Research Square

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# Development and Laboratory Evaluation of a Standardized Procedure for Environmental Sampling and Molecular Detection of Human Papillomavirus

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**Research Article**

**Keywords:** human papillomavirus, environmental surveillance, surface sampling, method standardization, positive detection rate, log-scale recovery index

**Posted Date:** October 23rd, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-11185926/v1

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**Additional Declarations:** No competing interests reported.

## Abstract

# Background

Environmental surveillance of infectious pathogens is increasingly important for disease prevention and control; however, heterogeneity in sampling, preservation, and transport procedures can substantially affect molecular detection results.

# Methods

Human papillomavirus (HPV) was used as a non-enveloped DNA virus model to evaluate six determinants of surface sampling performance: swab type, surface material, sampling interval, swab pre-moistening, ambient temperature, and preservation medium. An HPV-positive inoculum was prepared by pooling approximately 150 anonymized male urethral swab specimens, followed by saline elution, genotype confirmation, and quantitative testing. HPV DNA was quantified by real-time quantitative PCR (qPCR). Outcomes were the positive detection rate and a prespecified log-scale recovery index.

# Results

Polyester and sponge swabs achieved the highest positive detection rates (both 98.04% ± 3.40%), whereas polyester swabs had the highest mean log-scale recovery index. HPV DNA was more readily recovered from smooth, non-porous, non-absorbent surfaces (ceramic tile and polypropylene) than from wood or woven fabric. Dry polyester swabbing yielded a higher positive detection rate than PBS-pre-moistened swabbing (88.75% ± 2.80% vs 80.00% ± 5.23%; P = 0.032), although the log-scale recovery index did not differ significantly. Sampling from 1 to 24 h after inoculation did not significantly affect either outcome. Exposure to 35°C reduced both the positive detection rate and the log-scale recovery index compared with 15°C and 25°C. Dedicated viral transport media maintained HPV DNA detectability throughout 28 days of storage at 2–8°C. PBS first fell below the prespecified 70% detection threshold on day 7, whereas normal saline remained at or above the threshold through 3 h and fell below it at 6 h.

# Conclusions

Under the tested laboratory conditions, dry polyester swabs combined with dedicated viral transport media provided the most practical overall approach for molecular recovery of HPV from environmental surfaces. Surface properties and ambient temperature were the principal determinants of HPV DNA detectability. These findings support protocol standardization but do not establish viral infectivity or fomite transmission.

## Introduction

Environmental surfaces can serve as reservoirs for pathogen contamination and may contribute to indirect transmission in public and healthcare settings [1]. Frequently touched surfaces, including door handles, handrails, and countertops, are particularly vulnerable to cross-contamination by bacteria and viruses [2, 3]. Reliable environmental surveillance is therefore important for early warning, source tracking, and evaluation of infection-prevention interventions [4].

Despite this need, standardized procedures for environmental pathogen sampling remain limited. Sampling performance varies according to the target organism, swab material, surface characteristics, temperature, sampling interval, pre-moistening procedure, and transport medium [5–13, 15–19]. Polyester swabs have shown favorable performance for norovirus recovery [7], whereas flocked or sponge-based devices may be advantageous for selected bacterial targets [8, 9]. Smooth, non-porous surfaces generally permit greater recovery than porous or absorbent materials [10, 11]. Temperature can accelerate environmental DNA degradation [12, 13], and the value of swab pre-moistening remains inconsistent across studies [15–18]. Dedicated viral transport media may also reduce false-negative molecular results during storage and transport [19].

HPV is a highly prevalent non-enveloped DNA virus. HPV infection or DNA detection has been reported in children and in fingertip, bathroom, and other public-environment samples, supporting the plausibility of non-sexual exposure routes [14, 20–22]. However, detection of HPV DNA in environmental samples alone does not demonstrate viral infectivity or transmission. Standardized methods for environmental HPV sampling are lacking. We therefore used HPV as a model to systematically evaluate the major pre-analytical variables affecting surface sampling and DNA preservation, with the aim of defining a reproducible laboratory protocol for public-health environmental surveillance.

## Materials and Methods

# 1.1 Preparation of Experimental Materials

1.1.1 Preparation of the HPV-positive inoculum: Approximately 150 anonymized HPV-positive male urethral swab specimens were pooled and eluted with normal saline. The resulting suspension contained exfoliated epithelial cells, cell-associated HPV, extracellular viral particles, and cell-free HPV DNA. Two inoculum batches were prepared, genotyped, quantified using the commercial assay, aliquoted, and stored at -80°C until use. Batch 1 contained HPV genotypes 16, 18, 33, 59, 66, 51, 53, 52, 68, 56, 58, 31, 6, 81, 73, and 42; batch 2 contained genotypes 16, 33, 39, 59, 66, 51, 53, 52, 56, 58, 31, 6, 81, 11, and 42. All specimens were anonymized before pooling.

