

S3 Table. Quantitative methylation in tested samples

Sample ^a	HPV family	HPV type	Cytopathology ^b	Mean % methyl L1 I ^c	PyroMark Q24 score ^d	Mean % methyl L1 II ^e	PyroMark Q24 score ^d	Mean % methyl L2 ^f	PyroMark Q24 score ^d
1	16	16	NILM	5	passed	10	Passed	16	Check
2	16	16	HSIL	8	passed	3	Passed	5	Passed
3	16	16	HSIL	5	Passed	7	Passed	4	Passed
4	16	16	HSIL	47	Passed	48	Passed	38	Passed
5	16	16	HSIL	10	Passed	12	Passed	11	Passed
6	16	16	HSIL	12	Passed	18	Passed	11	Passed
7	16	16	HSIL	9	Passed	6	Passed	6	Passed
8	16	16	HSIL	19	Passed	12	Passed	35	Check
9	16	16	HSIL	20	Passed	21	Passed	6	Passed
10	16	16	NILM	22	Passed	4	Passed	10	Check
11	18	18	NILM	26	Passed	9	Passed	12	Passed
12	18	18	LSIL	34	Passed	31	Check	26	Passed
13	18	18	NILM	25	Passed	26	Check	22	Passed
14	18	18	LSIL	20	Passed	12	Passed	21	Passed
15	18	18	NILM	14	Passed	5	Check	4	Check
16	18	18	NILM	18	Passed	16	Passed	9	Check
17	18	18	LSIL	11	Passed	15	Passed	5	Passed
18	18	18	ASCUS	29	Passed	20	Passed	22	Check
19	18	18	NILM	20	Passed	13	Passed	9	Passed
20	18	18	NILM	26	Passed	34	Check	5	Passed
21	16	31	NILM	13	Passed	8	Passed	14	Check
22	16	31	NILM	5	Passed	4	Passed	4	Passed
23	16	31	HSIL	6	Passed	5	Passed	4	Passed
24	16	31	HSIL	10	Passed	7	Passed	14	Passed
25	16	31	HSIL	30	Check	29	Passed	24	Check
26	16	31	HSIL	18	Check	7	Passed	11	Passed
27	16	31	HSIL	19	Check	9	Passed	23	Check
28	16	31	HSIL	8	Passed	6	Passed	7	Passed
29	16	31	HSIL	14	Passed	12	Passed	16	Check
30	16	31	LSIL	9	Passed	12	Passed	10	Check
31	16	33	NILM	19	Passed	10	Passed	29	Passed
32	16	33	LSIL	7	Passed	19	Passed	11	Passed
33	16	33	NILM	10	Passed	13	Passed	13	Passed
34	16	33	NILM	18	Check	2	Passed	3	Passed
35	16	33	NILM	6	Passed	7	Passed	12	Passed
36	16	33	NILM	4	Passed	2	Passed	3	Passed
37	16	33	HSIL	12	Passed	61	Passed	26	Passed
38	16	33	ASCUS	5	Passed	2	Passed	9	Passed
39	16	33	HSIL	34	Passed	30	Passed	56	Passed
40	16	33	NILM	16	Check	40	Passed	52	Passed
41	16	35	LSIL	3	Check	3	Passed	1	Passed
42	16	35	NILM	11	Passed	4	Passed	3	Passed
43	16	35	NILM	8	Passed	11	Passed	7	Passed
44	16	35	NILM	9	Passed	9	Check	1	Passed
45	16	35	NILM	6	Passed	13	Passed	5	Passed

