Electronic Supplementary Material (ESI) for Analytical Methods.

Screening and detection of multivalent human papillomavirus antibodies using a high-throughput liquid chip fluoroimmunoassay system

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Table S1. Correspondence between magnetic beads and antigens

Magnetic beads	Capture antigens
#26	HPV 6
#35	HPV 11
#44	HPV 16
#52	HPV 18

Table S2. Correspondence between different value and monoclonal antibody

	Monoclonal antibody ID		Monoclonal antibody ID
Anti-HPV-6	39G2	Anti-HPV-16	2A1
	39G7		4G12
	42G5		5A6
	43C7		6C7
	44B11		7B9
Anti-HPV-11	34C9	Anti-HPV-18	1B1
	34D10		3A2
	34E5		3A4
	35C1		4H5
	58F8		7H8

Table S3. Detailed concentrations for the 4-PL fit

Concentration	Concentration/ pg/mL			
	Anti-HPV-6	Anti-HPV-11	Anti-HPV-16	Anti-HPV-18
256	5.00×10^{3}	5.00×10^{4}	5.00×10^{2}	2.50×10^{4}
128	2.50×10^{3}	2.50×10^{4}	2.50×10^{2}	1.25×10^{4}
64	1.25×10^{3}	1.25×10^{4}	1.25×10^{2}	6.25×10^{3}
32	6.25×10^{2}	6.25×10^{3}	62.5	3.13×10^{3}
16	3.13×10^{2}	3.13×10^{3}	31.3	1.56×10^{3}
8	1.56×10^{2}	1.56×10^{3}	15.6	7.81×10^{2}
4	78.1	7.81×10^{2}	7.81	3.91×10^{2}
2	39.1	3.91×10^{2}	3.91	1.95×10^{2}
1	19.5	1.95×10^{2}	1.95	97.7

Table S4. The RSD value for the repeatability experiment

Dilution / RSD / Antiserum antibody	1:8000	1:4000	1:2000	1:1000
Anti-HPV-6	4.4%	2.0%	5.7%	8.5%
Anti-HPV-11	6.6%	3.4%	5.1%	6.6%
Anti-HPV-16	10.6%	10.9%	9.1%	8.7%
Anti-HPV-18	6.3%	2.6%	3.5%	6.8%

