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Validation of the CyMol Medium for the ability to preserve HPV DNA for amplification assays

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ABSTRACT
Objectives: A variety of commercial molecular amplification assays for the detection of Human Papilloma viruses (HPV) are currently available or in the process of being CE or FDA approved. A collection and transportation system, that supports multiple diagnostic methods, like cell morphology for cytology and nucleic acids stability for the diagnosis of infectious agents, compatible with all commercial platforms would be beneficial. Copan has developed a new pre-analytical medium, the CyMol, which preserves cellular morphology and stabilizes DNA, and has been tested previously for compatibility with molecular assays available commercially. The objective of this study was to validate the Copan CyMol Medium's (CM) ability of stabilizing HPV DNA from clinical specimens stored at room temperature.
Materials and Methods: To prove CM's ability of preserving different HPV genotypes 3 specimens were taken from 8 species of the alpha-papillomavirus genus according to deVilliers et al. 2004. Clinical specimens were collected into 3 ml PBS. From each sample 4x0.2 ml was taken, spanned down and resuspended in 3x2 ml CM and once into PreservCyt. Samples were incubated at room temperature for 3 weeks. DNA was extracted with Roche's AmpliLute Liquid Media Kit from 0.25ml specimen weekly. At the zero sampling point the extracted DNA was diagnosed for HPV-High Risk/Low Risk by GenoID's FullSpectrum System and genotyped with Roche's LinearArray System and GenoID's genotyping assay. At every measuring point, HPV DNA was quantified by genotype specific TaqMan assay. **Results:** HPV DNA genotypes from the different subfamilies were stable after 3 weeks storage at room temperature without any significant DNA loss, according to our studies. **Conclusions:** The Copan CyMol medium stabilizes and preserves HPV DNA from clinical specimens for up to 3 weeks at room temperature.

INTRODUCTION

The effect of HPV to cause malignant lesions in women's genital areas is a well known and widely studied fact. This confirms the importance of the screening of gynecological samples for HPV besides traditional cytology screening. There are several commercially available and in-house made HPV detection assays. A collection and transportation system, that supports multiple diagnostic methods (such as cell morphology for cytology and nucleic acid stability for the diagnosis of infectious agents) compatible with all commercial platforms would be beneficial.
Copan Italia Spa. developed a new pre-analytical medium, the CyMol (CM), which is CE marked for transportation, preservation of epithelial cells collected by scraping and nucleic acids. It preserves cellular morphology and stabilizes DNA, and has been previously tested for compatibility with commercially available molecular assays.
The success of proper HPV detection depends a lot on the DNA preserving ability of the transport medium and the quality of the isolated DNA from the specimen. Accordingly, an extensive study is needed to validate the transport medium DNA preserving abilities. In this study we investigated CM's HPV DNA preserving efficacy from clinical specimens stored at room temperature for three weeks.

MATERIALS AND METHODS

All the samples used in this study were collected with ethical approval (ad.459/PI/2007. ad.22-279/2007-1018EKU). Gynecologists collected cervical samples with Cyto Brush Plus (SUMMAMED BT. Gödöllő, Hungary) placed into 3 ml PBS. Samples were shipped frozen to GenoID diagnostic laboratory. Before processing the samples, 1 ml aliquot was stored at -80˚C. Routine diagnostic assay for HPV was performed with the rest of the samples at GenoID laboratory. To prove CyMol's medium ability to preserve different HPV genotypes, based on the results obtained, 3 specimens were selected from each of the eight species of the alpha-papillomavirus genus, according to deVilliers et al. 2004 (Table 2.). Four 0.25 ml aliquots were prepared from the corresponding samples stored at -80˚C. After a brief centrifugation, 3 of the aliquots were resuspended in tubes with 2 ml CyMol medium (Copan Italia spa, Brescia, Italy) and as gold standard, one aliquot was resuspended in a tube with 2 ml PreservCyt solution (Cytyc Corp., Malborough, MA). The prepared samples were stored at room temperature for three weeks. Weekly, DNA was extracted from 0.25 ml sample, using the Roche's AmpliLute Liquid Media Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. At zero time, the extracted DNA was analyzed with GenoID's Full Spectrum HPV DNA Amplification and Detection System for the presence of high-risk and low-risk HPV. HPV genotyping was done with GenoID Genotyping test and, as a gold standard, with the Roche's Linear Array HPV Genotyping Test (Roche). Genotyping tests were done according to the manufacturers' instructions. HPV DNA was quantified at each testing time with an in-house type-specific TaqMan reactions. TaqMan assays were performed on an ABI StepOnePlus Real Time PCR instrument (Applied Biosystems, Foster City, CA). For copy number determination, duplicate dilution series of the target were used in each type specific TaqMan reaction. ABI StepOnePlus Standard Quantitation Curve software was used to analyze the data. Two parallel reactions were performed from each DNA sample. The mean value of the two parallel reactions is the estimated copy number of the DNA sample. (Figure 1.)

