

Table S1 p16 ICC interpretation standard

Number	Result interpretation	Result determination	Clinical recommendation
1	p16(+) cell number ≥ 1 independent cell morphology	Positive	The presence of high-grade lesions is more likely, and colposcopy is recommended
2	p16(-) independent cell morphology	Negative	The presence of advanced lesions is less likely, and routine cervical cytological screening is recommended

ICC, immunocytochemical.

Table S2 p16/Ki-67 TNS interpretation standard

Number	Staining condition	Result determination	Clinical recommendation
1	p16(+)/Ki-67(+), independent of cell morphology, double staining positive cells ≥ 1	High risk	There is a high probability of high-grade lesions (similar to HSIL or ASC-H), and colposcopy, a negative initial biopsy, an immediate second colposcopy review, or more aggressive clinical interference is recommended
2	p16(-)/Ki-67(+), abnormal morphology of squamous cell (Ki-67 monochromic squamous cell nuclear area ≥ 2.5 times the normal middle squamous cell nuclear area) ≥ 1	Low risk	There is a high possibility of low-grade lesions (similar to LSIL risk), a single test, colposcopy, is recommended. Dual screening, combined with LBC and/or HPV test results can determine the triage protocol
3	p16(-)/Ki-67(+), abnormal squamous cell morphology (Ki-67 monochromic positive squamous epithelial hollow cells or Ki-67 monochromic positive squamous cell with binucleation) ≥ 1		
4	p16(-)/Ki-67(+), abnormal glandular epithelial cells ≥ 1		There is a possibility of low-grade lesions (similar to ASC-US). Combining the results of LBC and/or HPV to determine the triage protocol is recommended
5	p16(-)/Ki-67(+), without abnormal squamous epithelial cell (Ki-67 monochromic squamous epithelial cell nuclear area < 2.5 times the normal midlayer squamous epithelial cell nuclear area)	No risk	The presence of lesions is less likely (similar to NILM), and routine cervical cytological screening is recommended
6	p16(-)/Ki-67(+), without abnormal glandular epithelial cell		
7	p16(+)/Ki-67(-) or p16(-)/Ki-67(-), independent of cell morphology		

TNS, type-number-strength; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; ASC-US, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; NILM, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions, HSIL, high-grade squamous intraepithelial lesions; +, positive; -, negative.

Table S3 p16/MCM2 TNS interpretation standard

Number	Staining condition	Result determination	Clinical recommendation
1	p16 (+)/MCM2 (+), independent of cell morphology, ≥ 50 double-stained-positive cells	High risk	There is a high probability of high-grade lesions (risk similar to HSIL), and colposcopy, a negative initial biopsy, a recent second colposcopy review, or more aggressive clinical interference is recommended
2	p16 (+)/MCM2 (+), independent of cell morphology, double-stained-positive cells ≥ 5 and < 50		There is a high probability of high-grade lesions (risk similar to HSIL), and colposcopy, a negative initial biopsy, and immediate second colposcopy review are recommended
3	p16 (+) /MCM2 (+), independent of cell morphology, double-stained-positive cells < 5		There is the possibility of high-grade lesions (risk similar to ASC-H), and colposcopy, a negative initial biopsy, and cervical cytology follow-up review are recommended
4	p16 (-)/MCM2 (+), abnormal cell morphology (nuclear area of MCM2 monochromic positive cells ≥ 3 times the nuclear area of normal midlayer squamous epithelial cells and ≥ 3 monochromic abnormal cells). Hollowed out cells and/or binucleated cells ≥ 1 regardless of nucleus size	Low risk	There is a high possibility of low-grade lesions (similar to LSIL risk), a single test, colposcopy, is recommended. Dual screening, combined with LBC and/or HPV test results, can determine the triage protocol
5	p16 (-)/MCM2 (+), abnormal cell morphology (nuclear area of MCM2 monochromic positive cells ≥ 3 times the nuclear area of normal midlayer squamous epithelial cells and monochromic abnormal cells < 3)		There is a possibility of low-grade lesions (risk similar to ASC-US), and combining the results of LBC and/or HPV to determine the triage protocol is recommended
6	p16 (-)/MCM2 (+), abnormal cell morphology (the nuclear area of MCM2 monochromic cells is 2.5 to 3 times that of the normal midlayer squamous epithelial cells)		There is a possibility of low-grade lesions (similar to ASC-US), and combining the results of LBC and/or HPV to determine the triage protocol is recommended
7	p16 (-)/MCM2 (+), the cell morphology is normal (the nuclear area of MCM2 monochromatic-positive cells is less than 2.5 times the nuclear area of normal intermediate squamous epithelial cells)	No risk	The presence of lesions is less likely (similar to NILM), and routine cervical cytological screening is recommended
8	p16 (+)/MCM2 (-) or p16 (-)/MCM2(-), independent of cell morphology		The presence of lesions is less likely (similar to NILM), and routine cervical cytological screening is recommended

TNS, type-number-strength; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; ASC-US, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; NILM, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions, HSIL, high-grade squamous intraepithelial lesions; +, positive; -, negative.

