# Rapid, high level inactivation of infectious HPV16 and HPV18 using hypochlorous acid (HOCl)

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## INTRODUCTION

High-risk human papillomaviruses (HPV 16 & 18) are causal agents of cervical, anal, and oropharyngeal cancers.1,2 Oral HPV infections and associated malignancies have increased alarmingly in recent years in the US, even as the rates of cervical cancer have begun to decline. Our previous studies using suspension and carrier tests demonstrated strikingly poor efficacy of common disinfectant agents in inactivating high-risk HPV (Table 1), 3 even with FDA-approved chemical sterilants, such as GTA and OPA. The potential for iatrogenic transmission in healthcare environments, including dental offices, from inadequate disinfection practices has therefore become a concern. Risk potentials and prior efficacy determinations have commonly been based on outdated criteria or inappropriate test systems.

**Objective:** To establish the efficacy of pure, stable preparations of HOCl (from Briotech Inc.) in the inactivation of HPV 16 & 18. Our approach to quantifying inactivation depends on use of large amounts of authentic infectious virus, and well-established methods of replication and testing of high-risk HPVs.

## METHOD & MATERIALS

Infectious high titer stocks of HPV16 and HPV18 were produced, titered, and infectivity-tested as previously described.1-5 Both suspension and carrier tests were performed with contact times spanning 15 seconds to 20 minutes. Following contact remaining HOCl was neutralized using a Tween 80/peptone/cysteine/Tris buffer formulation (pH 7.5). Residual HPV was isolated and measured by our published infectivity methods.1-5

## RESULTS

HOCl treatment contact times generally and quickly produced >99.99% reduction in infectivity of HPV16 and HPV18, comparable to the efficacy of 0.87% sodium hypochlorite. Exposure of cell fractions enriched by vector expression of L1 and L2 capsid proteins of BPV to HOCl for 30 seconds resulted in rapid aggregation of these and other extracted proteins on SDS PAGE gels.

## CONCLUSION

HOCl is a highly effective disinfectant for HPV 16 & 18, even after contact times as short as 15 seconds. Rapid changes in capsid proteins may be responsible for this decline.

Significance: More than 70% of oropharyngeal cancers are caused by high risk HPV. Infection rates range from 3-10%,6 and while most are eventually shed, some persist to cause cancerous lesions. Effective inactivation of HPV on surfaces in the dental office can clearly be accomplished using HOCl solutions that are safe for topical application to skin and mucosa. This raises the potential for HOCl use not only as a disinfectant for dental environments, but also for direct applications to oral and other tissues, e.g., as an oral rinse.

## REFERENCES

1. Ryndock E, Robison R, Meyers C. Susceptibility of HPV16 and 18 to high level disinfectants indicated for semi-critical ultrasound probes. J Med Virol. 2016;88(6):1076–1080.doi:10.1002/jmv.24421

2. Meyers C, Milici J, Robison R (2017) UVC radiation as an effective disinfectant method to inactivate human papillomaviruses. PLoS ONE 12(10): e0187377.

3. Jordan Meyers, Eric Ryndock, Michael J. Conway, Craig Meyers, Richard Robison, Susceptibility of high-risk human papillomavirus type 16 to clinical disinfectants, Journal of Antimicrobial Chemotherapy, Volume 69, Issue 6, June 2014, Pages 1546–1550.

4. Meyers, C. Methods Cell Sci (1996) 18: 201.

5. Biryukov, J., Cruz, L., Ryndock, E. J., & Meyers, C. (2015). Native human papillomavirus production, quantification, and infectivity analysis. Methods in Molecular Biology, 1249, 317-331.

6. https://www.cdc.gov/cancer/hpv/statistics/index.htm

Table 1. Reduction of HPV 16 infectivity with clinical disinfectants.*

| Disinfectant | Native virion ($log_{10}$ reduction) | Quasivirion ($log_{10}$ reduction) |
|---|---|---|
| 70 % Ethanol | -0.789 | -0.197 |
| 95 % Ethanol | -0.076 | -0.307 |
| 95 % Isopropanol | -0.272 | -4.435 |
| 3.4 % GTA | -0.306 | -0.145 |
| 0.55 % OPA | 0.017 | 0.200 |
| 0.525% Hypochlorite | 4.862 | 0.623 |

