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 United States (US) alone, according to the Centers for Disease Control and Prevention (CDC) and National Cancer Institute (NCI). 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 (L1) 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. This study aimed to develop an experimental methodology that will allow for the safe and effective detection of HPV among members of Coastal Carolina University (CCU) campus. Genomic isolation techniques were developed for the purpose of generating inactive, noninfectious viral particles, while preserving their genomic fingerprint for future characterization. Bacteriophage T4 are highly robust viral particles that are found naturally in the external environment, serving as a model virus for the initial establishment of safe and effective isolation techniques. Consensus primers were identified for a PCR-based HPV detection assay, targeting the conserved L1 and E6/E7 ORF (Open Reading Frame) regions of the HPV genome. The developed methodology provided effective and reproducible viral characterization, enabling the future applications of these techniques to be applied for the detection of HPV amongst members of the Coastal Carolina community.

Introduction

Papillomaviridae is a family of nonenveloped DNA viruses containing over 200 genetically distinct species, or types, known as papillomaviruses. Human papillomavirus (HPV) is the most common sexually transmitted infection, accounting for approximately 4.5% of all cancers worldwide (630,000 new cancer cases per year)\(^1\). Papillomaviruses are nonenveloped double stranded DNA (dsDNA) viruses composed of a small 8kb genome enclosed by two outer structural proteins: Late(L1) and L2\(^2\). Over the last century, there have been over 200 different HPV genotypes identified. Of those 15 types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68, -82) have been reported to possess an innate ability to induce oncogenic malignancies within cervical, vulvar, vaginal, anal, penile squamous epithelia, and more recently, in head and neck squamous cells\(^3\). These virions share indistinguishable genomic structures and organizations but vary widely in their epithelial tropism and life-cycle differentiations. The highest level of viral replication occurs in the granular layer of stratified epithelia where keratinocytes are terminally differentiated and are in the process of enucleation and death; the connective tissue found lining the top of the tongue and the esophageal track\(^4\). Enhanced activity of the E6 and E7 viral oncoproteins increase the oncogenic capacity of these high-risk types (HR-HPV), promoting active cell proliferation within the basal and well differentiated epithelium. While most HPV infections are transient, persistent carcinogenic-type infections induce the formation of precancerous lesions and neoplasms.

Papillomavirus research fostering methods of disease prevention and communal protection have been sparked ever since the 1983 postulation made by Harald zur Hausen- German virologist and professor emeritus-regarding the role of papillomavirus in cervical carcinogenesis\(^5\). Hausen was awarded the Nobel prize in 2008 for his discovery of HPV-16, and later HPV-18 in cervical cancer\(^6\). This acknowledged not only the importance of his discovery in the etiological understanding of HPV within specific oncogenic malignancies, but also the importance of this discovery for the clinical application of HPV screening and development of the prophylactic vaccine\(^6\).

In 2006 the FDA (Food and Drug Administration) approved the licensing of the first prophylactic vaccination against HPV. It contained two L1 (major capsid proteins) virus-like particles and additional adjuvants that enhanced both the major histocompatibility class I and class II, to activate cytotoxic [CD8\(^+\)] and T-Helper [CD4\(^+\)] lymphocyte presentation pathways, respectively\(^7\). Girls ages 11 and 12 served as the primary focus group for vaccination because the vaccine would only serve as an effective preventative measure if administered before the age of potential HPV exposure; its therapeutic application requires further investigation\(^8\). The initial isolation towards female vaccination over males was in large part due to the former discoveries made by Hausen and others, in addition to a lack of research in the effects that HPV has within men. It wasn’t until 2011 that boys were then considered as candidates to receive prophylactic HPV vaccination\(^9,10\). At the time of the policy, the United States was the only country to consider boys as eligible candidates for HPV vaccination and remained so for several remaining years. The quadrivalent HPV vaccine (qHPV) provides an effective method for individual protection against persistent HPV associated infections against HPV-6, 11, 16, and 18. Its administration has been documented to minimize the risk of HPV associated high-grade cervical intraepithelial neoplasia, anal condyloma- grade 1 anal intraepithelial neoplasia variant-, and other malignancies such as head and neck squamous cell carcinomas (NHSCC) in both women and men respectively; though the latter is not limited to the male population\(^10,11\). Specifically, the quadrivalent vaccine protects against HPV 16/18, which cause nearly 70% of all invasive cervical cancers\(^12\). There is a tremendous number of benefits for the administration of the qHPV vaccine; it is crucial to enhance the available data for HPV’s prevalence within both the male and female populations to further prevent its spread via methods of education and communal awareness.