Table S4 p16/Ki-67/MCM2 TNS interpretation standard

Number	Dyeing condition	Result determination	Clinical recommendation
1	p16/Ki-67 double staining (+), independent of cell morphology, double-stained-positive cells ≥ 50	High risk	There is a high probability of high-grade lesions (risk similar to HSIL), and colposcopy, a negative initial biopsy, a recent second colposcopy review, or more aggressive clinical interference is recommended
2	p16/Ki-67 double staining (+), independent of cell morphology, double-stained-positive cells ≥ 5 and < 50		There is a high probability of high-grade lesions (risk similar to HSIL), colposcopy, a negative initial biopsy, and immediate second colposcopy review are recommended
3	p16/Ki-67 double staining (+), independent of cell morphology, double-stained-positive cells < 5		There is a possibility of high-grade lesions (risk similar to ASC-H), colposcopy, negative initial biopsy, and cervical cytology follow-up review are recommended
4	p16/Ki-67 double staining (-), independent of cell morphology; MCM2 (+), abnormal cell morphology (nuclear area of MCM2 monochromic positive cells ≥ 3 times the nuclear area of normal midlayer squamous epithelial cells and ≥ 3 monochromic abnormal cells). Hollowed out cells and/or binucleated cells ≥ 1 regardless of nucleus size	Low risk	There is a high possibility of low-grade lesions (similar to LSIL risk), a single test, colposcopy is recommended. Dual screening, combined with LBC and/or HPV test results, can determine the triage protocol
5	p16/Ki-67 double staining (-), independent of cell morphology; MCM2 (+), abnormal cell morphology (nuclear area of MCM2 monochromic positive cells ≥ 3 times the nuclear area of normal midlayer squamous epithelial cells and monochromic abnormal cells < 3)		There is a possibility of low-grade lesions (risk similar to ASC-US), and combining the results of LBC and/or HPV to determine the triage protocol is recommended
6	p16/Ki-67 double staining (-), independent of cell morphology; MCM2 (+), abnormal cell morphology (nuclear area of MCM2 monochromic cells 2.5 to 3 times that of normal midlayer squamous epithelial cells)		There is a possibility of low-grade lesions (similar to ASC-US), and combining the results of LBC and/or HPV to determine the triage protocol is recommended
7	p16/Ki-67 double staining (-), independent of cell morphology; MCM2(+), normal cell morphology (nuclear area of MCM2 monochromic positive cells less than 2.5 times the nuclear area of normal intermediate squamous epithelial cells)	No risk	The presence of lesions is less likely (similar to NILM), and routine cervical cytological screening is recommended
8	p16/Ki-67 double staining (-), independent of cell morphology; MCM2(-), independent of cell morphology		

TNS, type-number-strength; ASC-H, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; ASC-US, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; NILM, atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions, HSIL, high-grade squamous intraepithelial lesions; +, positive; -, negative.

