Means	0.335	0.329	0.333			
	C/V (%)	2.60	2.40	5.03			

Table 5. Coincidence rate in clinical samples between the DAS-ELISA and HC2

		HC2	
		+	-
DAS-ELISA	+	42	8
	-	5	202
X^2		179.379	
P		0.0001	
kappa		0.835	

es between the strains. We observed differences in the secondary structure of the protein and B-cell antigen epitopes through bioinformatic analysis, and these may affect the specificity and effectiveness of a vaccine or alter disease diagnosis [15]. These differences also provide a theoretical basis for the development of HPV 16 vaccines and disease diagnoses suitable for the province of Xinjiang.

The purpose of HPV testing is to detect patients with cervical precancerous lesions and not viral carriers; therefore, the sensitivity of the detection method should match the clinical sensitivity. There is a specific relationship between viral load and clinical pathological changes. If the clinical sensitivity of the HC2 is 5000 copies/mL, then the PCR is too sensitive, and an analytical sensitivity of 10 copies/mL cannot truly reflect clinical pathological changes [16]. ELISA is widely used in disease detection because of its high sensitivity, convenient operation, rapidity, economy of use and other advantages. Since ELISA comprises an antigen-antibody binding reaction, there is no PCR amplification of a small sample in vitro, and the sensitivity of detection will be lower than that of PCR. However, whether ELISA test sensitivity can reach clinical sensitivity is unknown. We developed DAS-ELISA by using conserved HR-HPV L1 sequences [6] and three unique B-cell epitopes in Xinjiang strain KU721788 L1 (^{7}YILVITCYENDVNVYH^{22}, ^{26}QMSLWLPSEATVYLPP^{41} and ^{13}CYENDVNVYHIFFQMS^{28}). We observed no statistical difference between our ELISA and the approved HC2 assay ($P = 0.0001 < 0.05$), and the former was highly consistent in diagnostic results (kappa = 0.835 > 0.75), indicating that the test sensitivity of the DAS-ELISA reached clinical sensitivity.

Cytologic screening and HPV testing are the two primary preventive approaches to cervical cancer. During the early stages, cytologic screening plays a very important role in the clinic; however, it is suitable only in patients with detectable lesions, and is ineffective for those with HPV infection who have no detectable lesions [17]. The US FDA has approved only four HPV tests related to cervical cancer currently: Hybrid Capture 2 based on hybridisation capture, Cervista HPV based on enzyme-digestion signal amplification, Cobas HPV based on real-time PCR, and Aptima HPV based upon transcription-mediated isothermal amplification [18]. The first three HPV tests all detect HPV DNA and belong to the first generation of high-risk HPV tests. Although they are clinically sensitive and achieve detection of high-grade precancerous lesions (CIN2+), their specificity is low, and the majority of positive results are false-positives due to transient infection (i.e., with no high-grade histologic lesions). This is mainly because HPV DNA is a structural gene that can be detected when an infection occurs. These false-positive results cause patients to

suffer unnecessary psychological pressure, which can lead to unnecessary and traumatic follow-up tests (such as colposcopy and tissue biopsy) that may harm patients, waste limited medical resources, cause social problems among family members, and may damage doctor-patient relationships [19]. Aptima HPV is a second-generation detection method based on E6/E7 mRNA [20]; its clinical sensitivity, negative predictive value and safe screening interval are consistent with first-generation HPV DNA tests, and hence Aptima HPV can also be used in first-line population screening [21, 22]. In terms of clinical specificity and positive predictive value-and compared with the first generation of HPV DNA tests-false positives can be reduced by ~40% in first-line screening, thus reducing subsequent and unnecessary shunts and referrals for colposcopy and histologic testing.

The DAS-ELISA we established was consistent with the HC2 assay in terms of detection sensitivity and specificity, but the object of detection was different. HC2 detects HPV DNA, while DAS-ELISA detects HPV L1 protein, and HPV L1 protein is of more clinical significance.

Recent studies have shown that HPV L1 DNA and HPV L1 protein expression levels can reflect the replicative status of cells, and that the expression levels of the HPV L1 protein indicate the progression and regression of cervical lesions [23]. The presence of the HPV L1 protein indicates that HPV DNA is in a free state consistent with a transient HPV infection phase, and that there exists a good correlation with abnormal cellular regression. Detection of the L1 capsid protein allows effective assessment of the progression of cervical cancer and precancerous lesions, and some data indicate that its positive expression rate in HSIL and cervical cancer patients is significantly lower than that in LSIL and healthy individuals [24]. As the grade of cervical lesions increases, the positive expression rate of HPV L1 capsid protein exhibits a downward trend; and for these reasons, we selected the L1 protein as a target for screening HPV infection.