1.1.2 Environmental surface coupons: Four frequently touched materials were evaluated: ceramic tile and polypropylene (smooth, non-porous, and non-absorbent), and wood and woven fabric (rough, porous, and absorbent). Each material was prepared as an independent 10 cm × 10 cm coupon. Coupons were aseptically cleaned and air-dried before inoculation.

1.1.3 Surface inoculation: Frozen inoculum was thawed in a 25°C water bath and mixed by vortexing. A total of 100 μL was distributed as 100 separate 1-μL droplets over each coupon to promote uniform coverage. Inoculated coupons were held in a biosafety cabinet for 60 min until visibly dry before sampling.

# 1.2 Instruments and Reagents

Nucleic acid extraction and purification kit (lot AS002498; magnetic-bead method; Fosun Diagnostics [Shanghai] Co., Ltd., Shanghai, China); HPV nucleic acid genotyping and quantification kit (lot 25002-1; fluorescent-probe qPCR; Sansure Biotech Inc., Changsha, China); Smart LabAssist SLA-32 nucleic acid extraction analyzer (serial no. E13200042; Shanghai Xingyao Medical Technology Development Co., Ltd., Shanghai, China); and ABI 7500 Real-Time PCR System (serial no. 275054149; Applied Biosystems, USA).

Cotton swab (lot 20250610, Jiangsu Kangjie Medical Devices Co., Ltd.); flocked swab (lot 20250625, Jiangsu Kangjie Medical Devices Co., Ltd.); polyester swab (lot 20250410, Shijiazhuang Jiayi Yuan Technology Co., Ltd.); sponge swab (lot 20240822, Shijiazhuang Jiayi Yuan Technology Co., Ltd.); CG viral transport medium (with sampling tube, lot 20250126, Hangzhou Yiguo Ren Biotechnology Co., Ltd.); MH viral transport medium (with sampling tube, lot 20241219, Hangzhou Yiguo Ren Biotechnology Co., Ltd.); 0.1 M PBS (lot 250625213, Fuzhou Xijing Biotechnology Co., Ltd.); normal saline (lot 15B25030304, Shandong Qidu Pharmaceutical Co., Ltd.); and autoclaved borosilicate glass test tubes with silicone stoppers.

The CG viral transport medium contained purified water, NaCl, Tris, EDTA, sodium dodecyl sarcosinate, phenol red, and other proprietary components. The MH viral transport medium contained purified water, Tris, EDTA, sodium dodecyl sarcosinate, guanidine isothiocyanate, phenol red, and other proprietary components.

# 1.3 Experimental Design

1.3.1 Standardized wiping procedure: Each 10 cm × 10 cm coupon was wiped 10 times horizontally and 10 times vertically. The swab tip was kept in continuous contact with the surface and rotated during wiping to maximize coverage. Immediately after sampling, the swab was transferred to the designated collection tube.

# 1.3.2 Evaluation of Different Sampling Swabs

Four swab types (polyester, sponge, cotton, and flocked) were compared on inoculated ceramic-tile coupons using PBS-pre-moistened sampling. Three independent experiments were performed for each swab type (total n = 12).

# 1.3.3 Evaluation of Different Environmental Surface Materials

Ceramic tile, polypropylene, wood, and woven fabric were inoculated as described above and sampled with PBS-pre-moistened polyester swabs. Three independent experiments were performed for each material

(total n = 12).

## 1.3.4 Evaluation of Different Sampling Time Points

Inoculated ceramic-tile coupons were maintained at 25°C and 60%–65% relative humidity and sampled at 1, 3, 6, 12, and 24 h using polyester swabs. Three independent experiments were performed at each time point (total n = 15).

## 1.3.5 Evaluation of Different Pre-sampling Treatments (Wet and Dry Sampling)

Two pre-sampling procedures were compared on ceramic tile: (1) PBS-pre-moistened sampling, using a polyester swab wetted with sterile 0.1 M PBS; and (2) dry sampling, using an otherwise identical unwetted polyester swab. The standardized horizontal/vertical wiping procedure was used in both groups. Five independent experiments were performed per group (total n = 10).