This study aims to develop experimental methods of viral inactivation that will allow for the retention and subsequent detection of HPV’s viral genome in solution. This will promote the later analysis of HPV’s prevalence in both men and women comparatively, specifically within a college demographic. Peak prevalence of HPV occurs between 20 and 25 years of age, making college aged individuals the primary focus group\(^6,10\). There is an abundance of literature discerning the prevalent effects of HPV within females, yet the data remains scarce when analyzing men\(^13\). While the oncogenic effects of HR-HPV types are mitigated through childhood vaccination, low-risk types (LR-HPV) remain transient and may confer unpleasant viral indications. The most common manifestation of HPV is genital warts, or condyloma acuminate (CA)\(^12\). An estimated 1% to 2% of all sexually active individuals in the United States have CA, and the incidence has continued to increase steadily since the 1950s\(^13\). While LR-HPV infections are unlikely to result in malignant HPV-associated tumors, its effects may still illicit negative connotations within the individual.

Papillomaviruses are highly transmissible DNA viruses that infect epithelial cells. This indicates the need to develop methodology that will ensure adequate viral inactivation, while rendering all viral DNA detectible in solution. Inactivating all detectible HPV among members of Coastal Carolina University (CCU) campus. Genomic isolation techniques were developed for the purpose of generating inactive, noninfectious viral particles, while preserving their genomic fingerprint for future characterization. Bacteriophage T4 are highly robust viral particles that are found naturally in the external environment, serving as a model virus for the initial establishment of safe and effective isolation techniques. Consensus primers were identified for a PCR-based HPV detection assay, targeting the conserved L1 and E6/E7 ORF (Open Reading Frame) regions of the HPV genome. The developed methodology provided effective and reproducible viral characterization, enabling the future applications of these techniques to be applied for the detection of HPV amongst members of the Coastal Carolina community.

References

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

Undergraduate Research Article

Developing a Safe and Effective Papillomavirus Screen to be used on College Students at a South Carolina University

Owen R. Smith, Nicole E. Frantz, Emily E. Bishop, Rahjai G. Thomas, and Paul E. Richardson

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effectivity of the developed methodology, bacteriophage T4 was used as a model viral substitution. Bacteriophages, otherwise known as phages, are non-pathogenic, naturally occurring viruses that infect and kill bacterial cells. Phages possess their innate bactericidal activity by directly injecting their viral genome into the bacterial host cell, hijacking its cell machinery to produce viral copies. As mentioned, phages are found naturally within the environment. They can be found abundantly in fecal-contaminated water and within the human microbiome. Phages are amongst the most abundant and ubiquitous nucleic acid-based entities vital to maintaining the biological homeostasis of earth and likewise humans. The abundance and nonpathogenic nature of phages makes them optimal candidates for laboratory substitution. Additionally, the subject to continuous environmental stressors renders phages highly robust much more so than host dependent papillomaviruses—meaning, they are much more resistant to viral inactivation. Confidently, if methods of complete phage inactivation are proven with high efficacy, the application of these procedures towards HPV inactivation can be asserted.

This study was conducted through an initial viral inactivation stage, followed by molecular characterization. It is hypothesized that if viral inactivation is sufficient with a robust virus such as phage, then it will work just as well for a much more host dependent and stress-susceptible virus, such as HPV. To prevent additional unwanted degradation, a temperature-controlled heat treatment was utilized. Microbial testing was used to confirm active phage particles’ presence, while molecular testing confirmed viral DNA retention. This study’s purpose was to further develop viral HPV sampling methods that ensure efficiency and safety of all participating parties. Additionally, the viral detection limit for the primer sets E6/7 and L1 need to be investigated to determine their sensitivity to various genomic concentrations. This led to the following questions: 1. What methodology will allow for complete viral inactivation of all virions in a sample? 2. Do the methods of viral inactivation retain detectable genomic material? 3. What is the detection threshold of the papillomavirus primers sets E6/7 and L1?