*Data from Reference 3

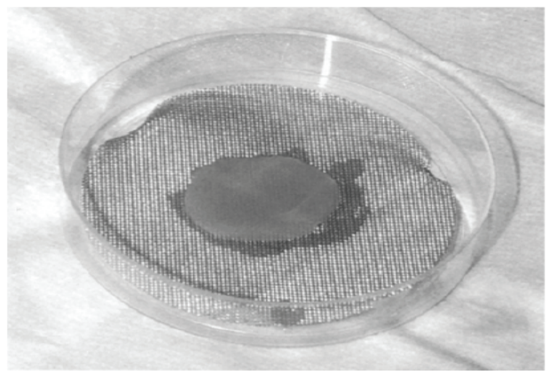

Figure 1. Image of in vitro organotypic (raft) culture of human skin

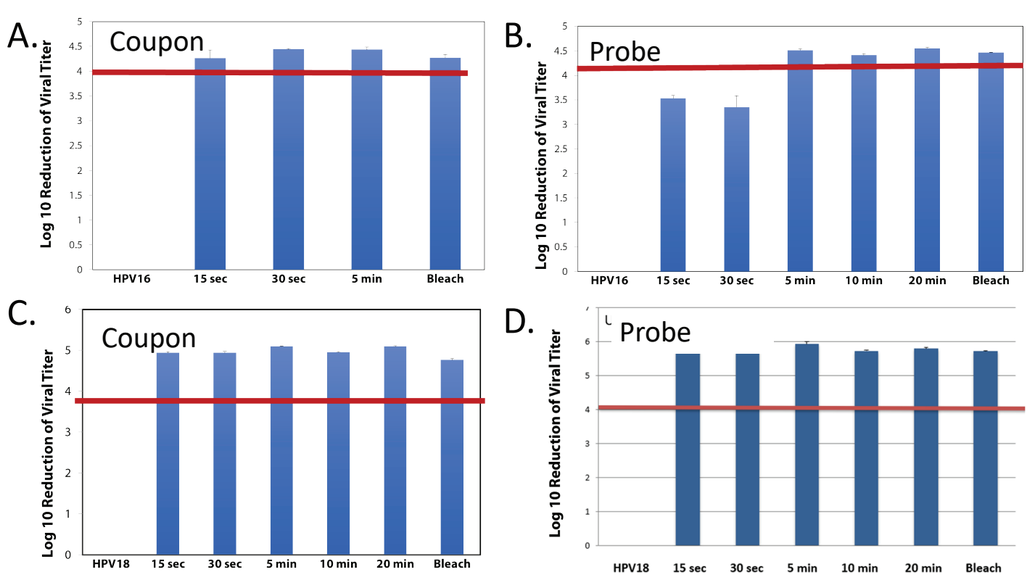


Figure 2. Inactivation of HPV16 and HPV18 after exposure to HOCl for different contact times. **A.** HPV16 inactivation on coupons of butadiene styrene, and **B** on ultrasonic probes. **C.** HPV18 inactivation on coupons of butadiene styrene, and **D** on ultrasonic probes. HOCl was used at 250-300 ppm. Bleach controls (~9000 ppm) were exposed for 10 minutes.

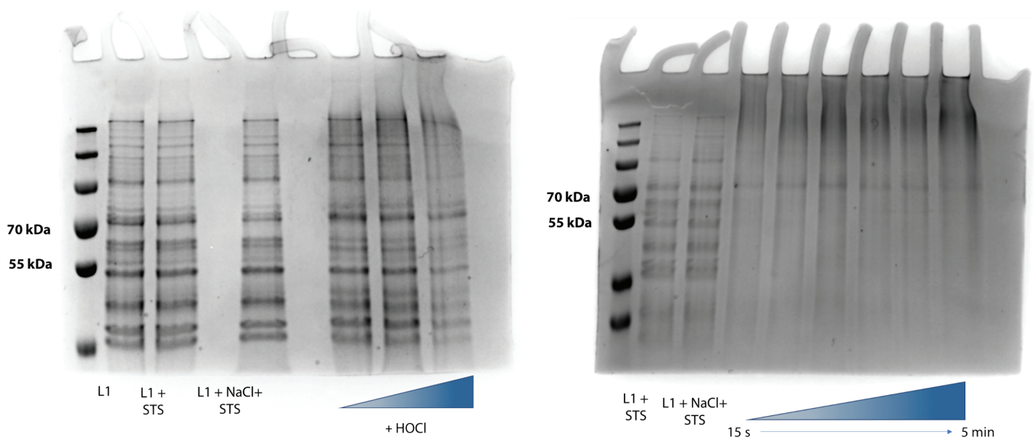


Figure 3. SDS-PAGE of L1 bovine papilloma virus (BPV). BPV was incubated with various concentrations of HOCl (0.6-3.0 mM) for 5 minutes (left). BPV was incubated with 3 mM HOCl for various times (right). All samples were quenched with sodium thiosulfate (STS) and denatured prior to loading on the gel.

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