## 1.3.6 Evaluation of Different Ambient Temperatures

Inoculated ceramic-tile coupons were incubated at 15, 25, or 35°C, with temperature variation maintained within ± 1°C, at 50%–60% relative humidity. After 2 h, coupons were sampled using PBS-pre-moistened polyester swabs. Three independent experiments were performed per temperature (total n = 9).

## 1.3.7 Evaluation of Different Preservation Solution Types

Four preservation systems were evaluated: non-inactivating CG viral transport medium, inactivating MH viral transport medium, 0.1 M PBS, and normal saline (NS). A 100-μL aliquot of HPV-positive inoculum was added directly to 3 mL of each medium and stored at 2–8°C. Aliquots (200 μL) were tested after 1, 3, 6, and 24 h and after 3, 7, 14, and 28 days. Three technical replicate wells were analyzed at each time point (4 media × 8 time points × 3 replicates; total n = 96).

# 1.4 Sample Pretreatment and PCR Detection

## 1.4.1 Sample Pretreatment

For swabs collected without transport medium, 500 μL of normal saline was added to the tube. Samples were vortexed, transferred to 1.5-mL tubes, and centrifuged at 13,000 rpm for 10 min. A 200-μL mixture containing both supernatant and resuspended sediment was used for nucleic acid extraction.

## 1.4.2 PCR Detection

Samples were tested by qPCR using 40 μL of PCR master mix and 10 μL of extracted DNA. The assay targeted 23 HPV genotypes (16, 18, 33, 39, 45, 59, 35, 66, 51, 52, 53, 68, 31, 56, 58, 6, 26, 81, 82, 11, 42, 43, and 73), with β-globin as the internal control. Concentrations were generated by the kit software from an assay standard curve. The analytical limit of detection was 400 copies/mL, defined as the concentration detected in at least 95% of ≥ 20 replicate measurements. A genotype was considered positive when Ct ≤ 39 and the β-globin control was positive (Ct ≤ 40), with an acceptable sigmoidal amplification curve.

Cycling conditions were 50°C for 2 min (UDG reaction), 94°C for 5 min, followed by 45 cycles of 94°C for 15 s and 57°C for 30 s. Testing and result interpretation followed the manufacturers' instructions.

## 2. Statistical Analysis

Analyses were performed using IBM SPSS Statistics version 27. Shapiro–Wilk tests indicated that the principal outcomes were not normally distributed; therefore, Mann–Whitney U tests were used for two-group comparisons and Kruskal–Wallis H tests for comparisons of three or more groups. Significant omnibus tests were followed by Dunn pairwise comparisons with Bonferroni adjustment. Because each condition comprised only three to five independent observations, descriptive results are presented as mean ± standard deviation to remain consistent with the prespecified analysis and figures; non-parametric tests were used for inference. Two-sided P values < 0.05 were considered statistically significant. Figures were generated using GraphPad Prism 10.1.2 and Origin 2025.

Two outcomes were prespecified. The positive detection rate quantified genotype recovery, whereas a log-scale recovery index summarized the retained log10 concentration relative to the inoculum. The latter was treated as a supplementary comparative index rather than a conventional mass-balance recovery percentage.

Positive detection rate (%) = (Ndetected / Ninoculum) × 100

where Ndetected is the number of HPV genotypes detected after sampling and Ninoculum is the number of HPV genotypes detected in the inoculum.

Log-scale recovery index (%) = [log10(Crecovered) / log10(Cinoculum)] × 100

where Crecovered is the measured post-sampling HPV concentration and Cinoculum is the initial quantified HPV concentration. This metric should not be interpreted as the absolute proportion of viral material recovered.

## Results

# 3.1 Evaluation of Factors Before HPV Sampling

The effects of swab type, surface material, sampling interval, and swab pre-moistening were evaluated before subsequent temperature and preservation experiments.

# 3.1.1 Evaluation of Swab Types

Polyester and sponge swabs produced the highest positive detection rates (both 98.04% ± 3.40%), followed by cotton (96.08% ± 3.40%) and flocked swabs (94.12% ± 0.00%) (Table 1; Fig. 1A). The log-scale recovery index differed among swab types (H = 24.132, P < 0.001). Dunn tests with Bonferroni adjustment showed that flocked swabs had lower index values than the other three swabs (all adjusted P < 0.01),

whereas polyester, sponge, and cotton swabs did not differ significantly (all adjusted $P > 0.05$) (Fig. 1B). Polyester swabs were selected for subsequent experiments because they combined high detection performance with practical availability.

