

Genotyping 50 Types of Human Papillomavirus (HPV) with PlexBio Barcoded Magnetic Bead Multiplex Assay

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introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection. Some HPV types cause genital warts, while others can lead to precancerous lesions and invasive cancers. Infection of HPV types that are high-risk for developing into cervical and other cancers tend to be symptomless until the cancers are advanced, making early detection and treatment of HPV infections crucial.

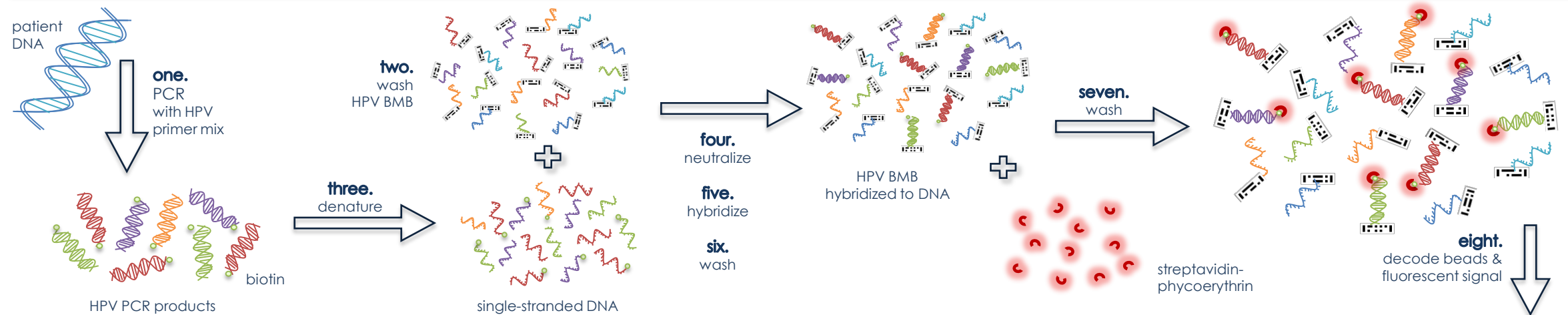
Using our Barcoded Magnetic Bead (BMB) technology, we developed a multiplex *in vitro* HPV DNA test that can detect and identify infections of 50 HPV types, both high- and low-risk, with a single assay. Each type of BMB is coupled to type-specific HPV DNA probes, which hybridize with DNA samples amplified and biotinylated through PCR. After reacting the mixture with streptavidin-phycoerythrin, the BMB Analyzer decodes each bead and measures its fluorescent signal in order to identify HPV types in the DNA sample.

Our data showed that the BMB genotyping assay produces clear and reliable results due to its high sensitivity and specificity to HPV DNA. Positive signals are significantly higher than background values, and the system is demonstrated to produce results that match HPV types as determined by DNA sequencing. While some HPV types cross-react, they can be identified through data analysis.

materials & methods

DNA is extracted from cervical cell samples, then amplified and biotinylated through PCR with the HPV-specific primer set. The BMB reagent and Wash Buffer I are added to a 96-well plate. After washing the beads with Wash Buffer I, the PCR product and Denature Buffer are added. The plate is shaken at 1000rpm, 52°C for 5 minutes before the Neutralization Buffer is added for a 20-minute hybridization time, at the same conditions, followed by two washes with Wash Buffer II 5 minutes apart. Once the supernatant is removed, streptavidin-phycoerythrin (SA-PE) is added and the plate is moved to room temperature for a 10-minute incubation at 1000rpm, followed by three washes with Wash Buffer I and the addition of the Detection Buffer. The beads are then decoded with the PlexBio BMB Analyzer, which identifies the barcode and fluorescent signal of each bead, genotyping up to 96 samples at a time.

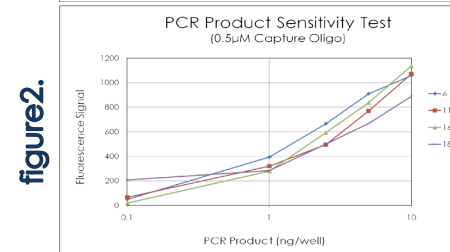
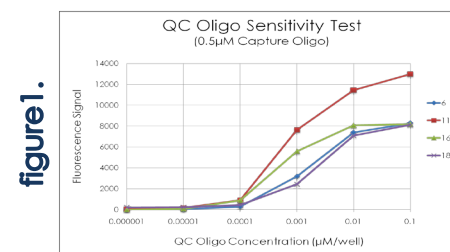
multiplex genotyping assay procedure



