

# **AS-EPA Nearshore Marine Water Quality Monitoring Plan**

**(Revision 7)**



**Prepared by:**

**American Samoa Environmental Protection Agency**

**Revised January 14, 2011**

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## **I Introduction**

American Samoa assesses water quality by comparing monitoring data to the narrative and numeric criteria in the American Samoa Water Quality Standards (ASWQS). The comparisons are used to determine whether the designated uses assigned to the waterbody are supported and to issue weekly public beach advisories. Where uses are impaired, the Territory identifies the pollutants causing water quality impairments, and the sources of those pollutants.

Main pollutants that cause water quality impairments in American Samoa include:

- Pathogen indicators
- Nutrients
- Siltation
- Turbidity

Main sources of these pollutants in American Samoa include:

- On Site Disposal System (OSDS) failures
- Piggeries
- Agriculture
- Urban runoff

American Samoa has determined that all threatened or impaired designated uses in the Territory are due to non point sources. There are only seven identified point sources in the Territory. Analysis of NPDES monitoring data has demonstrated that these point sources have little or no contribution to water quality impairment.

The Nearshore Marine Water Quality Monitoring Plan was developed to address the need to monitor for non point source pollution in American Samoa. Monitoring parameters were chosen to measure concentrations of the main pollutants causing impairment. The parameters also measure the effectiveness of Best Management Practices (BMP's) that have been implemented to prevent impairment and restore impaired uses.

## **II Monitoring Goal**

To provide ambient water quality data for designated use support determinations in support of AS-EPA's Coastal Nonpoint Source Monitoring Strategy.

### **III Monitoring Objectives**

1. To determine whether nearshore marine water quality meets ASWQS for enterococci.
2. To inform the public when recreation waters do not meet ASWQS for enterococci, and of the potential risks associated with the polluted waters.

### **IV Sampling Stations and Sampling Frequencies**

Sampling locations and sampling frequencies are provided in Appendix A.

Locational data for AS-EPA water quality monitoring sites were collected using a Trimble GeoExplorer XT GPS unit. Point data were collected along the waterfront at each monitoring station at 5 second intervals for a period of at least 120 seconds. PDOP maximum filter was set at 6.0 and the elevation mask was set at 15 degrees. During field data collection reference site data at NGS (National Geodetic Survey) monument points were also collected to assure accuracy. Error in NGS point reference was 0.5 meters or less. Field GPS data was subsequently differentially corrected using Pathfinder Office Software and downloading base files from the ASPA CORS station on the NGS web site ([http://www.ngs.noaa.gov/CORS/Islands/islands\\_aspa.html](http://www.ngs.noaa.gov/CORS/Islands/islands_aspa.html)). Following differential correction, point files were exported into an ArcView Shapefile format with the following attribute data:

```
> COMMENT MAX_PDOP MAX_HDOP CORR_TYPE RCVR_TYPE GPS_DATE  
GPS_TIME UPDATE_STA STD_DEV GPS_HEIGHT HORZ_PREC  
VERT_PREC NORTHING EASTING POINT_ID
```

Locational data was provided by Hope Anderson, AS-EPA, in conjunction with Troy Curry, American Samoa Department of Commerce (AS DOC) GIS Specialist.

### **V Monitoring Parameters**

Monitoring parameters and associated ASWQS are provided in Appendix B.

### **VI Analytical Methods and Procedures**

Analytical methods and procedures are provided in Appendix C.

## **VII Data Analysis**

Data is screened, entered into a spreadsheet database, and assessed for compliance with ASWQS. The assessments are used to issue weekly public beach advisories, and to make annual designated use support determinations. Specific criteria used to determine attainment of these individual uses are in accordance with federal guidance Guidelines for Preparation of the Comprehensive State Water Quality Assessment 305(b) Reports and Electronic Updates (USEPA 1997). Specific criteria are as follows:

AS-EPA Decision Guidelines for Microbiological Parameters Used to Assess Whole Body Contact Recreation Use Support in Marine Waters