Methods

Study Design

Bacteriophage T4 was used as a model viral substitution for the developmental progressions of these procedures due to their accessibility, robust, and non-pathogenic nature. Three samples were individually collected from a prepared viral culture and were processed under microbial and molecular testing. Microbial testing ensured whole sample inactivation, while molecular testing was used to discern the presence/absence of viral genomic material. Five consecutive positive DNA detections were required to discern the established methods’ efficiency. All samples were processed with adequate controls and under controlled conditions to ensure the accuracy of the results.

Viral Inactivation

Viral cultures were prepared by adding 10µL of bacteriophage T4 culture and 1mL of phosphate buffered saline (PBS) (PBS Tablets, Calbiochem) solution into a 2.0mL microcentrifuge tube (Thermo Fisher). A viral buffer was then prepared by combining 50µL of Proteinase K (Thermo Fisher) and 950µL of PBS solution. 10µL of viral culture and 390µL of viral buffer was combined in a sterile 2.0mL microcentrifuge tube. It was then incubated at room temperature for 1-hour on a shaking incubator. Samples were then processed under heat denaturation in a 95°C heat block for 30 minutes. These steps were repeated, substituting the PBS solution for deionized water (DI H2O) and nuclease free water (NFW) (Promega Corporation).

Plaque Assays

Plaque assays were prepared by adding 200µL of E. coli B liquid culture to three sterile 2.0mL microcentrifuge tubes, followed by a 20-minute 37°C incubation period. Fifty microliters (50µL) of buffer treated sample was added to each tube and let incubate for 30 minutes at 37°C in a shaken incubator. Fifty microliters (50µL) of E. coli B liquid culture and untreated bacteriophage T4 were used as a negative and positive control, respectively. Standard top agar was prepared and stored in a temperature-controlled water bath set to 47°C. Standard 2x LB Agar plate were incubated for 10 minutes at 37°C. Two-hundred microliters (200µL) of microbial sample was then added to a sterile 15mL centrifuge tube, followed by 11mL of prepared top agar. Samples were mixed by careful inversions and were slowly poured onto the plate. Plates were allowed to dry for 15 minutes and were placed upside down and were incubated overnight. Plaque assays were analyzed the following day to determine the presence of lytic activity. Results were classified as having no lytic activity, definite lytic activity, or non-determinate (ND) activity (sample contaminants obstructed visualization of potential lytic zones). All ND samples were processed again to obtain clearer results.

Crude DNA Extraction

Positive control samples were prepared using standard DNA extraction techniques. One hundred microliters (100µL) of phage containing sample were placed into a 2.0mL microcentrifuge tube and centrifuged at 2,500 rpm for 5 minutes. The supernatant was transferred to a new microcentrifuge tube. Five microliters (5µL) of proteinase K (Thermo Fisher) were added and the sample incubated at room temperature on a shaking incubator for 60 minutes. Samples were then placed in a heat block for five minutes at 95°C and stored at -20°C until further use.

Polymerase Chain Reaction (PCR)

Following DNA extraction, polymerase chain reaction (PCR) was conducted. Twenty-five microliters (25µL) of Gotaq Green Master Mix (Promega Corporation), 21µL template (DNA extraction), and 4µL of the corresponding primer set were added to a 0.2 mL PCR tube (VWR International). PCR analysis was conducted in the BioRad T100 Thermocycler. PCR analysis was conducted as follows: an initial 4-minute DNA unwinding step at 95°C, followed by 39 cycles of DNA denaturation (30 seconds at 94°C), primer annealing (1 minute at 55°C), and DNA extension (72°C for 2 minutes). After completion, PCR products were held at 4°C for short-term storage. The primer set for coliphage comprised of CPO, primer sets for papillomavirus comprised of E6/7 and L1 are delineated in Table 1.