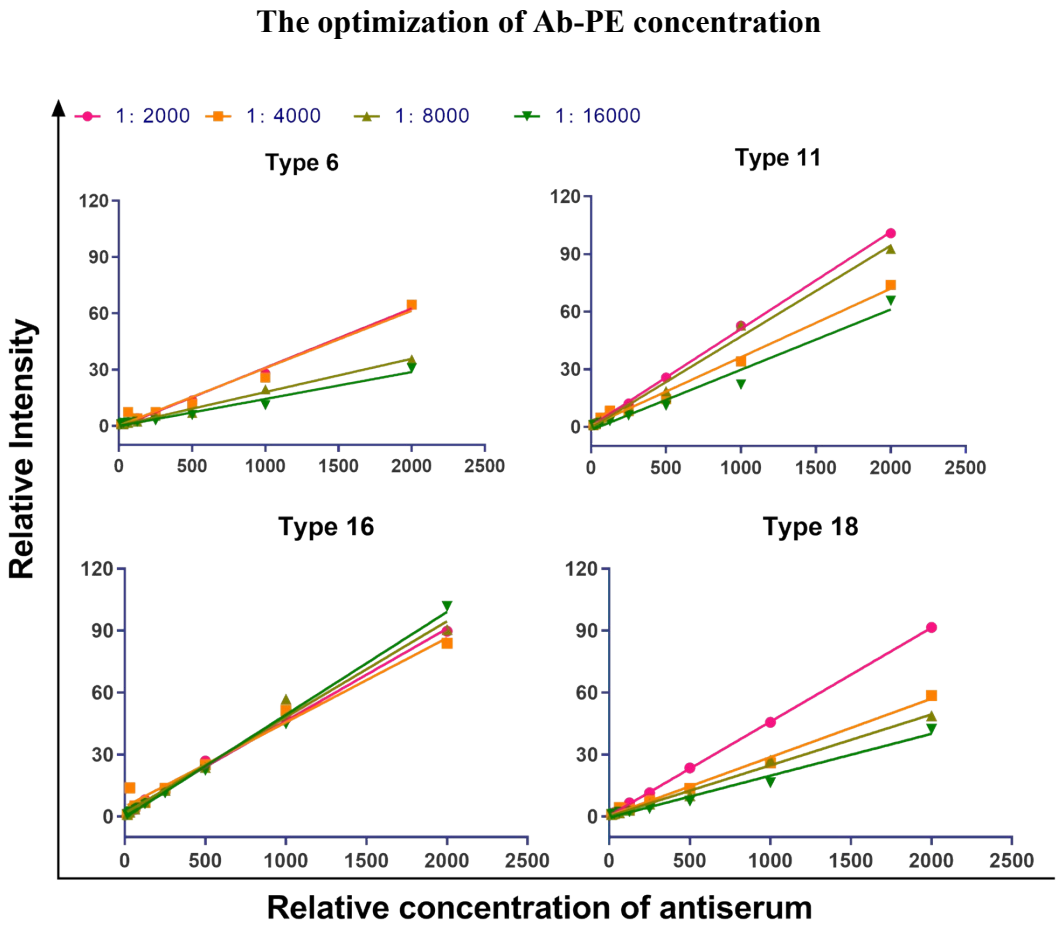

Fig. S1 The optimization of Ab-PE concentration (at four dilutions: 1:2000, 1:4000, 1:8000, 1:16000) for the four anti-HPV antibodies.

The screening of monoclonal antibody on IMBs

The screening of monoclonal antibodies was based on two principles: one is lower signal-crosstalk and the other is similar fluorescence signal ratio to the antiserum detection.