Sample ^a	HPV family	HPV type	Cytopathology ^b	Mean % methyl L1 I ^c	PyroMark Q24 score ^d	Mean % methyl L1 II ^e	PyroMark Q24 score ^d	Mean % methyl L2 ^f	PyroMark Q24 score ^d
46	16	35	NILM	6	Check	6	Passed	1	Passed
47	16	35	NILM	5	Passed	6	Passed	1	Passed
48	16	35	HSIL	27	Passed	12	Passed	26	Passed
49	16	35	LSIL	3	Passed	1	Passed	1	Passed
50	16	35	LSIL	7	Check	2	Passed	2	Passed
51	18	39	NILM	43	Check	10	Passed	14	Passed
52	18	39	LSIL	11	Passed	10	Passed	6	Passed
53	18	39	NILM	24	Passed	9	Passed	10	Passed
54	18	39	NILM	12	Passed	7	Passed	3	Passed
55	18	39	NILM	10	Passed	10	Passed	8	Passed
56	18	39	NILM	30	Check	18	Passed	1	Passed
57	18	39	LSIL	15	Passed	8	Passed	18	Passed
58	18	39	LSIL	8	Passed	7	Passed	6	Passed
59	18	39	NILM	7	Passed	8	Passed	6	Passed
60	18	39	NILM	12	Passed	11	Check	16	Passed
61	18	45	NILM	73	Passed	55	Passed	18	Passed
62	18	45	NILM	43	Passed	41	Passed	8	Passed
63	18	45	NILM	26	Passed	28	Passed	9	Passed
64	18	45	NILM	52	Passed	46	Passed	21	Check
65	18	45	HSIL	92	Passed	80	Passed	86	Passed
66	18	45	HSIL	94	Passed	89	Passed	88	Passed
67	18	45	NILM	24	Passed	6	Passed	5	Passed
68	18	45	NILM	28	Passed	22	Passed	16	Passed
69	18	45	NILM	24	Passed	25	Check	15	Passed
70	18	45	HSIL	18	Passed	11	Passed	5	Passed
71	18	45	NILM	30	Passed	28	Passed	13	Passed
72	18	51	NILM	11	Check	23	Passed	6	Passed
73	18	51	LSIL	3	Passed	7	Passed	2	Passed
74	18	51	NILM	8	Passed	28	Passed	19	Passed
75	18	51	LSIL	5	Check	4	Passed	2	Passed
76	18	51	NILM	17	Passed	28	Passed	10	Passed
77	18	51	LSIL	5	Check	8	Passed	4	Passed
78	18	51	NILM	25	Passed	78	Passed	17	Check
79	18	51	NILM	4	Passed	18	Passed	4	Passed
80	18	51	NILM	5	Check	20	Passed	1	Passed
81	18	51	NILM	21	Check	19	Passed	4	Passed
82	18	51	NILM	1	Passed	11	Check	2	Passed
83	16	52	NILM	14	Passed	2	Check	16	Passed
84	16	52	NILM	18	Passed	44	Passed	6	Passed
85	16	52	NILM	7	Passed	8	Passed	1	Passed
86	16	52	NILM	10	Passed	14	Passed	5	Passed
87	16	52	NILM	24	Passed	27	Passed	6	Passed
88	16	52	NILM	24	Passed	38	Passed	17	Passed
89	16	52	HSIL	10	Passed	21	Passed	9	Passed
90	16	52	NILM	24	Passed	43	Passed	7	Passed
91	16	52	NILM	18	Passed	41	Passed	4	Check
92	16	52	HSIL	24	Passed	39	Passed	11	Passed