Gel Electrophoresis

PCR products were imaged by gel electrophoresis using 2% agarose (Agarose I, VWR) gels and 1x Tris-acetate EDTA (TAE) buffer. Five microliters (5µL) of ethidium bromide were used as a staining agent within the 2% agarose gel. Seven microliters (7µL) of 1 kb DNA ladder (Promega Corporation) and 10µL of PCR product were loaded into the corresponding wells. Gel electrophoresis was conducted at 100 volts for 90 minutes (1 and a half hours) before being imaged under UV light with the Molecular Imager ChemiDoc XRS+ Imaging System from BioRad Laboratories, Inc.

PCR Primers

Coliphage primers consisted of the CPO primers set, cultivated by Dr. Paul E. Richardson’s lab at Coastal Carolina University. CPO is comprised of ORF23 and ORF43. Papillomavirus primers consisted of the E6/7 and L1 primer sets; obtained from a 2019 Universidad Mayor de San Simón (UMSS) dissertation by Pedro Surriabre. Human β-globin primer set included PC100 and GH100, which were derived from R.K. Saiki, et al. Coliphage primer set CPO comprised of 0.67µL ORF23 Forward (“For”), 0.67µL ORF43 Reverse (“Rev”), 0.67µL ORF43 For, 0.67µL ORF43 Rev, and 1.33µL of nuclease-free water. Papillomavirus primer set papL1 consisted of 1.33µL papL1 For, 1.33µL papL1 Rev, and 1.33µL of nuclease-free water. Papillomavirus primer set papE6/7 comprised of 1.33µL papE6/7 For, 1.33µL papE6/7 Rev, and 1.33µL of nuclease-free water. The above CPO, papL1, papE6/7, and β-globin primers each totaled 4.0µL to be used individually in the PCR reactions.

Primer Detection Threshold

Papillomavirus p53 DNA (American Type Culture Collection) at an initial concentration of 1E5 copies per microliter of solution, was used
for the establishment of the detection threshold for primers E6/7 and L1. Ten microliters (10μL) of pap DNA were added to 40μL of NFW. Serial dilutions were performed up until 2 copies of pap DNA remain in solution. Standard PCR and gel electrophoresis procedures were performed as previously described. The results were analyzed under UV light with the Molecular Imager ChemiDoc XRS+ Imaging System from BioRad Laboratories, Inc. Coliphage primer set CPO has a detection limit of 2 viral particles in solution, which was previously confirmed by Cannon et al. in 201325.

### Results

#### Microbial Conformation

30 samples were processed and analyzed to determine the efficiency of viral inactivation. Of the 30 samples, 0% formed lytic zones when subjected to plaque assays. This was demonstrated in the PBS, DI H₂O, and NFW conditions (Table 2). No observable difference was indicated between the three testing conditions. Comparatively, the bacteriophage T4 positive control dilutions (10⁻³, 10⁻⁴) showed numerous lytic zones at an initial coliphage concentration of sixty-two thousand plaque forming units per milliliter of solution (6.2E5 PFU/mL). 18 additional samples were processed by viral inactivation but were omitted from microbial plaque assays due to the repeated absence of active viral indicators.

#### Molecular Conformation

48 samples were processed through molecular PCR techniques. PBS buffer condition indicated a complete absence, 0% (0/6; samples 1-6), of positive viral detection using the CPO primer set. Buffer formulation supplemented with DI H₂O resulted in the positive detection of viral DNA 73.3% (11/15; samples 7-21) of the time. DI H₂O resulted in inconsistencies that needed to be mitigated for when employing these procedures in experimental settings. Positive detection of viral DNA in the NFW buffer condition resulted in accurate detection 100% (27/27; samples 22-48) of the time (figure 1). Trials were repeated consecutively to ensure accurate results.