Table 1
Positive detection rate and log-scale recovery index according to sampling variable

| Research object | Variable classification | Positive detection rate(%) | Log-scale recovery index(%) |
|---|---|---|---|
| Swab type | Polyester swab | 98.04 ± 3.396 | 85.68 ± 15.53 |
| | Sponge swab | 98.04 ± 3.396 | 84.40 ± 14.87 |
| | Cotton swab | 96.08 ± 3.396 | 83.30 ± 19.33 |
| | Flocked swab | 94.12 ± 0.00 | 77.41 ± 21.67 |
| | H | 3.457 | 24.132 |
| | P value | 0.326 | < 0.001 |
| Environmental surface material | Ceramic tile | 98.04 ± 3.40 | 85.68 ± 15.53 |
| | Polypropylene | 94.12 ± 0.00 | 83.65 ± 22.37 |
| | Wood | 86.28 ± 3.40 | 52.43 ± 21.73 |
| | Woven fabric | 68.63 ± 3.40 | 28.61 ± 20.78 |
| | H | 10.275 | 137.916 |
| | P value | 0.016 | < 0.001 |
| Sampling time point | 1H | 85.42 ± 3.61 | 67.05 ± 31.09 |
| | 3H | 79.17 ± 13.01 | 58.61 ± 31.86 |
| | 6H | 91.67 ± 3.61 | 66.33 ± 23.55 |
| | 12H | 89.58 ± 7.22 | 65.52 ± 27.21 |
| | 24H | 91.67 ± 3.61 | 64.52 ± 26.87 |
| | H | 4.430 | 3.702 |
| | P value | 0.351 | 0.448 |
| Pre-sampling treatment | WET | 80.00 ± 5.229 | 61.19 ± 32.81 |
| | DRY | 88.75 ± 2.795 | 69.14 ± 28.59 |
| | Z | -2.356 | -1.496 |
| | P value | 0.032 | 0.135 |
| Ambient temperature | 15℃ | 85.42 ± 3.61 | 57.10 ± 27.07 |
| | 25℃ | 81.25 ± 0.00 | 56.68 ± 29.30 |
| | 35℃ | 56.25 ± 0.00 | 36.27 ± 33.59 |
| | H | 7.086 | 14.419 |

| Research object | Variable classification | Positive detection rate(%) | Log-scale recovery index(%) |
|---|---|---|---|
| | P value | 0.029 | < 0.001 |

## 3.1.2 Evaluation of Environmental Surface Materials

Both outcomes declined as surface roughness, porosity, and absorbency increased (positive detection rate: H = 10.275, P = 0.016; log-scale recovery index: H = 137.916, P < 0.001) (Table 1). Ceramic tile and polypropylene yielded substantially higher positive detection rates and log-scale recovery index values than wood and woven fabric (Fig. 1C,D). The log-scale recovery index did not differ significantly between ceramic tile and polypropylene, whereas wood had a higher index than woven fabric (adjusted P < 0.01).

## 3.1.3 Evaluation of Sampling Time Points

Across the five sampling intervals from 1 to 24 h after inoculation, neither positive detection rate (H = 4.430, P = 0.351) nor log-scale recovery index (H = 3.702, P = 0.448) differed significantly (Table 1; Fig. 1E,F).

## 3.1.4 Evaluation of Pre-sampling Treatments (Dry and Wet Sampling Methods)

Dry polyester swabbing yielded a higher positive detection rate than PBS-pre-moistened sampling (88.75% ± 2.80% vs 80.00% ± 5.23%; Z = -2.356, P = 0.032). The log-scale recovery index was numerically higher with dry swabbing but did not differ significantly (69.14% ± 28.59% vs 61.19% ± 32.81%; Z = -1.496, P = 0.135) (Table 1; Fig. 1G,H).

## 3.2 Evaluation of Factors during HPV Sampling

Based on the initial experiments, ceramic tile, polyester swabs, and sampling 1 h after inoculation were used in subsequent studies. Although dry swabbing yielded a higher positive detection rate, PBS-pre-moistened sampling was retained for the temperature and preservation experiments to maintain comparability with prevailing laboratory practice.

## 3.2.1 Evaluation of Ambient Temperatures

Ambient temperature significantly affected positive detection rate (H = 7.086, P = 0.029) and log-scale recovery index (H = 14.419, P < 0.001). At 35°C, the positive detection rate (56.25% ± 0.00%) and index (36.27% ± 33.59%) were lower than at 15°C (85.42% ± 3.61%; 57.10% ± 27.07%) and 25°C (81.25% ± 0.00%; 56.68% ± 29.30%). No significant difference was observed between 15°C and 25°C (Table 1; Fig. 1I,J).