Although there are vaccines available against cervical cancer in China, these are too expensive for ordinary families. Therefore, HPV screening is still an effective measure for preventing cervical cancer, at least for a period of time, and it is therefore important to develop convenient and inexpensive assays that are highly sensitive and accurate and suitable for remote and less economically developed areas.

ELISA is widely used in disease detection due to its high sensitivity, convenient operation, rapidity, and cost-effectiveness [25]. Since ELISA is based on an antigen-antibody binding reaction, the primary challenge is ensuring the specificity and accuracy of the antigen and antibody. The DAS-ELISA not only ensures specificity and accuracy, but it is also suitable for screening HPV infection in low-grade cervical lesions since it is based upon the HPV L1 protein. This is especially important for underdeveloped areas such as Xinjiang, as it achieves early screening, early treatment, reduces treatment costs, and improves the cure rate.

Conclusions

Based on the recombinant HPV L1 sequence, we herein prepared a monoclonal antibody that contains species-specific conserved peptides, and using HPV16 Xinjiang epidemic strain (KU721788) epitope polymorphism peptides, established a DAS-ELISA. Compared with HC-2, the DAS-ELISA possesses the advantages of simple specimen processing, less antigen loss, low reagent cost, and simple operation. The assay is therefore especially suitable for cervical cancer screening in developing countries.

Acknowledgements

This work was supported by grands from the National Natural Science Foundation of China (Grant No: 81760386, 81560053). The Youth Science and Technology Innovation Leading Talents Project of Corps (2017CB004). The International Science and Technology Cooperation Promotion Plan of Shihezi University (GJH201805). The Project Sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2016LX011).

Ethical approval was obtained from the Shihezi University School of Medicine Research Ethics Committee. Written informed consent was obtained from each patient.

Disclosure of conflict of interest

None.

Address correspondence to: Chuangfu Chen, College of Animal Science and Technology, Shihezi University, Shihezi 832000, Xinjiang, China. E-mail: chuangfu_chen@163.com; Yan Ren, Department of Pathology, The First Affiliated Hospital, Shihezi University School of Medicine/Department of Pathology and Key Laboratory for Xinjiang, Endemic and Ethnic Diseases (Ministry of Education), Xinjiang 832000, China. Tel: +86-18609935597; E-mail: rycb1225@163.com

References

[1] Bao HL, Liu YN, Wang LJ, Fang LW, Cong S, Zhou MG and Wang LH. Analysis on mortality of cervical cancer and its temporal trend in women in China, 2006-2012. Zhonghua Liu Xing Bing Xue Za Zhi 2017; 38: 58-64.

[2] Yan X, Huang Y, Zhang M, Hu X, Li K and Jing M. Prevalence of human papillomavirus infection and type distribution among Uyghur females in Xinjiang, Northwest China. Oncol Lett 2020; 20: 25.

[3] Milliet F, Bozec A, Schiappa R, Viotti J, Modesto A, Dassonville O, Poissonnet G, Guelfucci B, Bizeau A, Vergez S, Dupret-Bories A, Garrel R, Fakhry N, Santini L, Lallemant B, Chambon G, Sudaka A, Peyrade F, Saada-Bouzid E, Benezery K, Jourdan-Soulier F, Chapel F, Sophie Ramay A, Roger P, Galissier T, Coste V, Ben Lakdar A, Guerlain J, Temam S, Mirghani H, Gorphe P, Chamorey E and Culié D. Synchronous primary neoplasia in patients with oropharyngeal cancer: impact of tumor HPV status. A GETTEC multicentric study. Oral Oncol 2021; 112: 105041.

[4] Mohsen MO, Zha L, Cabral-Miranda G and Bachmann MF. Major findings and recent advances in virus-like particle (VLP)-based vaccines. Semin Immunol 2017; 34: 123-132.

[5] Frazer IH and Cox J. Finding a vaccine for human papillomavirus. Lancet 2006; 367: 2058-2059.

[6] Urquiza M, Guevara T, Espejo F, Bravo MM, Rivera Z and Patarroyo ME. Two L1-peptides are excellent tools for serological detection of HPV-associated cervical carcinoma lesions. Biochem Biophys Res Commun 2005; 332: 224-232.

[7] Lee SH, Vigliotti VS, Vigliotti JS and Pappu S. Validation of human papillomavirus genotyping by signature DNA sequence analysis. BMC Clin Pathol 2009; 9: 3.