patient sample results

Patient Sample	XX04	XX25	XX31	XX37	XX55	XX60	XX64	XX72	XX97	XX99
Sequencing Results	39, 52, 56	91	51, 54, 58	6, 62	67	18	51, 52	51	87	68
16	3	15	9	47	10	57	8	24	15	13
18	3	8	7	5	56	1912	8	8	8	5
26	3	8	7	5	7	4	8	8	8	6
30	3	8	7	4	7	4	8	8	8	6
31	3	8	7	8	7	4	8	13	7	6
33	3	8	7	4	7	4	8	8	7	6
35	3	8	7	5	7	7	8	8	8	6
39*	246	8	7	5	7	52	8	8	7	5
45	3	8	7	26	7	4	8	8	7	6
51	5	8	1270	4	75	4	246	1215	30	6
52	1341	8	16	4	14	4	1328	67	6	11
53	3	8	58	4	7	4	8	68	7	6
56	719	3	1781	18	7	4	8	13	6	6
58	4	3	227	5	7	4	8	9	7	6
59	3	3	7	4	7	4	10	8	8	6
64	3	3	7	4	7	4	8	8	5	6
67	3	3	11	4	1797	7	4	8	8	6
68	3	8	7	4	7	4	13	7	7	995
68a	3	3	8	4	7	4	8	8	5	8
69	3	3	7	4	7	4	8	8	5	8
70	3	3	7	5	7	4	8	8	8	8
73	3	3	7	4	7	4	8	8	5	8
82/S391	3	3	7	4	2	4	8	10	6	8
82M4	3	4	7	4	2	4	8	12	5	10
6	3	55	7	133	90	63	8	25	22	59
11	3	8	7	14	7	7	8	10	10	4
32	3	8	7	4	7	4	8	12	6	6
34	3	8	13	17	12	11	24	8	7	87
40	4	8	7	5	7	4	8	8	12	7
42	9	8	6	29	7	4	8	8	6	6
43	3	7	7	7	7	4	8	8	6	6
44	3	8	16	4	7	4	8	8	6	6
54	3	8	7	4	7	4	8	8	6	6
55	3	3	7	4	7	4	8	46	7	6
61	3	3	7	4	2	4	15	9	2	6
62	3	3	7	1050	7	4	8	11	5	6
64	3	3	12	34	12	6	17	11	10	41
71	3	3	7	4	7	4	8	8	5	8
72	3	3	7	23	7	4	8	8	7	8
74	3	3	7	4	4	4	8	8	5	8
81	4	3	7	4	2	4	8	8	6	8
83	3	3	7	4	2	4	8	8	5	8
84	3	3	7	4	2	4	8	8	8	8
85	3	3	7	4	2	4	8	8	5	8
87	3	3	7	4	2	4	8	8	1288	8
89	3	3	7	4	2	4	8	8	24	8
90	3	3	7	16	2	4	12	8	10	10
91	4	383	7	4	2	4	8	8	6	8
94	3	3	7	4	2	4	8	8	5	28
102	3	3	7	4	2	4	8	8	7	8
ACTB	1079	608	592	358	866	972	471	513	910	807
GAPDH	795	514	405	329	423	463	320	273	621	517
Negative	3	3	7	4	2	4	8	8	5	8

sensitivity tests



QC Conc. in µM (50 µL/well)	Fluorescent Signal			
	HPV Type 6	HPV Type 11	HPV Type 16	HPV Type 18
10 ⁻¹	8286	12982	8184	8133
10 ⁻²	7398	11451	8073	7083
10 ⁻³	3198	7645	5589	2444
10 ⁻⁴	305	904	889	444
10 ⁻⁵	39	169	81	236
10 ⁻⁶	13	67	9	232

PCR Product (ng /well)	Fluorescent Signal			
	HPV Type 6	HPV Type 11	HPV Type 16	HPV Type 18
15	1424	1286	1302	1065
10	1059	1072	1137	889
5	911	770	837	669
2.5	667	496	594	500
1	394	321	280	283
0.1	49	66	19	209
0	5	15	3	129

table1. We selected DNA samples positive for HPV through southern blotting, then sequenced each sample to confirm positive HPV types. We then tested the DNA samples with the PlexBio HPV genotyping assay and found that our data perfectly matched both southern blot and sequencing results, demonstrating the accuracy and reliability of our multiplex assay. Signals under 100 are considered background. The assay is able to accurately identify multiple HPV types in one sample. Internal controls test for housekeeping genes beta-actin and GAPDH.

cross-reactivity

We observed cross-reactivity between a few HPV genotypes, such as types 34 & 64 and 43 & 45, due sequence similarity. As fluorescent signals due to cross-reactivity are consistently lower than positive signals, we can check data for cross-reactive pairs and determine the true positive for accurate results.

Bead	Bead Count	HPV Type	Fluorescent Signal
	45	52*	1564
	32	56*	741
	55	58*	598
	39	18*	352
	58	94	7
	46	11	5

table4. Each barcode is specific to one HPV type. Fluorescent signals are the average of the middle 60% of all beads of the same type in each well. Signal levels significantly higher than background indicate the presence of type-specific HPV DNA in the sample. *Denotes high-risk HPV types.

conclusions

one. The PlexBio multiplex HPV DNA testing system is demonstrated to be an effective method for detecting and genotyping up to 50 HPV types in a single assay.

two. The entire process to obtain results for 96 samples, including PCR, hybridization, and decoding, can be completed in under five hours for high-throughput HPV testing.

three. This multiplex assay exhibits high sensitivity and specificity, producing clear and accurate results comparable to that of DNA sequencing.