## 3.2.2 Evaluation of Preservation Solution Types

The four media differed over the 28-day storage period (Table 2; Fig. 1M,N). CG and MH maintained mean positive detection rates above 70% and mean log-scale recovery index values above 40% through day 28. PBS first fell below the prespecified 70% positive-detection threshold on day 7 (66.67% ± 3.61%), whereas

NS remained at or above 70% through 3 h and fell below the threshold at 6 h (64.58% ± 3.61%). Thus, PBS may serve as a short-term alternative for storage or transport for < 7 days, whereas NS should be limited to very short transport intervals, preferably within 3 h. Overall performance ranked CG > MH > PBS > NS.

Table 2
Positive detection rate and log-scale recovery index of HPV samples stored in different media over time

| | | 1H | 3H | 6H | 24H | 3DAYS | 7DAYS | 14DAYS | 28DAYS |
|---|---|---|---|---|---|---|---|---|---|
| Positive detection rate(%) | CG | 81.25 ± 11.83 | 81.25 ± 6.250 | 81.25 ± 10.83 | 79.17 ± 7.217 | 83.33 ± 9.547 | 85.42 ± 13.01 | 83.33 ± 9.547 | 75.05 ± 6.250 |
| | MH | 81.25 ± 6.250 | 85.42 ± 3.608 | 81.25 ± 0.00 | 79.17 ± 3.608 | 83.33 ± 3.608 | 81.25 ± 6.250 | 81.25 ± 6.250 | 72.92 ± 7.217 |
| | PBS | 85.42 ± 3.608 | 70.83 ± 9.547 | 70.83 ± 7.217 | 79.17 ± 3.608 | 72.92 ± 9.547 | 66.67 ± 3.608 | 62.50 ± 0.000 | 50.00 ± 0.00 |
| | NS | 83.33 ± 13.01 | 70.83 ± 3.608 | 64.58 ± 3.608 | 60.42 ± 7.217 | 50.00 ± 0.00 | 41.67 ± 3.608 | 50.00 ± 6.250 | 37.50 ± 6.250 |
| | H | 0.875 | 6.863 | 6.9734 | 6.766 | 8.091 | 9.492 | 9.560 | 9.703 |
| | P value | 0.692 | 0.076 | 0.073 | 0.080 | 0.044 | 0.023 | 0.023 | 0.021 |
| Log-scale recovery index(%) | CG | 63.58 ± 33.43 | 66.73 ± 37.19 | 64.11 ± 34.55 | 62.95 ± 33.84 | 68.81 ± 32.75 | 69.61 ± 30.72 | 65.78 ± 31.43 | 51.60 ± 31.43 |
| | MH | 58.90 ± 29.57 | 61.10 ± 27.19 | 56.70 ± 28.75 | 56.05 ± 29.58 | 60.83 ± 28.43 | 59.08 ± 29.94 | 57.70 ± 29.20 | 49.34 ± 30.79 |
| | PBS | 61.10 ± 26.78 | 51.49 ± 34.43 | 47.71 ± 31.54 | 55.22 ± 29.46 | 54.57 ± 34.41 | 41.20 ± 29.84 | 43.07 ± 34.05 | 24.97 ± 26.01 |
| | NS | 56.46 ± 26.72 | 51.00 ± 34.22 | 39.19 ± 30.37 | 34.98 ± 24.55 | 27.42 ± 28.09 | 21.18 ± 25.77 | 26.96 ± 28.09 | 17.74 ± 23.48 |
| | H | 11.517 | 13.574 | 30.842 | 41.494 | 60.612 | 80.285 | 57.781 | 53.977 |
| | P value | 0.009 | 0.004 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

# 3.3 Concentration-stratified analysis

HPV genotypes with initial concentrations ≥ 10^6 copies/mL were classified as high concentration because their positive detection rates approached 100%; genotypes with concentrations ≤ 10^5 copies/mL

were classified as low concentration. Preservation-medium comparisons used the 1-h results at 6–8°C. Kruskal–Wallis tests were used for concentration-stratified analyses.

All high-concentration genotypes were detected (100%), and most experimental factors had limited influence; surface material remained the dominant factor ($P < 0.001$). Among low-concentration genotypes, surface material and ambient temperature were the two strongest determinants (both $P < 0.01$; Fig. 2A,B). The log-scale recovery index generally decreased as inoculum concentration declined (Fig. 2C-H).