#### Papillomavirus Detection Threshold

Primer detection thresholds were determined using the HPV p52 template at an initial concentration of 1E5 copies of DNA per microliter of plasmid solution. Primer set E6/7 was determined to have a detection limit of less than 20 copies of DNA per microliter (4μL) of E6/7 primer (figure 2a). The L1 primer set was determined to have a detection limit of 2E4 (twenty thousand) copies of DNA per four microliters (4μL) of L1 primer (figure 2b). E6/7 was shown to have a heightened sensitivity to lower genomic concentrations compared to the L1 primer; variable sensitivity may provide insight towards the relative concentrations of viral DNA when sourced directly from consenting participants.

### Discussion

From September 2016 to November 2017, an epidemiological cross-sectional study of the prevalence of HPV in sexually active men and women was conducted in the Federal District of Brazil under the approval of the Moinhos de Vento Hospital human subjects research board21. This was, and remains, one of the only studies to have analyzed the prevalence of HPV in both men and women simultaneously, allowing the direct comparison of HPV rates between the genders. HPV infections are typically transient infections, HR-HPV infections can last up to 12-18 months before eventually being cleared by the immune system22. Studies indicate nearly 10% of women ineffectively clear HPV infections, resulting in persistent long-term infections; a primary risk factor of HPV-associated carcinogenesis23. Targeted interactive informational interventions are an effective method of increasing the awareness HPV and the willingness of adolescents to get vaccinated within the targeted range of prophylactic administration24. Further discerning the comparative prevalence of HPV within a college demographic requires supporting methods of viral inactivation and genomic isolation.

#### Microbial Conformation

Microbial plaque assays signifying the activity of virions within a sample indicated the complete absence of active virions in all tested samples. 100% (30/30; samples 1-30) of the samples processed under microbial testing demonstrated an absence of lytic viral activity. Active coliphage T4 possesses a distinct bactericidal potentiality with E. coli B bacterium. Coliphage T4 functions by injecting their genomic material into the host E. coli bacterial cell, hijacking its cellular machinery for the production of more viral copies. The accumulated result of this process is delineated through lytic zones. Meaning, the absence of these zones is a direct measure of the buffer's efficiency in whole sample capsid degradation, inactivating all subsequent virions. Viral inactivation was shown to be independent of the buffer formulation. All three conditions—PBS, DI H₂O, and NFW—produced equivalent results. Repeated succession of these results prompted the omission of future microbial conformations, as all samples regardless of buffer condition demonstrated satisfactory inactivation. The ability to render all virions within a sample inactive significantly increases the safety of the developed procedures. It completely mitigates the risk of accidental infections and eliminates the possibility of ever handling live HPV within a sample; further improving the safety of the methodology.

#### Molecular Conformation

Differences in formulation efficiency arose during molecular testing and genomic characterization. Samples processed with the PBS diluent indicated a complete absence of detectable genomic material. 0.00% (0/6; samples 1-6) of viral samples produced amplified PCR fragments of ORF23 and ORF43. PBS is often considered an efficacious medium for the storage and transport of viral specimens. Conversely, studies...

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Table 1: Primer set CPO aided in the identification of retained coliphage DNA. CPO was derived from L. Pieterse, et al.17. Pap E6/7 and L1 were used in the identification of HPV p52 DNA at 10-fold concentration differences. The dissertation of Pedro Surriabre aided in the attainment of E6/7 and L1 primer sets18.

