# Monitoring of Hawaiian Beach Water Quality Using Enteric Viruses As Alternative Indicators

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## Abstract

Recreational waters contaminated with human fecal pollution are a public health concern, and ensuring the safety of recreational waters for public use is a priority of both the Environmental Protection Agency and the Centers for Disease Control and Prevention. Current recreational water standards rely on fecal indicator bacteria levels as indicators of human disease risk. However present evidence indicates that levels of FIB do not always correspond to the presence of other potentially harmful organisms, such as viruses. Thus, enteric viruses are currently tested as water quality indicators, but have yet to be successfully implemented in routine monitoring of water quality.

This study utilized enteric viruses as possible alternative indicators of water quality to examine 18 different fresh and offshore recreational waters on O'ahu, Hawai'i, by using newly established laboratory techniques including highly optimized polymerase chain reaction (PCR), real time PCR, and viral infectivity assays. All sample sites were detected positive for all four viruses tested in this study by PCR including enterovirus, norovirus genogroups I and II, and male specific FRNA coliphage. A six time-point seasonal study of enteric virus presence indicated significant variation in virus detection between the rainy and dry seasons. Quantitative PCR detected the presence of norovirus genogroup II at levels at which disease risk may occur, and there was no correlation found between enteric virus presence and FIB counts. Under the present laboratory conditions, no infectious viruses were detected from the samples PCR-positive for enteric viruses.

These data emphasize the need for improved monitoring of Hawaii recreational water quality for safe use, and support the notion of using human enteric viruses as potential alternative indicators

## Background

Current water quality monitoring standards rely solely on fecal bacterial indicators. However, it has been shown that these bacterial indicators do not reflect the presence of viral pathogens in the water. Enteric viruses have been associated with waterborne disease outbreaks worldwide, and are of public health concern, due to their resilience in the environment, low infectious dose, and high degree of host specificity. Enteric viruses persist and propagate in the gastrointestinal tract, are shed via feces, and are spread through fecal-oral contact. Due to these factors, human enteric viruses should be tested for the use as potential alternative indicators for recreational water quality monitoring. Our laboratory has recently established effective methods of enhanced concentration and detection of viral pathogens from environmental water samples; this study utilizes these methods to conduct a seasonal study of enteric virus presence in beach waters around Oahu.

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## Materials & methods

**Sample Collection and Processing:** 18 sites corresponding to four different water types were sampled at six different time points; three during rainy season and three during dry season. Five milliliters of 5M $MgCl_2$ was added to freshwater sample and left to sit for five minutes. Samples were filtered over type HA negatively charged 0.45µm filter membranes (Millipore).

**Nucleic Acid Extraction:** Nucleic acids were extracted using the MoBio PowerWater kit RNA isolation kit using a modified protocol designed for both RNA and DNA extraction. RNA was treated with DNAse-I solution to remove any trace of DNA contamination, and was stored at -80°C until further processing. RNA samples were subjected to RT-PCR and cDNA was stored at 4 °C .

**PCR Detection:** cDNA was subjected to previously established highly optimized PCR conditions to test for four different viruses: enterovirus, norovirus genogroup I, norovirus genogroup II, and male-specific FRNA coliphage. Results were visualized using gel electrophoresis on a 2% agarose gel stained with ethidium bromide solution.

**Statistical Analysis:** Statistical analysis was done using Microsoft Excel 2010. T-tests were used to determine significant differences between wet and dry season for overall detection and percent of possible detections. ANOVA was used to determine differences between types of water and viruses detected. Alpha was set at 0.05 for all analyses.

## Results

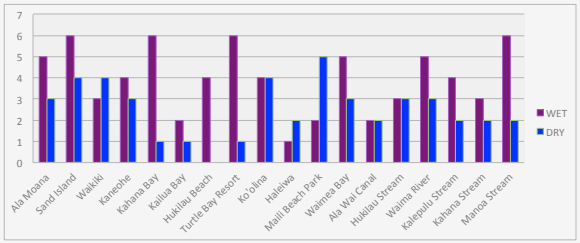


**Figure 1. Total detections in rainy and dry seasons, by site.** All sites tested positive at least three times over the course of the study; the site with the most positive detections was Sand Island with a total of 10 detections throughout the study period.

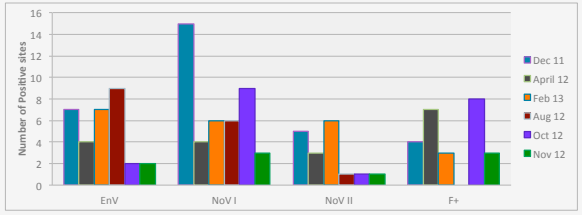


**Figure 2. Total number of detections of each virus type, by month**. Four different viruses were tested at six different time points; this graph shows the total number of detections of a particular virus at one time point.