RESULTS

27 samples were chosen for this evaluation. During the six months study period, no HPV43 positive sample (representing species 8) was found. After resuspension in CyMol and analysis of the extracted DNA. 3 samples were found to be species 5, 3 samples were species 10 and another 3 samples were species 7. Only 2 samples were found to be species 6, and 4 samples species 9. Samples infected with HPV42, representing species 1, had low DNA copy number for investigation after resuspension in the transport media (Table 1.). Since the HPV DNA preservation ability of the media is not related to the genotype species, the abscence of some species does not affect the result. There was no significant difference in the DNA copy numbers between the samples stored in CyMol and in PreservCyt. There were some variations in both media, due to sampling and DNA extraction process. This explains why, in some cases, a 5% increase of DNA copy numbers was noted during storage in both media. There was no significant difference in DNA preserving ability of the two transport media. (Figure 2.)

CONCLUSION

Copan CyMol medium (CE marked) preserves HPV DNA up to 3 weeks at room temperature as efficiently as PreserCyt does.

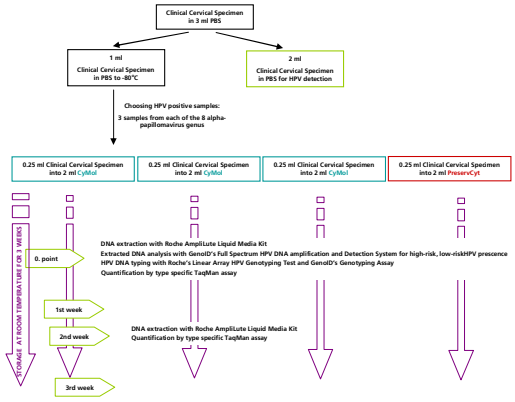

Figure 1. Study workflow

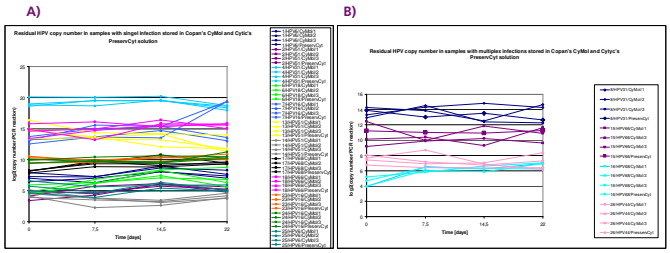

Figure 2. A) Representation of the residual HPV copy numbers during the incubation in CyMol at room temperature for three weeks in case of single infections, and **B)** in case of multiplex infections. Parallel incubations, started from the same sample, are represented with the same colour. Data from aliquots stored in PreservCyt is labelled with thicker line.

Sample Number	Detected Genotypes By GenoID's Full Spectrum HPV System from CyMol and PreservCyt	Detected Genotypes by Roche's Linear Array HPV Genotyping Test from CyMol and PreservCyt	Species	Sample status in the study
1	HPV6	HPV6	10	Investigated
2	HPV51	HPV51	5	Investigated
3	HPV56	HPV56	6	Low copy number for investigation
4	HPV31	HPV31	9	Investigated
5	-	-	-	negative
6	HPV18	HPV18	7	Investigated
7	HPV16	HPV16	9	Investigated
8	HPV31	HPV31; 61	9	Multiplex infection
9	-	-	-	Investigated
10	HPV42; 59; 6	HPV42	1	Low copy number for investigation
11	HPV42	HPV42	1	Low copy number for investigation
12	HPV42	HPV42	1	Low copy number for investigation
13	HPV51	HPV51	5	Investigated
14	HPV51	HPV51	5	Investigated
15	HPV66; 59; 51	HPV66; 59; 51	6	Multiplex infection
16	HPV68; 6	HPV68; 6	7	Multiplex infection
17	HPV68	68	7	Investigated
18	HPV66	66	6	Investigated
19	HPV68	-	7	negative
20	HPV42	42	1	Low copy number for investigation
21	HPV42	42	1	Low copy number for investigation
22	HPV42	42	1	Low copy number for investigation
23	HPV16	16	9	Investigated
24	HPV16	16	9	Investigated
25	HPV6	6	10	Investigated
26	HPV64	44; 61	10	Multiplex infection
27	HPV66	66; 73	6	Low copy number for investigation

Table 1. Status of the samples analyzed in the study Investigated samples are highlighted in blue (singel infections) and green (multiplex infections). Samples with low or no copy number are presented with black colour.

HPV species	HPV types
γ-papillomavirus species 1	HPV-42
γ-papillomavirus species5	HPV-51
γ-papillomavirus species 6	HPV-56; -66
γ-papillomavirus species 7	HPV-18; -39; -45; -59; -68
γ-papillomavirus species 8	HPV-43
γ-papillomavirus species 9	HPV-16; -31; -33; -35;-52;-58
γ-papillomavirus species 10	HPV-6; -11; -44-

Table 2. Classification of alpha papillomavirus types according to deVilliers et al. 2004.

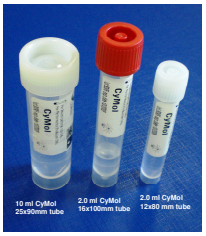

Figure 3. The three different formats of CyMol medium