Sample ^a	HPV family	HPV type	Cyto logy ^b	Mean % methyl L1 I ^c	PyroMark Q24 score ^d	Mean % methyl L1 II ^e	PyroMark Q24 score ^d	Mean % methyl L2 ^f	PyroMark Q24 score ^d
93	16	56	NILM	14	Passed	7	Passed	39	Passed
94	16	56	NILM	23	Passed	11	Check	1	Passed
95	16	56	ASCUS	26	Passed	14	Passed	42	Passed
96	16	56	LSIL	4	Passed	4	Passed	2	Passed
97	16	56	NILM	13	Passed	7	Passed	8	Passed
98	16	56	NILM	11	Passed	7	Passed	15	Passed
99	16	56	LSIL	5	Passed	12	Passed	3	Passed
100	16	56	NILM	5	Passed	48	Check	5	Passed
101	16	56	NILM	4	Passed	7	Passed	3	Passed
102	16	56	NILM	18	Passed	9	Passed	18	Check
103	16	56	NILM	17	Passed	19	Passed	30	Passed
104	16	56	NILM	5	Passed	4	Passed	4	Check
105	16	56	NILM	13	Passed	11	Passed	1	Check
106	16	56	NILM	31	Check	41	Passed	43	Passed
107	16	58	NILM	19	Passed	13	Passed	32	Passed
108	16	58	LSIL	6	Passed	3	Passed	12	Passed
109	16	58	NILM	12	Passed	8	Passed	21	Passed
110	16	58	NILM	67	Passed	65	Check	75	Passed
111	16	58	NILM	10	Passed	6	Passed	16	Passed
112	16	58	NILM	7	Check	5	Passed	21	Passed
113	16	58	NILM	16	Passed	14	Passed	24	Passed
114	16	58	NILM	16	Passed	9	Passed	35	Passed
115	16	58	NILM	10	Passed	5	Passed	27	Passed
116	16	58	NILM	10	Passed	7	Passed	12	Passed
117	18	59	NILM	18	Passed	11	Passed	2	Passed
118	18	59	NILM	15	Passed	14	Passed	2	Passed
119	18	59	LSIL	15	Passed	19	Passed	2	Passed
120	18	59	LSIL	11	Passed	11	Passed	2	Passed
121	18	59	NILM	17	Passed	8	Passed	4	Passed
122	18	59	NILM	37	Passed	31	Passed	4	Passed
123	18	59	NILM	11	Passed	10	Passed	3	Passed
124	18	59	LSIL	11	Passed	14	Passed	2	Passed
125	18	59	NILM	17	Passed	10	Passed	2	Passed
126	18	59	NILM	12	Passed	11	Passed	1	Passed
HPV16 Pl. U ^g	16	16	-	4 (1-6)	Passed	3 (3-7)	Passed	2 (2-3)	Passed
HPV18 Pl. U ^g	18	18	-	5 (4-9)	Passed	5 (3-9)	Passed	3 (1-6)	Passed
HPV16 Pl. M ^g	16	16	-	93 (88-95)	Passed	95 (93-96)	Passed	87 (85-92)	Passed
HPV18 Pl. M ^g	18	18	-	84 (80-86)	Passed	86 (80-96)	Passed	94 (90-95)	Passed
SiHA ^g	16	16	-	92 (90-93)	Passed	98 (97-98)	Passed	na	-
HeLA ^g	18	18	-	nd	-	48 (45-57)	Passed	nd	-

^aSamples collected in STM from women who participated at the pilot project in Turin.

^bNILM, Negative for Intraepithelial Lesion or Malignancy; ASCUS Atypical Squamous Cells of Undetermined Significance; LSIL, Low-Grade Squamous Intraepithelial Lesion; HSIL, High-Grade Squamous Intraepithelial Lesion.

^c Mean percentage of methylation among all the target CpGs in the L1 I region released by PyroMark Q24 software (reference HPV16 CpG 5601, 5606, 5609, 5016)

^d Colour-based score for result quality: Passed,(blue); Check, (yellow); Failed (red). No red score was assigned by the automatic PyroMark Q24 software.

^e Percentage of methylation released by PyroMark Q24 software for the targeted CpG in the L1 II region (reference HPV16 CpG 6457). For HPV56 and HPV 58 mean percentage of methylation between the two targeted CpGs is reported

^f Percentage of methylation released by PyroMark Q24 software for the targeted CpG in the L2 region (reference HPV16 CpG 4261)

^g Mean (and range) of at least ten replicates: Pl=plasmid; U= unmethylated; M=methylated; na=not applicable, for L2 only synthetic plasmids were used as controls; nd = not detectable for deletion upon virus integration [Schwartz E, et al. *Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature 1995;314: 111-114*]