Criteria	
<b>Level of Recreation Use Support</b>	All Other Embayments, Open Coastal Waters
	Embayments: Pago Pago Harbor, Fagatele Bay, Pala Lagoon
Fully Supporting	<u>Enterococci</u> : A geometric mean of 35 <i>enterococci</i> per 100mL is not exceeded AND the single sample density does not exceed 104 <i>enterococci</i> per 100mL more than once per assessment period.
Partially Supporting	<u>Enterococci</u> : The single sample density of 104 MPN/100mL is exceeded during the year AND a geometric mean does not exceed 35 MPN/100mL.
Not Supporting	<u>Enterococci</u> : The geometric mean standard of 35 MPN/100mL is not met.
	<u>Enterococci</u> : A geometric mean of 35 <i>enterococci</i> per 100mL is not exceeded AND the single sample density does not exceed 124 <i>enterococci</i> per 100mL more than once per assessment period.
	<u>Enterococci</u> : The single sample density of 124 MPN/100mL is exceeded during the year AND a geometric mean does not exceed 35 MPN/100mL.
	<u>Enterococci</u> : The geometric mean standard of 35 MPN/124mL is not met.

## **VIII Quality Assurance and Quality Control**

### Quality Assurance (QA) Program

The goal of the QA Program at the AS-EPA laboratory is to provide data which meets or exceeds the data quality objectives associated with each project that passes through the laboratory. This is achieved through the implementation of quality assurance and quality control measures designed to improve the level of quality of all operations within the laboratory, from sample acceptance to sample handling, and from analysis to reporting.

AS-EPA laboratory staff recognizes that the data they generate must be legally defensible. To ensure data is legally defensible, the QA Program emphasizes the implementation of quality control processes, which identify, control, correct, and prevent quality problems rather than simply detect and make subsequent corrections. The QA Program is used to demonstrate attainment of a state of statistical control, and to demonstrate that the data generation system produces data that are scientifically valid, traceable and retrievable.

AS-EPA laboratory is implementing the following practices as part of its QA program:

- Strict adherence to principles of good laboratory practice such as the use of legible handwriting; the use of indelible black ink; and single line, initialed and dated corrections.
- The consistent use of Standard Operating Procedures. The laboratory uses program specific approved methodologies (e.g., approved drinking water methods for the drinking water program). Standard Operating Procedures specific to the laboratory instrumentation and equipment are written for each method and are updated every two years or sooner if needed.
- The use of qualified personnel.
- Reliable and well maintained equipment.
- Appropriate calibrations and standards; including the use of traceable or certified reference materials.
- The implementation of a comprehensive, organized and straightforward documentation system.
- A program of “in house” training and proficiency of the analysts on analytical procedures, methods, and instrumentation. The documentation of training is maintained in individual training files.
- Appropriate reagents and supplies.
- The close supervision of all operations by the QA Officer, management and senior personnel.

### Quality Control (QC) Program

QC consists of the techniques used to assess and ensure the quality of the analytical measurement process. The AS-EPA lab is implementing the following practices as part of its QC program:

QC assessment tools:

- Evaluation of accuracy through the use of spiked samples (matrix spikes and matrix spike duplicates, blank spikes and blank spike duplicates, and surrogate spikes) for each analytical batch or for each sample matrix, whichever is more frequent. The spiked results are calculated and a percent recovery determination is calculated by the analyst. The percent recovery is compared to the appropriate statistically based control limits to assess method performance and the effect the sample matrix has on the analysis.
- The use of duplicate samples (sample duplicates, matrix spike duplicates and blank spike duplicates) enable the laboratory staff to assess the precision of the analytical batch. The relative percent difference (RPD) between the original sample and its duplicate is calculated by the analyst. The RPD is compared to the appropriate statistically based control limit to assess method reproducibility and the sample homogeneity.

In addition, the following QC tools are used by the laboratory to ensure data meets the overall QA objectives:

- The use of peer and/or supervisory review of all data inputs, calculations, and reports. A knowledgeable and well-trained analyst or supervisor reviews all data prior to release.
- The use of second source check standards to ensure reliability of the primary source.

## ASEPA MONITORING PARAMETERS

Parameters	American Samoa Water Quality Standards		
	Open Coastal Waters	Embayments	Pala Lagoon Embayment Pago Harbor Embayment
Water Temperature (°C)	-not to deviate more than 1.5 °F from ambient and not to fluctuate more than 1 °F on an hourly basis or to exceed 85 °F (except when due to natural causes)		
Specific Conductivity (mS/cm)	-no standards currently exist for this parameter		
Salinity (ppt)	-no standards currently exist for this parameter		
Dissolved Oxygen (% Saturated)	not < 80% saturation	not < 70%	not < 80% saturation not < 70% saturation
Dissolved Oxygen (mg/L)	not < 5.5 mg/L	not < 70%	not < 5.5 mg/L not < 5.0 mg/L
Water Depth (ft)	N/A		
pH	6.5 - 8.6 range (+/- 0.2 pH units of that which would naturally occur	6.5 - 8.6 range (+/- 0.2 pH units of that which would naturally occur	6.5 - 8.6 range (+/- 0.2 pH units of that which would naturally occur
Turbidity (NTU)	not > 0.25 NTU	not > 0.35 NTU	not > 0.75 NTU not > 0.75 NTU
Enterococcus	geometric mean not > 35 MPN/100 mL and instantaneous sample not > 124 MPN/100 mL	geometric mean not > 35 MPN/100 mL and instantaneous sample not > 124 MPN/100 mL	geometric mean not > 35 MPN/100 mL and instantaneous sample not > 104 MPN/100 mL