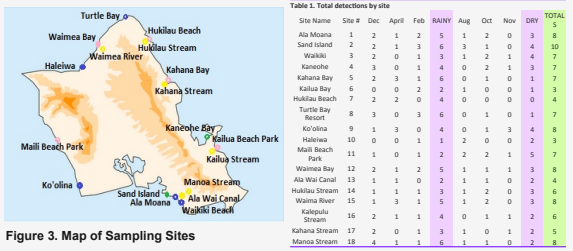


**Figure 3. Map of Sampling Sites**

**Table 1. Total detections by site**

| Site Name | Site # | Dec | April | Feb | RAINY | Aug | Oct | Nov | DRY | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| Ala Moana | 1 | 2 | 1 | 2 | 5 | 1 | 2 | 0 | 3 | 8 |
| Sand Island | 2 | 2 | 1 | 3 | 6 | 3 | 1 | 0 | 4 | 10 |
| Waikiki | 3 | 2 | 0 | 1 | 3 | 1 | 2 | 1 | 4 | 7 |
| Kaneohe | 4 | 3 | 0 | 1 | 4 | 0 | 2 | 1 | 3 | 7 |
| Kahana Bay | 5 | 2 | 3 | 1 | 6 | 0 | 1 | 0 | 1 | 7 |
| Kailua Bay | 6 | 0 | 0 | 2 | 2 | 1 | 0 | 0 | 1 | 3 |
| Hukilau Beach | 7 | 2 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| Turtle Bay Resort | 8 | 3 | 0 | 3 | 6 | 0 | 1 | 0 | 1 | 7 |
| Ko'olina | 9 | 1 | 3 | 0 | 4 | 0 | 1 | 3 | 4 | 8 |
| Haleiwa | 10 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 2 | 3 |
| Maili Beach Park | 11 | 1 | 0 | 1 | 2 | 2 | 2 | 1 | 5 | 7 |
| Waimea Bay | 12 | 2 | 1 | 2 | 5 | 1 | 1 | 1 | 3 | 8 |
| Ala Wai Canal | 13 | 1 | 1 | 0 | 2 | 1 | 1 | 0 | 2 | 4 |
| Hukilau Stream | 14 | 1 | 1 | 1 | 3 | 1 | 2 | 0 | 3 | 6 |
| Waima River | 15 | 1 | 3 | 1 | 5 | 1 | 2 | 0 | 3 | 8 |
| Kalepulu Stream | 16 | 2 | 1 | 1 | 4 | 0 | 1 | 1 | 2 | 6 |
| Kahana Stream | 17 | 2 | 0 | 1 | 3 | 1 | 0 | 1 | 2 | 5 |
| Manoa Stream | 18 | 4 | 1 | 1 | 6 | 1 | 1 | 0 | 2 | 8 |

Statistical Analysis revealed significant differences in detections between rainy and dry seasons, with significantly higher mean total detections in rainy season as compared to the dry season ($p<0.05$). Additionally, there were significant differences in mean detection between rainy and dry season based on type of water ($p<0.05$).

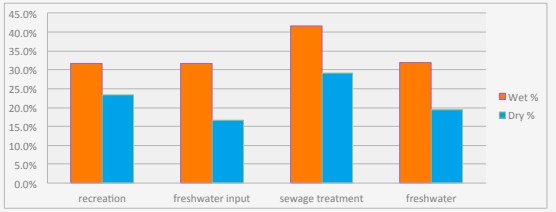


**Figure 4. Percent of total possible detections in rainy and dry season, by water type.** Four different water types were tested; Sites near sewage treatment outfalls had generally higher rates of detection than other types of sites.

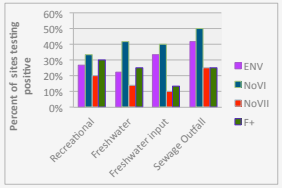


**Table 2.** Correlation between bacterial counts and viral presence determined in six sampling sites

| | | Enterococci (MPN/100 mL) | C. perfringens (CFU/100 mL) | EnV[a] | NoVI[b] | NoVII[c] | F+[d] |
|---|---|---|---|---|---|---|---|
| Test 1 | Ala Wai | 30 | <1 | - | + | - | - |
| | Sand Island | <10 | <1 | - | - | - | - |
| | Kailua | 10 | <1 | - | + | - | - |
| | Haleiwa | <10 | <1 | + | + | - | + |
| | Manoa Stream | 262 | 42 | - | - | - | - |
| | Ala Moana | 10 | <1 | - | + | + | - |
| Test 2 | Ala Wai | 836 | 10 | + | + | - | + |
| | Sand Island | <10 | <1 | - | - | - | - |
| | Kailua | <10 | <1 | + | - | - | - |
| | Haleiwa | <10 | <1 | + | - | - | - |
| | Manoa Stream | 4884 | 2 | + | - | + | - |
| | Ala Moana | <10 | <1 | + | + | - | - |

## Conclusions

- Enteric viruses can be utilized effectively as indicators of fecal contamination of beaches and streams in Hawaii.
- Substantial fecal contamination of Hawaii's recreational beaches and streams exists, and this contamination appears to vary significantly with season or rainfall.
- Detection of human enteric viruses was not correlated with and FIB counts.
- Future studies are warranted to investigate the infectivity of these viruses and the sources of fecal contamination.