[8] Nahar F, Hossain MA, Paul SK, Ahmed MU, Khatun S, Bhuiyan GR, Nasreen SA, Haque N, Ahmed S, Kobayashi N, Akter SN and Begum H. Molecular diagnosis of human papilloma virus by PCR. Mymensingh Med J 2019; 28: 175-181.

[9] Saha S and Raghava GP. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins 2006; 65: 40-48.

[10] Rost B, Yachdav G and Liu J. The PredictProtein server. Nucleic Acids Res 2004; 32: W321-326.

[11] Yue Y, Yang H, Wu K, Yang L, Chen J, Huang X, Pan Y, Ruan Y, Zhao Y, Shi X, Sun Q and Li Q. Genetic variability in L1 and L2 genes of HPV-16 and HPV-58 in Southwest China. PLoS One 2013; 8: e55204.

[12] Li S, Fu Z, Zhang H, Hu Y, Wang R, Chen J, Mao D, Liu Z, Wang J and Yang L. Consistency evaluation of human serum 25-hydroxyvitamin D using liquid chromatography-tandem mass spectrometry and enzyme-linked immunosorbent assay.Wei Sheng Yan Jiu 2020; 49: 447-457.

[13] Kringelum JV, Nielsen M, Padkjær SB and Lund O. Structural analysis of B-cell epitopes in antibody: protein complexes. Mol Immunol 2013; 53: 24-34.

[14] Kozlova E, Viart B, de Avila R, Felicori L and Chavez-Olortegui C. Classification epitopes in groups based on their protein family. BMC Bioinformatics 2015; 16 Suppl 19: S7.

[15] Ren J, Song J, Ellis J and Li J. Staged heterogeneity learning to identify conformational B-cell epitopes from antigen sequences. BMC Genomics 2017; 18: 113.

[16] Nindl I, Greinke C, Zahm DM, Stockfleth E, Hoyer H and Schneider A. Human papillomavirus distribution in cervical tissues of different morphology as determined by hybrid capture assay and PCR. Int J Gynecol Pathol 1997; 16: 197-204.

[17] Bhatla N and Singhal S. Primary HPV screening for cervical cancer. Best Pract Res Clin Obstet Gynaecol 2020; 65: 98-108.

[18] Arbyn M, Snijders PJ, Meijer CJ, Berkhof J, Cuschieri K, Kocjan BJ and Poljak M. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect 2015; 21: 817-826.

[19] Schiffman M and de Sanjose S. False positive cervical HPV screening test results. Papillomavirus Res 2019; 7: 184-187.

[20] Chorny JA, Frye TC, Fisher BL and Remmers CL. Human papillomavirus detection with genotyping by the cobas and Aptima assays: significant differences in HPV 16 detection? Diagn Cytopathol 2018; 46: 568-571.

[21] Akagi K, Li J, Broutian TR, Padilla-Nash H, Xiao W, Jiang B, Rocco JW, Teknos TN, Kumar B, Wangsa D, He D, Ried T, Symer DE and Gillison ML. Genome-wide analysis of HPV integration in human cancers reveals recurrent, focal ge-

nomic instability. Genome Res 2014; 24: 185-199.
[22] Ebisch RM, Siebers AG, Bosgraaf RP, Massuger LF, Bekkers RL and Melchers WJ. Triage of high-risk HPV positive women in cervical cancer screening. Expert Rev Anticancer Ther 2016; 16: 1073-1085.
[23] Bosch FX, Broker TR, Forman D, Moscicki AB, Gillison ML, Doorbar J, Stern PL, Stanley M, Arbyn M, Poljak M, Cuzick J, Castle PE, Schiller JT, Markowitz LE, Fisher WA, Canfell K, Denny LA, Franco EL, Steben M, Kane MA, Schiffman M, Meijer CJ, Sankaranarayanan R, Castellsagué X, Kim JJ, Brotons M, Alemany L, Albero G, Diaz M and de Sanjosé S; Authors of the ICO Monograph 'Comprehensive Control of HPV Infections and Related Diseases' Vaccine Volume 30, Supplement 5, 2012. Comprehensive control of human papillomavirus infections and related diseases. Vaccine 2013; 31 Suppl 6: G1-31.
[24] Hu H, Zhao J, Yu W, Zhao J, Wang Z, Jin L, Yu Y, Han L, Wang L, Zhu H and Li F. Human papillomavirus DNA, HPV L1 capsid protein and p16INK4a protein as markers to predict cervical lesion progression. Arch Gynecol Obstet 2019; 299: 141-149.
[25] Wang Y and Liang Y. Clinical significance of serum calprotectin level for the disease activity in active rheumatoid arthritis with normal C-reactive protein. Int J Clin Exp Pathol 2019; 12: 1009-1014.