**American Samoa Environmental Protection Agency**  
**BEACH SAMPLING STATIONS AND SAMPLING FREQUENCIES FY2011**

ID #	Location	Watershed No.	Latitude <sup>1</sup>	Longitude <sup>1</sup>	Frequency	Tiers	Miles
1W	Nu'uuli Pala Lagoon	27	-14.321721857	-170.714181340	Weekly	1	2.92
2W	Nu'uuli Pala Spring	27	-14.319707000	-170.715294000	Weekly	1	2.92
3W	Nu'uuli Coconut Point	27	-14.316862768	-170.700381984	Weekly	1	2.92
4W	Nu'uuli Avau	26	-14.307335428	-170.694573971	Weekly	1	1.11
5W	Fatumafuti Beach	26	-14.297999425	-170.678319055	Weekly	1	1.11
6W	Faga'alu Beach	25	-14.292206351	-170.681509694	Weekly	1	0.67
7W	Gataivai Beach	25	-14.284021101	-170.677059742	Weekly	1	0.67
8W	Utulei Yacht Club Beach	24	-14.281110849	-170.681721405	Weekly	1	1.32
9W	Utulei Samoana Beach	24	-14.279128439	-170.682482466	Weekly	1	1.32
10W	Fagatogo Stream Beach	24	-14.277080928	-170.690022473	Weekly	1	1.32
11W	Aua Pouesi Beach	24	-14.270054236	-170.666689313	Weekly	1	1.32
12W	Aua Stream Beach	24	-14.270922763	-170.665093992	Weekly	1	1.32
13W	Aua Diosa Beach	24	-14.275633670	-170.664923426	Weekly	1	1.32
14W	Lauli'i Tuai Beach	23	-14.287891776	-170.652588993	Weekly	1	2.01
15W	Alega Stream Beach	22	-14.280109159	-170.637822950	Weekly	1	0.44
16W	Alega Beach	22	-14.280375287	-170.638302960	Weekly	1	0.44
17W	Avaio \$2 Beach	22	-14.281033442	-170.631565896	Weekly	1	0.44
1M	Aunu'u Wharf Beach	34	-14.284351045	-170.561312766	Monthly	2	3.37
2M	Ofu Beach	38	-14.174186111	169.677661111111	Monthly	2	4.05
3M	Olosega Beach	36	14.172683333	-169.627822222	Monthly	2	4.16
4M	Ta'u Beach	40	-14.240000000	-169.510455556	Monthly	2	6.24
5M	Fitiuta Beach	40	-14.214838889	-169.423494444	Monthly	2	6.24
6M	Faleasao Beach	40	-14.220655556	-169.515580556	Monthly	2	6.24
18W	Onoa Beach	16	-14.251609000	-170.581501000	Weekly	3	0.90
19W	Tula Beach	17	-14.254213000	-170.564216000	Weekly	3	2.50
20W	Alao Beach	18	-14.263676000	-170.563593000	Weekly	3	0.70
21W	Utumea East Beach	19	-14.271375320	-170.569526533	Weekly	3	0.56
22W	Auasi Wharf Beach	19	-14.271586669	-170.572921948	Weekly	3	0.56
23W	Aganoa Beach (before Auasi)	19	-14.274708453	-170.578366793	Weekly	3	0.56
24W	Aoa Bridge Stream Beach	15	-14.261424593	-170.586382218	Weekly	3	1.49
25W	Amouli Beach	20	-14.274277000	-170.585368000	Weekly	3	2.41
26W	Alofau Asasama Stream Beach	21	-14.273389000	-170.604501000	Weekly	3	1.23
27W	Masefau Bridge Stream Beach	12	-14.255765320	-170.631701781	Weekly	3	4.53
28W	Sa'ilele LMS Beach	14	-14.256291787	-170.597749685	Weekly	3	1.48
29W	Fagaitua HS Beach	21	-14.268869759	-170.615740084	Weekly	3	1.23
30W	Auto Vikings Mart Beach	21	-14.278144357	-170.628074435	Weekly	3	1.23
31W	Afono Beach	11	-14.258540000	-170.351631000	Weekly	3	3.44
32W	Vatia Bridge Stream Beach	10	-14.250567996	-170.675219554	Weekly	3	4.01
33W	Fagasa-Fagalea Stream Beach	8	-14.285481939	-170.720604358	Weekly	3	1.15
34W	Fagasa-Fagatele Boat House Beach	8	-14.258234000	-170.723971000	Weekly	3	1.15
35W	Tafuna Plain Beach (Maliu Mai)	28	-14.341446000	-170.721677000	Weekly	3	3.48