## Discussion

This laboratory study systematically evaluated six pre-analytical determinants of molecular recovery of HPV DNA from environmental surfaces: swab type, surface material, sampling interval, swab pre-moistening, ambient temperature, and preservation medium. Positive detection rate was the primary endpoint, and the log-scale recovery index was used as a supplementary comparative measure. The findings support a standardized workflow centered on polyester swabs, smooth non-absorbent surfaces, controlled transport conditions, and dedicated viral transport media.

Polyester swabs combined high HPV DNA detection with the highest numerical log-scale recovery index and were therefore selected for subsequent experiments. Their performance is consistent with previous swab-sampling studies [7, 23, 25, 26]. Sponge swabs performed similarly but are generally more costly. Flocked swabs yielded lower index values, underscoring that swab performance depends on both the target material and surface properties [27–29, 40].

Surface material was one of the strongest determinants of molecular recovery. Smooth, non-porous, non-absorbent materials such as ceramic tile and polypropylene yielded higher positive detection rates and log-scale recovery index values than wood and woven fabric. Porous or absorbent matrices can sequester cells, viral particles, and nucleic acids below the accessible surface, thereby reducing swab recovery [28, 30, 31]. Consequently, frequently touched smooth surfaces may be particularly suitable sentinel sites for environmental HPV surveillance.

No significant change was observed over the 1–24-h sampling window. Previous studies indicate that environmental nucleic acid detectability and viral persistence are influenced by exposure conditions [13, 24, 32–34]; therefore, the short-term stability observed here should not be interpreted as evidence that infectious HPV virions remained viable. More broadly, PCR detection of viral material on healthcare surfaces indicates environmental contamination but does not by itself establish transmission of viable virus [35]. Future studies incorporating capsid-integrity assays or cell-based infectivity testing are required before conclusions regarding transmission risk can be drawn.

Dry swabbing improved genotype detection relative to PBS pre-moistening, although the secondary index did not differ significantly. Pre-moistening may dilute low-abundance material or alter adhesion between swab fibers and the contaminated surface. Published studies have used or directly compared different swab-moistening approaches across organisms and matrices, with variable effects on recovery [15, 17, 18,

23, 25, 36–39, 41]. The pre-moistening procedure should therefore be validated for the specific target and surface rather than assumed to be universally advantageous.

Ambient temperature substantially influenced HPV DNA recovery. Exposure to 35°C for 2 h reduced both endpoints, potentially because accelerated drying and stronger adsorption impeded recovery or because extracellular DNA underwent more rapid degradation [12, 13, 42]. The lack of a significant difference between 15°C and 25°C indicates that both conditions were acceptable within the tested interval; it does not establish superior long-term stability at 15°C.

Dedicated CG and MH media best maintained HPV DNA detectability during refrigerated storage. This finding is consistent with reports showing that dedicated transport or preservation media can support reliable molecular detection [43, 44]. Because qPCR measures nucleic acid rather than infectivity, these findings should be described as preservation of molecular detectability, not preservation of viral integrity or viability. The 70% detection threshold was prespecified as a practical criterion: PBS crossed this threshold on day 7, while NS remained at or above 70% through 3 h and crossed the threshold at 6 h. These data support dedicated media for prolonged storage, PBS for short-term storage or transport for < 7 days, and NS only for very short intervals [45–47].

The study has several limitations. The inoculum was a heterogeneous pooled clinical suspension containing infected epithelial cells, extracellular particles, and cell-free HPV DNA; therefore, the relative contribution of each component could not be determined. The experiments were performed under controlled laboratory conditions and did not include ultraviolet exposure, cleaning residues, competing microbiota, blank-swab controls, or uninoculated-surface controls. The log-scale recovery index is not a conventional absolute recovery percentage and requires external validation. In addition, infectivity was not assessed, and the findings should not be interpreted as direct evidence of fomite transmission.

Strengths include the systematic evaluation of six pre-analytical variables, use of multiple HPV genotypes spanning a wide concentration range, independent replication for the principal sampling experiments, and explicit molecular quality-control procedures. Each run included an extraction-negative control and the kit-provided PCR-positive control. Contamination-prevention measures included physically separated work areas, filtered pipette tips, routine environmental decontamination, and a UDG/dUTP carryover-control system.

In conclusion, dry polyester swabs and dedicated viral transport media provided the best overall molecular sampling performance under the tested conditions. Smooth, non-porous surfaces and moderate ambient temperatures favored HPV DNA recovery, whereas 35°C and absorbent materials reduced detection. The proposed workflow is suitable for further validation in real-world settings, but additional field sampling, conventional recovery calculations, and infectivity-related assays are needed before it can inform transmission-risk assessment or public-health policy.

