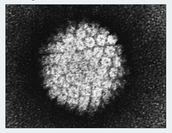
Developing a Safe and Effective Papillomavirus Screen to be used on College Students

Owen R. Smith[1], Nicole E. Frantz[1], Emily E. Bishop[2], Rahjai G. Thomas[2], and Dr. Paul E. Richardson

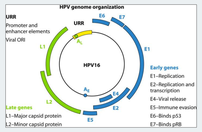
[1]*Department of Chemistry,* [2]*Department of Biology*

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection that accounts for approximately 5% of all cancers worldwide and affects more than 80 million people in the US alone, according to the CDC and National Cancer Institute. Human papillomaviruses are small, nonenveloped, icosahedral DNA viruses that infect squamous epithelial cells. The viral particles consist of a single double stranded DNA molecule bound to histones and contained within a protein capsid composed of structural proteins late (L)1 and L2. To date, over 100 different genotypes of HPV have been identified, and approximately 15 types are considered oncogenic in cervical, vulvar, vaginal, anal, penile squamous epithelia, and more recently, in head and neck squamous cells.



Electron micrograph of a papillomavirus



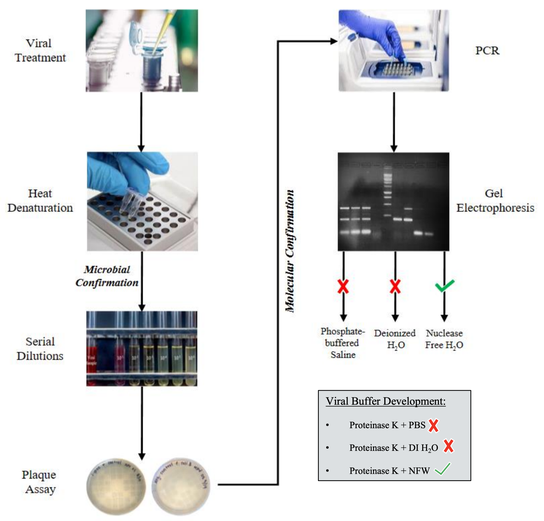
Genomic organization of HPV

Research Questions

1. **What methodology will allow for the complete inactivation of ALL virions in a given sample?**

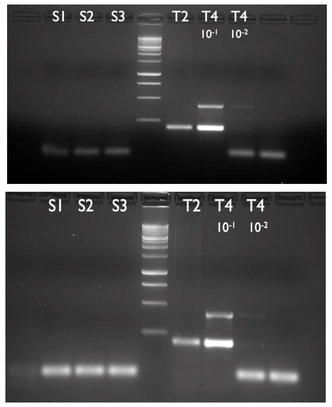
2. **How can we ensure the developed viral treatment will leave behind detectible genomic material?**
3. **What is the detection threshold of papillomavirus?**