**American Samoa Environmental Protection Agency  
BEACH SAMPLING STATIONS AND SAMPLING FREQUENCIES FY2011**

ID #	Location	Watershed No.	Latitude <sup>1</sup>	Longitude <sup>1</sup>	Frequency	Tiers	Miles
36W	Taputimu Sliding Rock	30	-14.359240763	-170.778352633	Weekly	3	2.47
37W	Leone Pala Bridge Beach	30	-14.336459091	-170.787326519	Weekly	3	2.47
38W	Asili LMS Beach	31	-14.331352000	-170.797026000	Weekly	3	1.24
39W	Utumea West Beach	32	-14.329360954	-170.815022751	Weekly	3	2.65
40W	Amanave LMS Beach	33	-14.326364580	-170.830318637	Weekly	3	1.85
41W	Malota Gurr Beach	3	-14.304100367	-170.816455845	Weekly	3	0.44
42W	Malota Stream Beach	3	-14.303693798	-170.815880565	Weekly	3	0.44
43W	Tafuna Plain Beach - Swimming Hole	28	-14.340956000	-170.721741000	Weekly	3	3.48
44W	Masausi Beach	13	-14.255236000	-170.611922000	Weekly	3	1.69

**NOTE<sup>1</sup>:** Latitude and Longitude measured using the Universal Transverse Mercator System

ID #	Location	Watershed No.	Latitude <sup>1</sup>	Longitude <sup>1</sup>	Frequency	Tiers <sup>2</sup>	Miles
1Nm	Poloa Beach	1	TBD	TBD	TBD	4	1.35
2Nm	Fagali'i Beach	2	TBD	TBD	TBD	4	1.81
3Nm	Fagamalo Beach	4	TBD	TBD	TBD	4	3.24
4Nm	Aoloau Sasae	5	TBD	TBD	TBD	4	3.33
5Nm	Aoloau Sisifo	6	TBD	TBD	TBD	4	2.62
6Nm	Aasu Beach	7	TBD	TBD	TBD	4	4.48
7Nm	Fagatuitui - Vaaogeoge	9	TBD	TBD	TBD	4	8.61
15Nm	Fagatele - Larsen	29	TBD	TBD	TBD	4	5.73
17Nm	Aunuu Sasae	35	TBD	TBD	TBD	4	0.11
18Nm	Ofu Saute	37	TBD	TBD	TBD	4	5.19
19Nm	Olosega Sasae	39	TBD	TBD	TBD	4	3.36
20Nm	Tau Saute	41	TBD	TBD	TBD	4	6.36

**NOTE<sup>1</sup>:** Latitude and Longitude measured using the Universal Transverse Mercator System

**NOTE<sup>2</sup>:** Recreational waters classified as Tier 4 beaches are not currently sampled. Tier 4 recreational waters will be sampled as resources become available.

\***TBD** - To be determined

# **ASEPA LABORATORY**

## **STANDARD OPERATING PROCEDURE**

**Enterolert®**

**Enterococci Water Analysis**

Revision 2

**Prepared by:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Revised by:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**Reviewed by:** \_\_\_\_\_

**Date:** \_\_\_\_\_

**ASEPA Laboratory Director**

## 1. **Scope and Application**

Enterolert® reagent is used for the detection of enterococci in fresh and marine water. The enterococcus group is a subgroup of the fecal streptococci that includes *S. faecalis* and *S. faecium*. Although the US EPA does not approve enterococci methods, Enterolert® is included in the proposed Ground Water Treatment Rule that, upon approval, will be the US EPA's first enterococci regulation.