Table S5 Diagnostic efficacy of the single screening methods

Method	Positive cutoff value	Sensitivity			Specificity			PPV			NPV			Kappa			Overall agreement			Youden index
		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		
LBC	LBC_HR	54.1%	0.443	0.637	97.9%	0.951	0.993	92.2%	0.827	0.974	82.1%	0.771	0.864	0.585	0.491	0.678	0.840	0.797	0.877	0.520
	LBC_LR	81.7%	0.731	0.884	44.3%	0.378	0.509	40.5%	0.339	0.473	83.9%	0.762	0.899	0.204	0.123	0.284	0.561	0.507	0.614	0.259
HR-HPV	HPV_HR	56.0%	0.461	0.655	63.4%	0.569	0.696	41.5%	0.334	0.499	75.6%	0.690	0.815	0.177	0.074	0.280	0.610	0.557	0.662	0.194
	HPV_LR	98.2%	0.935	0.998	8.5%	0.053	0.128	33.2%	0.281	0.387	90.9%	0.708	0.989	0.044	0.014	0.074	0.369	0.318	0.423	0.067
p16 ICC	P16_positive	99.1%	0.950	1.000	0.9%	0.001	0.030	31.7%	0.268	0.369	66.7%	0.094	0.992	-0.000	-0.014	0.013	0.320	0.271	0.372	-0.000
p16/Ki-67	p16/Ki-67_HR	68.6%	0.587	0.775	85.5%	0.800	0.900	70.0%	0.600	0.788	84.7%	0.791	0.893	0.544	0.444	0.644	0.799	0.750	0.843	0.541
	p16/Ki-67_LR	81.4%	0.724	0.884	61.8%	0.548	0.685	51.2%	0.433	0.592	87.1%	0.806	0.920	0.376	0.281	0.471	0.683	0.628	0.734	0.432
p16/MCM2	p16/MCM2_HR	85.3%	0.773	0.914	69.8%	0.635	0.756	56.7%	0.488	0.644	91.1%	0.860	0.948	0.485	0.397	0.574	0.747	0.698	0.792	0.551
	p16/MCM2_LR	97.2%	0.922	0.994	27.2%	0.216	0.334	38.3%	0.325	0.443	95.5%	0.875	0.991	0.173	0.120	0.227	0.494	0.440	0.548	0.245
p16/Ki-67/ MCM2	p16/Ki-67/MCM2_HR	66.1%	0.564	0.749	86.8%	0.818	0.909	69.9%	0.601	0.785	84.6%	0.795	0.890	0.537	0.440	0.633	0.802	0.756	0.843	0.529
	p16/Ki-67/MCM2_LR	94.5%	0.884	0.980	29.4%	0.236	0.356	38.3%	0.325	0.444	92.0%	0.834	0.970	0.171	0.113	0.230	0.500	0.446	0.554	0.239

HPV, human papillomavirus; HR, high risk; ICC, immunocytochemical; LBC, liquid-based cytology; LR, low risk; MCM2, minichromosome maintenance protein 2.

Table S6 Diagnostic efficacy of the combined screening methods

Method	Sample size	Sensitivity			Specificity			PPV			NPV			Kappa			Overall agreement			Youden index
	n	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		
LBC + HR-HPV	344	91.7%	0.849	0.962	26.4%	0.209	0.325	36.6%	0.309	0.426	87.3%	0.773	0.940	0.129	0.071	0.187	0.471	0.417	0.525	0.181
LBC + p16/Ki-67	309	84.3%	0.758	0.908	67.2%	0.603	0.735	55.8%	0.476	0.638	89.7%	0.838	0.940	0.456	0.362	0.549	0.728	0.675	0.777	0.515
LBC + p16/MCM2	344	96.3%	0.909	0.990	42.6%	0.361	0.491	43.8%	0.374	0.503	96.2%	0.904	0.989	0.294	0.226	0.363	0.596	0.542	0.648	0.389
LBC + p16/Ki-67/MCM2	344	89.9%	0.827	0.949	48.5%	0.420	0.551	44.8%	0.380	0.516	91.2%	0.848	0.955	0.302	0.225	0.380	0.616	0.563	0.668	0.384
HR-HPV + p16/Ki-67	309	91.2%	0.839	0.959	36.7%	0.301	0.437	41.5%	0.350	0.483	89.4%	0.808	0.950	0.214	0.141	0.287	0.547	0.490	0.603	0.279
HR-HPV + p16/MCM2	344	98.2%	0.935	0.998	18.7%	0.139	0.243	35.9%	0.305	0.416	95.7%	0.852	0.995	0.115	0.072	0.158	0.439	0.386	0.493	0.169
HR-HPV + p16/Ki-67/MCM2	344	96.3%	0.909	0.990	20.9%	0.158	0.266	36.1%	0.306	0.419	92.5%	0.818	0.979	0.119	0.071	0.166	0.448	0.394	0.502	0.172

HPV, human papillomavirus; HR, high risk; LBC, liquid-based cytology; MCM2, minichromosome maintenance protein 2.