# Declarations

**Ethics approval and consent to participate**

This study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine (Approval No. Yan 2024 – 0207). The requirement for informed consent was waived by the Ethics Committee. All clinical specimens were anonymized before pooling and analysis. All methods were performed in accordance with relevant guidelines and regulations.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

# Funding

This study was supported by the Science and Technology Program for Disease Prevention and Control of Zhejiang Province (grant no. 2026JKZ040) and the Medical and Health Research Science and Technology Plan Project of Zhejiang Province (grant no. 2024KY077).

# Author Contribution

Conceptualization, Zhihua Tao; methodology, Xiuzhi Duan, Meng Yang; software, Xuchu Wang; validation, Xuchu Wang; formal analysis, Meng Yang; investigation, Meng Yang; resources, Xiuzhi Duan; data curation, Xiuzhi Duan,Meng Yang, and Yiyi Xie; writing-original draft preparation,Meng Yang; writing-review and editing, Xiuzhi Duan, and Yiyi Xie; visualization, Yiyi Xie and Xuchu Wang; supervision, Liqing Xu; project administration,Zhihua Tao; funding acquisition, Meng Yang. All authors have read and agreed to the published version of the manuscript.

# Acknowledgements

Not applicable.

# Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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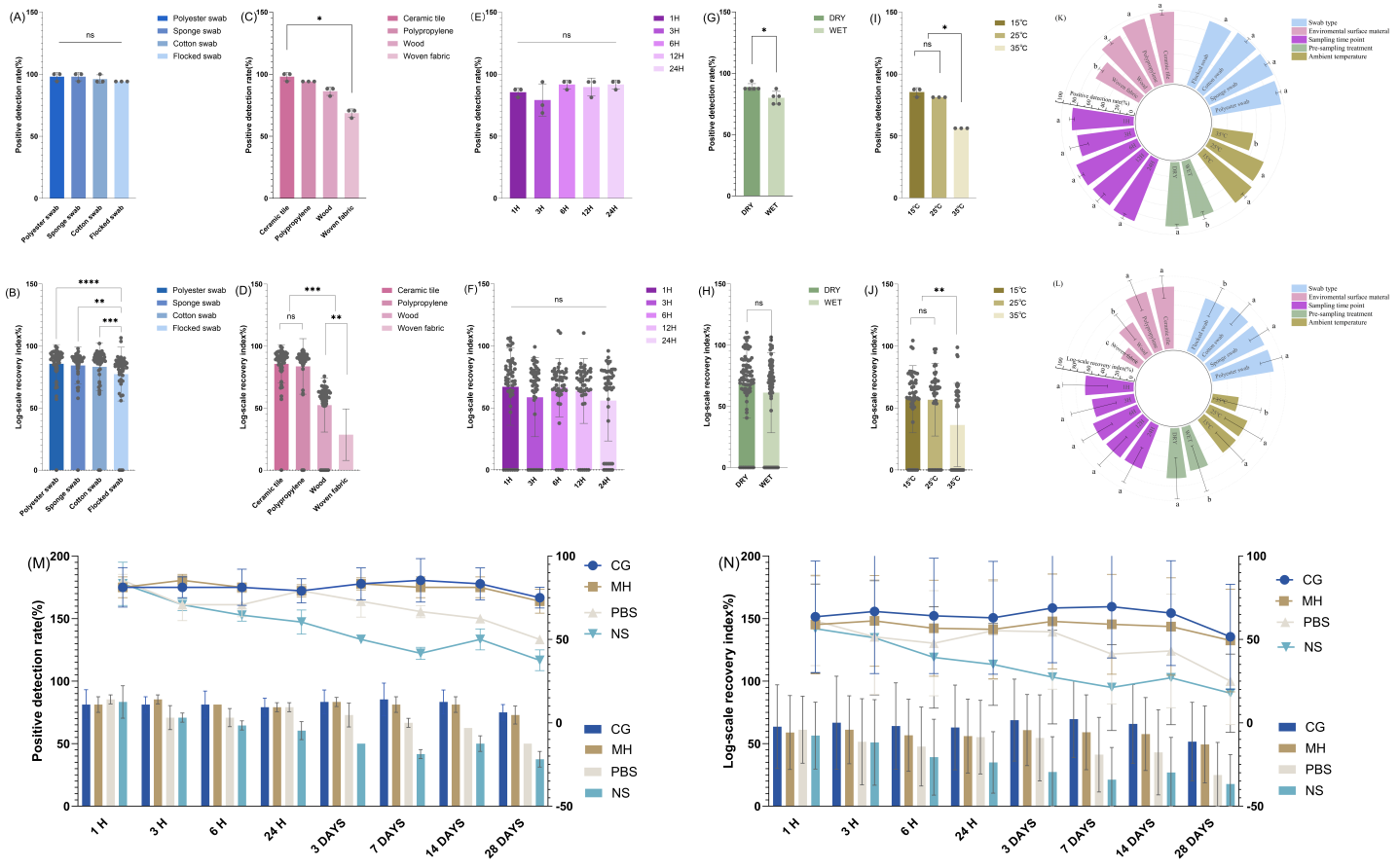
# Figures