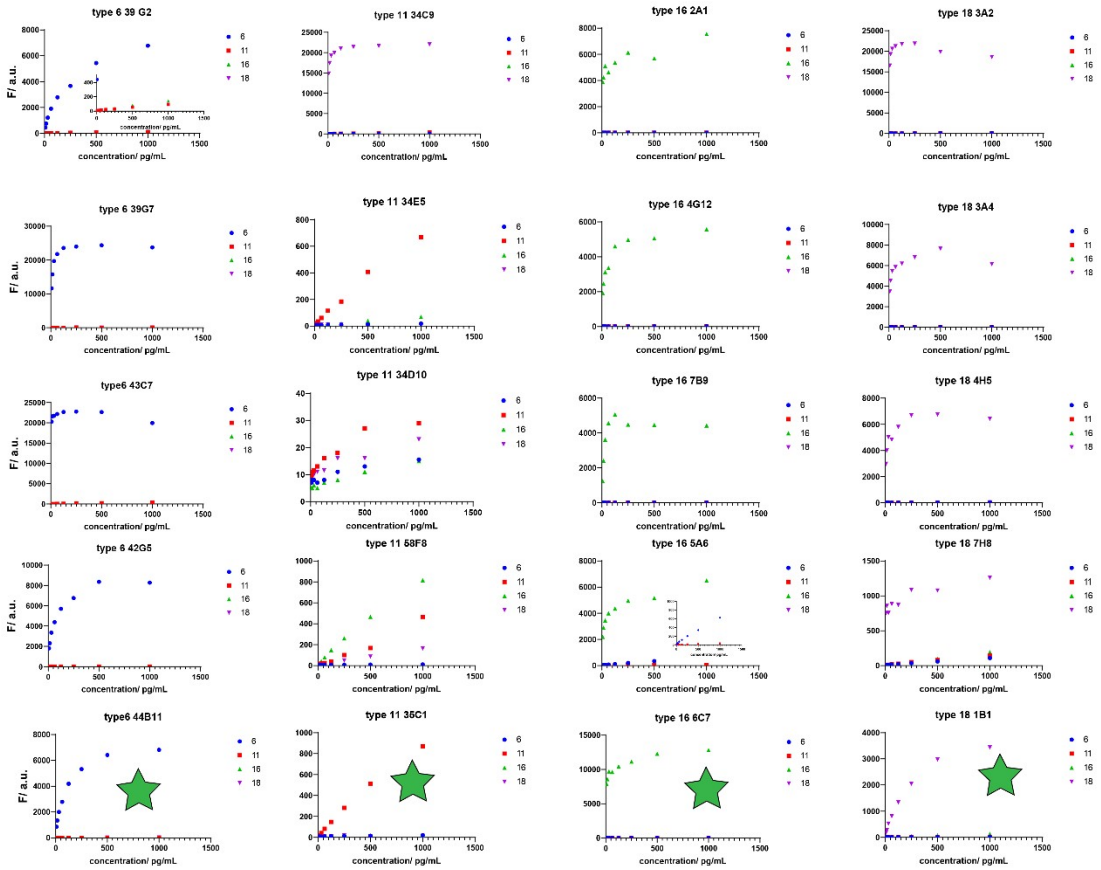

Fig. S2 The screening of 20 monoclonal antibodies by incubating with every antibody with a mixture of four IMBs.

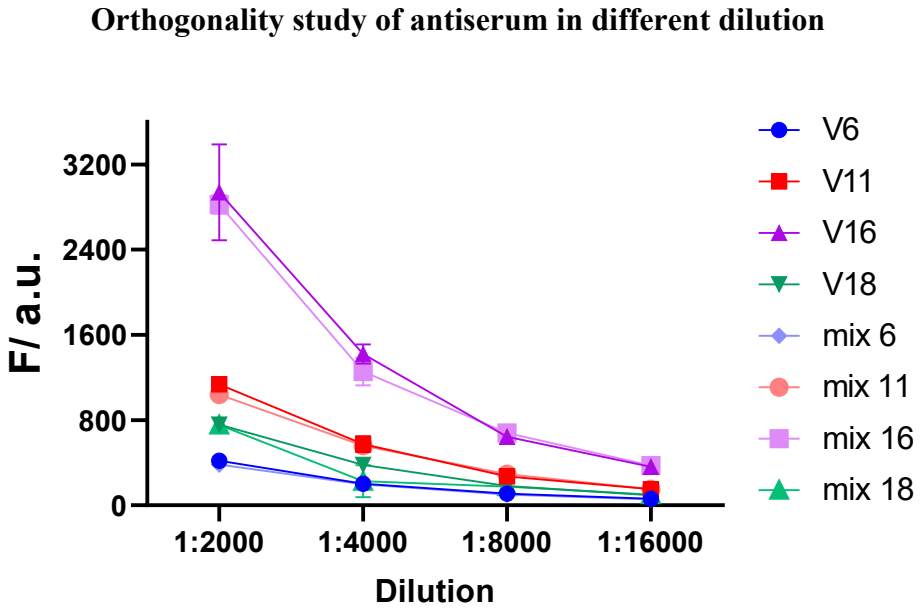

Fig. S3 Fluorescence intensity of HPV antiserum incubated with single and mixed IMBs at a series of four dilutions.

The Ratios of the results of the HIGH and ELISA methods

To obvious compare the performance of the two HIGH and ELISA methods, we calculated the ratios of the results of the two analytical methods (HIGH/ELISA). Detection of HPV-6, 11, 16, and 18 antibodies by HIGH was on average 6, 10, 18, and 9 times more sensitive than ELISA, with the highest ratios being 27, 34, 54, and 42.