<table>
<thead>
<tr>
<th>Primer Set</th>
<th>Target Family/ Organism</th>
<th>Gene target</th>
<th>PCR Fragment Length (bp)</th>
<th>Primer Name</th>
<th>Primer Size (bp)</th>
<th>Primer Sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPO</td>
<td>Coliphage T2 - T4</td>
<td>ORF 3</td>
<td>405</td>
<td>ORF23FRev</td>
<td>20</td>
<td>TGCGGACGATATCAGAATG</td>
</tr>
<tr>
<td></td>
<td>Coliphage T2 - T4</td>
<td>ORF 43</td>
<td>198</td>
<td>ORF23Rev</td>
<td>20</td>
<td>GCCAGCCTCCTAGTGTTT</td>
</tr>
<tr>
<td>Pap E6/7</td>
<td>Papilloma Virus</td>
<td>Oral E1</td>
<td>250-270</td>
<td>ORF3F</td>
<td>20</td>
<td>TCGCTGCTCTCTTAAT</td>
</tr>
<tr>
<td>Pap L1</td>
<td>Papilloma Virus</td>
<td>Oral E2</td>
<td>150</td>
<td>ORF4RRev</td>
<td>20</td>
<td>ATGCGAGAACGCTCCCAA</td>
</tr>
<tr>
<td>β Globin</td>
<td>Human Globin Gene</td>
<td>β Globin</td>
<td>260</td>
<td>BetaFRev</td>
<td>20</td>
<td>GAAGACAAGGACGACGTC</td>
</tr>
</tbody>
</table>
The L1 primer set was determined to have a detection threshold of 2E4 copies of DNA in solution. Positive viral indications were demonstrated in both the DI H2O and the NFW conditions. This was indicated by a 73.3% (11/15; samples 7-21) and a 100% (27/27; samples 22-48) detection rate, respectively. Inconsistencies within the DI H2O condition resulted in its immediate substitution for NFW. Impurities within DI H2O samples were presented as occasional molecular interference in amplification stages, whereas the purity of NFW prevented any substance level interference from occurring within the molecular amplification protocols. As a result, from the repeated positive detection of viral DNA, NFW will be used in all future experimental applications.

Papillomavirus Detection Threshold

Application of these methods for HPV characterization warrants a demand for an enhanced comprehension of the detection boundaries for HPV specific primers. Primer sequences E6/7 and L1 were sources from a 2019 dissertation by Pedro Surriabre at the Universidad Mayor de San Simón (UMSS)\(^\text{18}\). The oncogenicity of HPV is heavily dependent upon the continuous activity of the E6 viral oncoproteins. E6 and E7 are tumor-associated antigens (TAA) that directly alter interrelate cellular processes for the promotion of cell proliferation and tumor development\(^\text{26}\). They work by inhibiting p53 and pRB tumor suppressors, respectively, giving cancerous cells an opportunity to bypass the resolution of oncogenic signals and DNA damage to continue abnormal proliferation\(^\text{7,28}\). Targeting the E6/7 sequence for molecular detection is an enthralling option as these sequences are expressed in high-degree lesions and cancer. Likewise, the L1 primer sequence is an excellent target sequence for broad HPV detection. Transcribed late in the viral genome, the major capsid protein L1, a near 55kD structural protein, that can undergo spontaneous self-assembly into virus-like particles (VLP)\(^\text{29}\). It conceals the entire external surface of HPV virions, and plays a key role in stabilizing mature virions, in addition to mediating the attachment to host cells and tissues. Extensive studies in various clinical settings have been conducted using the L1 primer sequence, reporting sufficient type-independent detection of papillomavirus. Due to its pivotal role in overall survival, the L1 sequence is highly conserved among various HPV-types. Additionally, it is crucial to understand the sensitivity of the primers at hand, as the level of viral DNA may vary from person to person. The E6/7 primer sequence was experimentally determined to have a detection threshold of less than 20 copies of DNA, while the L1 primer set was determined to be 2E4 (twenty thousand) copies of DNA per four microliters (4μL) of L1 primer. Naturally acquired samples will vary significantly in the amount of detectible genomic material, and understanding the limitations of the material's used to detect the presence of viral DNA

Table 2: Viral inactivation summary of coliphage T4 microbial (plaque assay) and molecular (PCR) retention results by buffer condition. *Samples 30-48 were omitted from microbial testing as further verification for viral inactivation was not warranted.