## Figure 1

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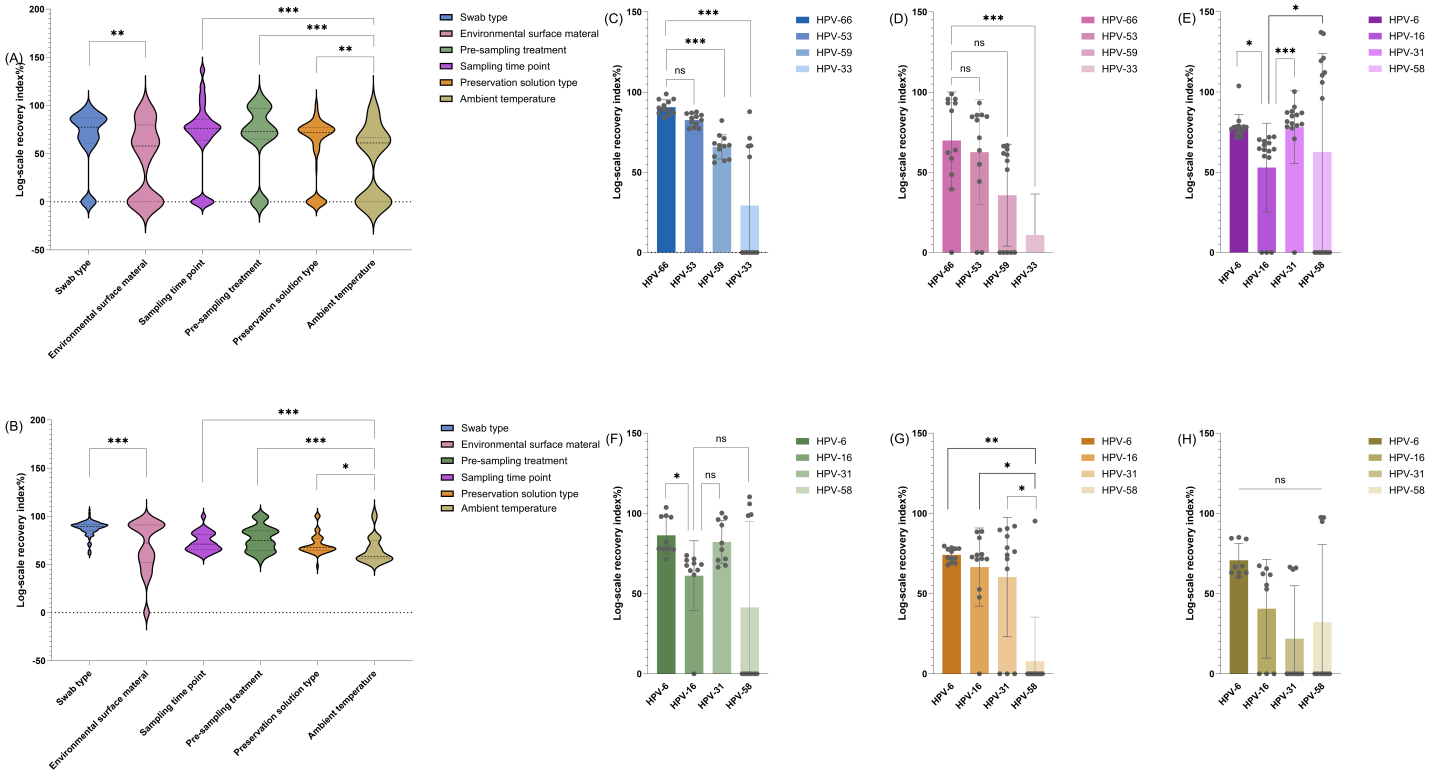


## Figure 2

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