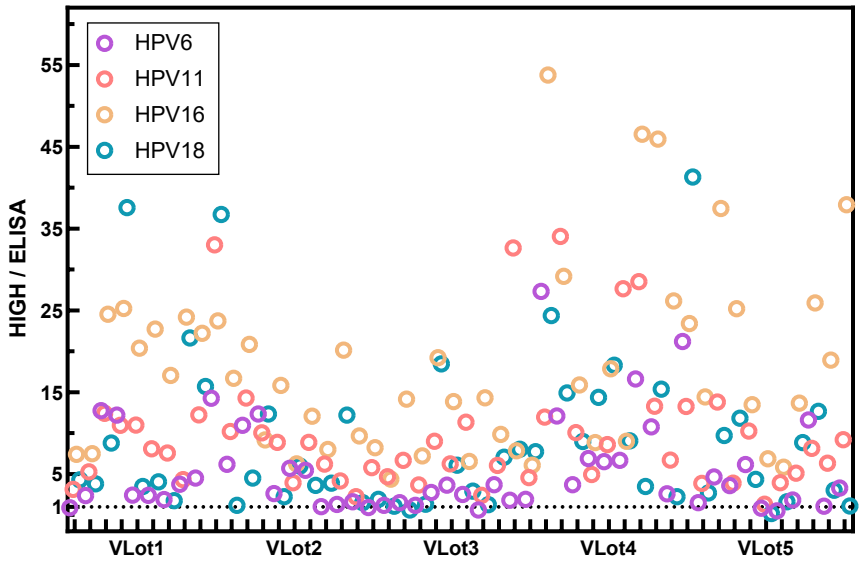

Fig. S4 Ratios of the results of the HIGH and ELISA methods for the quantification of four HPV antibodies.

The detection of antiserum by low concentration vaccine

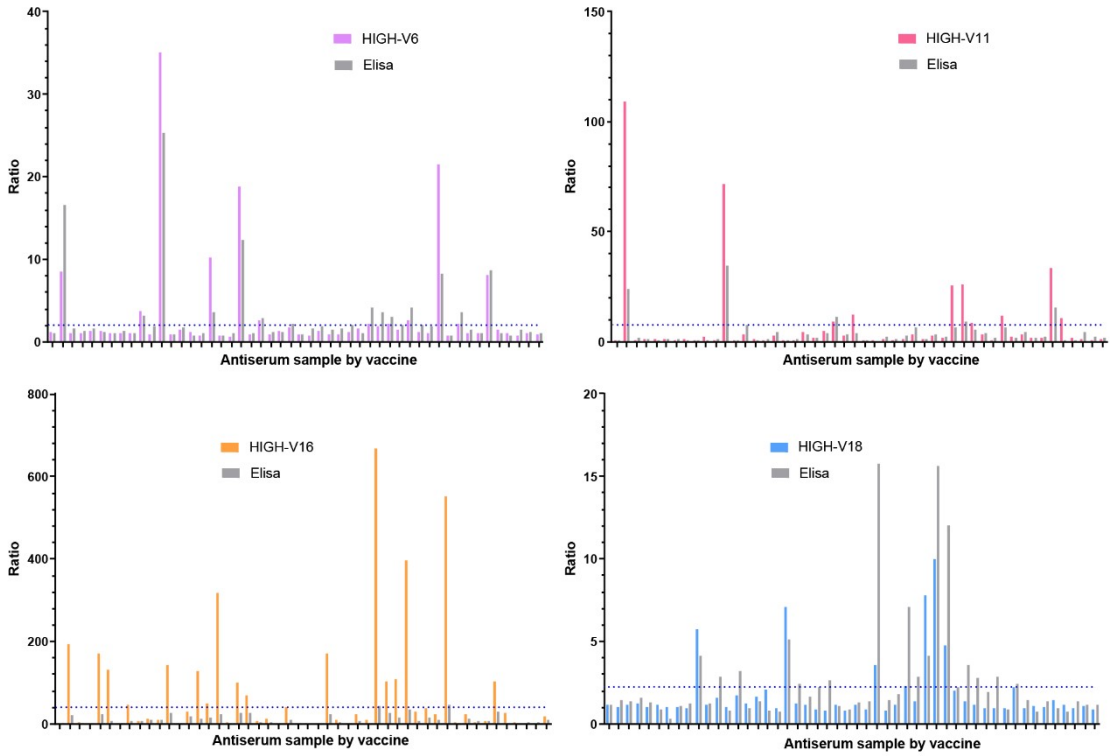

Fig. S5 Analysis of antisera from low concentration immunization for HPV antibodies by ELISA and HIGH.