Table S7 Diagnostic efficacy of p16 ICC as triage method after HRH-PV of LBC screening

Screening method	Triage method	Sample size	Sensitivity			Specificity			PPV			NPV			Kappa			Overall agreement			Youden index
		n	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		
HR-HPV	TCT_LR	175	80.4%	0.661	0.906	33.3%	0.253	0.422	30.1%	0.221	0.390	82.7%	0.697	0.918	0.089	-0.004	0.183	0.457	0.382	0.534	0.138
	p16/Ki-67_LR	154	80.5%	0.651	0.912	52.2%	0.426	0.617	37.9%	0.277	0.490	88.1%	0.778	0.947	0.241	0.118	0.364	0.597	0.515	0.676	0.327
	p16/MCM2_LR	175	95.7%	0.852	0.995	20.2%	0.136	0.281	29.9%	0.227	0.380	92.9%	0.765	0.991	0.093	0.034	0.152	0.400	0.327	0.477	0.158
	p16/Ki-67/MCM2_LR	175	93.5%	0.821	0.986	22.5%	0.156	0.307	30.1%	0.227	0.383	90.6%	0.750	0.980	0.095	0.030	0.161	0.411	0.338	0.488	0.160
LBC	HR-HPV_LR	156	100.0%	0.884	1.000	7.1%	0.033	0.131	20.4%	0.142	0.278	100.0%	0.664	1.000	0.029	0.008	0.050	0.250	0.184	0.326	0.071
	p16/Ki-67_LR	132	75.0%	0.551	0.893	51.0%	0.410	0.609	29.2%	0.190	0.411	88.3%	0.774	0.952	0.165	0.038	0.292	0.561	0.472	0.647	0.260
	p16/MCM2_LR	156	96.7%	0.828	0.999	15.9%	0.100	0.234	21.5%	0.149	0.294	95.2%	0.762	0.999	0.054	0.010	0.097	0.314	0.242	0.393	0.125
	p16/Ki-67/MCM2_LR	156	96.7%	0.828	0.999	18.3%	0.119	0.261	22.0%	0.152	0.300	95.8%	0.789	0.999	0.065	0.018	0.112	0.333	0.260	0.413	0.149

HPV, human papillomavirus; HR, high risk; ICC, immunocytochemical; LBC, liquid-based cytology; LR, low risk; MCM2, minichromosome maintenance protein 2; TCT, ThinPrep cytologic test.

Table S8 Overall diagnostic efficacy of HR-HPV or LBC screening with one of p16 ICC as triage method

Method	Sample size	Sensitivity			Specificity			PPV			NPV			Kappa			Overall agreement			Youden index
	n	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		
HR-HPV + LBC_triage	344	89.9%	0.827	0.949	26.8%	0.213	0.330	36.3%	0.306	0.423	85.1%	0.750	0.923	0.120	0.059	0.180	0.468	0.414	0.522	0.167
HR-HPV + p16/Ki-67_triage	309	90.2%	0.827	0.952	36.7%	0.301	0.437	41.3%	0.347	0.480	88.4%	0.797	0.943	0.207	0.133	0.281	0.544	0.486	0.600	0.269
HR-HPV + p16/MCM2_triage	344	96.3%	0.909	0.990	19.6%	0.147	0.252	35.7%	0.302	0.415	92.0%	0.808	0.978	0.109	0.063	0.156	0.439	0.386	0.493	0.159
HR-HPV + p16/Ki-67/MCM2_triage	344	95.4%	0.896	0.985	20.9%	0.158	0.266	35.9%	0.303	0.417	90.7%	0.797	0.969	0.113	0.064	0.161	0.445	0.391	0.499	0.163
LBC + HR-HPV_triage	344	81.7%	0.731	0.884	48.1%	0.415	0.547	42.2%	0.354	0.492	85.0%	0.777	0.906	0.238	0.155	0.320	0.587	0.533	0.640	0.297
LBC + p16/Ki-67_triage	309	75.5%	0.660	0.835	72.9%	0.664	0.789	57.9%	0.490	0.664	85.8%	0.797	0.906	0.450	0.350	0.549	0.738	0.685	0.786	0.484
LBC + p16/MCM2_triage	344	80.7%	0.721	0.877	52.8%	0.462	0.593	44.2%	0.372	0.514	85.5%	0.787	0.908	0.274	0.188	0.360	0.616	0.563	0.668	0.335
LBC + P16/Ki-67/MCM2_triage	344	80.7%	0.721	0.877	54.0%	0.474	0.605	44.9%	0.378	0.521	85.8%	0.791	0.910	0.286	0.200	0.373	0.625	0.571	0.676	0.348

HPV, human papillomavirus; HR, high risk; ICC, immunocytochemical; LBC, liquid-based cytology; MCM2, minichromosome maintenance protein 2.