<table>
<thead>
<tr>
<th>Buffer Type</th>
<th># of Samples</th>
<th># of Positive Microbial Indications</th>
<th>% of Positive Microbial Indications</th>
<th># of Positive Molecular Tests</th>
<th>% of Positive Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate Buffered Saline (PBS)</td>
<td>6 (Samples 1-6)</td>
<td>0/6</td>
<td>0.00%</td>
<td>0/6</td>
<td>0.00%</td>
</tr>
<tr>
<td>Deionized water (DI H2O)</td>
<td>15 (Samples 7-21)</td>
<td>0/15</td>
<td>0.00%</td>
<td>11/15</td>
<td>73.3%</td>
</tr>
<tr>
<td>Nuclease free water (NFW)</td>
<td>27 (Samples 22-48)</td>
<td>0/9</td>
<td>0.00%</td>
<td>27/27</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 1: Depicts positive molecular (PCR) indication of coliphage T4 in the NFW condition using primer set CPO. This is delineated by samples 33-35 (S33, S34, S45) producing PCR fragments at 198bp and 405bp. Coliphage T2 and T4 served as positive controls and NFW as a negative. Positive indication was absent in all PBS trials. Positive T4 detection was present in only 11/15 samples using the DI H2O formulation.

Figure 2a: Depicts the Pap E6/7 primer detection threshold, surpassing a 10\(^{-3}\) dilution; threshold was established below 2E1 copies of DNA in solution. 10\(^0\) = 2E5 copies, 10\(^-1\) = 2E4 copies, 10\(^-2\) = 2E3 copies, 10\(^-3\) = 2E2 copies, and 10\(^-4\) = 2E1 copies of DNA. Figure 2b: Depicts the Pap L1 primer detection threshold at a 10\(^{-3}\) dilution; threshold was established at 2E4 copies of DNA in solution. 10\(^0\) = 2E5 copies, 10\(^-1\) = 2E4 copies, 10\(^-2\) = 2E3 copies, 10\(^-3\) = 2E2 copies, and 10\(^-4\) = 2E1 copies of DNA.

have speculated that PBS has an optimal cation concentration for suppressing molecular interference, but no further investigation into this theory is warranted for the purpose of this study\(^\text{25}\). The repeated lack of detectible DNA in the PBS formulation resulted in its immediate dismissal from all experimental stages as it lacked the required ability to provide necessary molecular products. Positive viral indications were demonstrated in both the DI H2O and the NFW conditions. This was indicated by a 73.3% (11/15; samples 7-21) and a 100% (27/27; samples 22-48) detection rate, respectively. Inconsistencies within the DI H2O condition resulted in its immediate substitution for NFW. Impurities within DI H2O samples were presented as occasional molecular interference in amplification stages, whereas the purity of NFW prevented any substance level interference from occurring within the molecular amplification protocols. As a result, from the repeated positive detection of viral DNA, NFW will be used in all future experimental applications.
becomes ever important. Variance in detectability ensures that both low and elevated viral concentrations will be detected in actively collected samples.

**Future Developments**

Establishing safe and effective laboratory techniques for detecting HPV was the first step towards active HPV screening. The development of additional experimental measures for proper sampling of consenting participants is being investigated. HPV is a sexually transmitted infection, meaning there is an inherent social stigma surrounding its acquisition. Methods fostering values of noninvasiveness, privacy, and self-sampling serve as the foundation for sampling methodology. The administration of a behavioral survey to members of the Coastal Carolina community, obtaining empirical data on the frequency of HPV-risk associated behaviors, represents an additional effort to establish potential correlations between behavioral engagement and HPV prevalence. This survey is being created by members of the Coastal Carolina University Department of Health Sciences, ensuring proper ethicality of its employment to students. All consenting participants will be informed that this investigation is not being used for the substitution of clinically administered HPV screening. Rather, this project aims to enhance the available information regarding HPV’s prevalence for both men and women within a college demographic.

**Conclusion**

Limited prior data commands further investigations into the prevalence of HPV within both men and women comparatively. The immediate study demonstrated that a broad-spectrum serum proteinase-based buffer is an effective method for inactivating all virus-like particles within a sample. Microbial level testing did not show variance between samples regarding overall inactivation. There was, however, an unequivocal efficacy difference in genomic detection between the buffer formulations. NFW proved time after time to provide the most reliable particles within a sample. Microbial level testing did not show variance based buffer is an effective method for inactivating all virus stock, PCR-based strategy for high-risk human papillomavirus DNA detection for cervical cancer prevention. BMC infectious diseases. 2019;19(1):842.