Methods

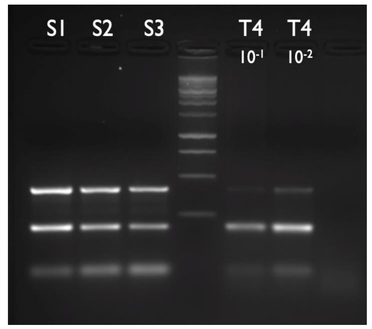


Developmental Results

Viral Buffer Development
Proteinase K + PBS/DI H_2O ✗

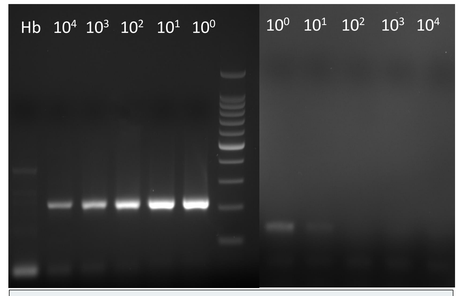


Viral Buffer Development
Proteinase K + NFW ✓

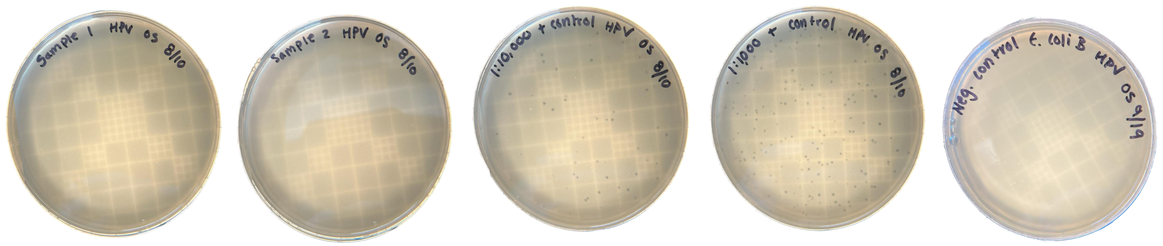


Gel with positive (+) bacteriophage T4 detection using CPO primer set

Papillomavirus Detection Threshold
E6/7 L1



L1 primer detection limit of 2E4 copies of papillomavirus DNA per 4µL pap L1; E6/7 detection exceeds 2E1 copies papillomavirus DNA per 4µL pap E6/7.



Microbial plaque assays for *E. coli* B strain from 2022. All 3 buffer formulations resulted in complete virial inactivation.

Papillomavirus Primers

Target type	Sequence	Target Gene(s)	Primer Size	PCR Fragment Size
Papillomavirus	CCGTTGTGTCCAGAAGAAAA	E6/7	20bp	**230-270bp**
	GAGCTGTCGCTTAATTGCTC	E6/7	20bp	
Papillomavirus	TTTGTTACTGTGGTAGATACTAC	L1	23bp	**150bp**
	GAAAAATAAACTGTAAATCATATTC	L1	25bp	
Control Gene (β Globin)	CAACTTCATCCACGTTCACC	β-Globin	20bp	**260bp**

Conclusion

What methodology will allow for the complete inactivation of ALL virions in a given sample?

- All 3 buffer formulations proved to efficiently inactivate all virions in each sample- demonstrated through the consistent absence of bacterial zones of lysis in all buffer treated plaque assays.
- Bacteriophage T4 served as an optimal model virus for method development
- Positive control dilutions verified lytic virions in untreated samples, at an initial concentration of 6.2E5 PFU/mL

How can we ensure the developed viral treatment will leave behind detectible genomic material?

- Initial molecular identification with PBS solution proved ineffective at providing detectible genomic results.
- Alterations using DI H_2O provided inconsistent results when processed under molecular verification
- Viral buffer supplemented with NFW provided consistent results; adequately detecting viral genomic material in all tested samples.
- Repeated positive molecular detection with NFW indicates its reliability in ensuring accurate results.

What is the detection threshold of papillomavirus?

- Papillomavirus primer set L1 has a detection threshold of 2.0E4 copies of papillomavirus DNA per 4µL of pap L1 primer.
- Papillomavirus primer set E6/7 has a detection threshold less than 2.0E1copies of papillomavirus DNA per 4µL of pap E6/7 primer.
- E6/7 was found to detect papillomavirus DNA (sourced from ATCC) at a 10^3 lower concentration than pap L1.

Future Directions

- Administer a behavioral survey to members of the Coastal Carolina community to obtain empirical data on the frequency of HPV-risk associated behaviors.
- Refine the collection methodology for sampling of the oral cavity, specifically from the squamous epithelial cells of the lingual and palatine tonsils.
- Develop ethical and practical procedures for participant self-collected samples of the genital areas. More specifically, samples of the vaginal epithelial cells and cells of the external penile epithelial cells.
- Statistical analysis of the quantitative and qualitative data with the intent of potentially establishing a correlation between risk behaviors and HPV infections, as well as determine the prevalence of infections amongst men and women in a comparative manner.
- In all, the investigation of HPV amongst college students is purposed around enhancing the available information on the infection's prevalence in both men and women. Specifically, closing the gap on the disparity of accessible data for men.
- This investigation is NOT being implemented as a substitution for clinically administered HPV testing. All consenting participants will be informed to consult with a provider if they are concerned with contracting HPV.