## 2. **Method Summary**

The Enterolert® test is based upon the ability of enterococci to produce the enzyme  $\beta$ -glucosidase which forms 4-methyl-umbelliferone, a fluorescent substance, when it metabolizes the nutrient-indicator 4-methyl-umbelliferyl. This Defined Substrate Technology® (DST™) allows detection of enterococci within 24 hours. According to the manufacturer, Enterolert® can detect bacteria down to one colony forming unit (CFU) in a 100 ml sample.

## 3. **Definitions**

Enterolert – Enterolert® is a product of IDEXX laboratories, Inc. (800-321-0207). The Enterolert® test is similar in principle to the MUG, or fluorogenic test, as discussed in Standard Methods 9223 B. However, SM 9223 B has been modified for Enterolert® use.

MPN – Enterolert® can be used as either a presence/absence test, or for enumeration of Most Probable Number (MPN) per 100 ml. Enumeration is possible using either a multiple-tube enzyme substrate technique, as in a traditional test, or using a multiple-well enzyme substrate format such as IDEXX's Quanti-trays.

## 4. **Health and Safety Warnings**

Microbiological analyses involve the culturing of potentially pathogenic organisms. Gloves, lab coats and safety glasses should be worn when handling samples, culturing media and equipment. All biologically contaminated materials in the laboratory, particularly media with growth, must be autoclaved prior to disposal. Contaminated media must never be discarded in the trash or poured down the drain prior to autoclaving. Laboratory equipment and benches should be disinfected daily.

All laboratory-acquired infections must be reported to the Laboratory Director, as must all accidents which may cause infection such as accidental spilling or spattering of potentially infectious materials on persons or floors, table tops and other surfaces.

A 6-watt ultraviolet light is used to read Enterolert results for enterococci. Care should be taken not to look directly at the light, and it should be pointed away from the analyst during readings.

## **5. Collection, Handling and Preservation**

Samples must be collected in polypropylene bottles with watertight polypropylene closures, and must be at least 125 ml volume for adequate sampling and good mixing. Sample bottles must be autoclavable, and must be contact clear to permit visible liquid level checks without opening. Discard bottles that have chips, cracks, or etched surfaces. Bottles must be cleansed thoroughly with laboratory detergent, and must undergo a final rinse with purified water at least three times. Sample bottles must be autoclaved at 121°C for 15 minutes. In the event that heat-resistant polypropylene bottles are not available, water samples may be collected in sterile Colilert® 120 ml bottles. The Colilert® bottles contain sodium thiosulfate, so bottles must be rinsed with the sample prior to collection to remove all traces of sodim thiosulfate.

Sampling will occur mainly between the morning to early afternoon hours (0800-1400), year round, and will occur weekly, monthly, and quarterly depending on the concentration of bathers and historical records of contamination. Weekly samples will be collected from areas that have the highest bather densities and are known to be chronically contaminated. Monthly samples will be collected from remote areas that are visited for the collection of monthly drinking water samples. Quarterly samples will be collected from remote areas where swimming occurs. One grab sample will be collected from each of the designated sites.

Samples for microbiological analysis must be collected using aseptic sampling procedures, and must be collected as follows:

1. Carefully wade into the water until water is knee-deep. Avoid kicking up bottom material at the sampling station. Sampler must be positioned downstream and facing any water current to take the sample from incoming flow.
2. Remove the cap from the bottle just before sampling. Avoid touching the inside of the bottle or the cap. If contact is made with any area inside of the bottle or cap, contamination may have occurred and a new sample bottle must be used.
3. Grasp the bottle at the base and plunge the bottle mouth downward in the water to avoid introducing surface scum. The sampling depth must be at least 6 to 12 inches below the water surface, or mid-way between the surface and the bottom.
4. Turn the bottle underwater into the current and away from sampling point.
5. Remove the bottle from the waterbody.
6. Leave a 1-2 inch air space above each sample for proper mixing of the sample before analysis.
7. Recap the bottle carefully, and avoid touching the inside.
8. Label the bottle with the sample location, and place in cooler with an icepack.
9. Complete the Weekly, Monthly, or Quarterly "Sample Collection Record", depending on the sampling period.

Samplers are encouraged, but not required, to hold bacteriological samples at 1-4°C during transit to the laboratory. Samples should be examined as soon as possible after collection. Do not hold samples longer than 6 hours between collection and initiation of analysis (USEPA, 2000). Do not analyze samples that exceed holding time limits.

## 6. **Interferences**

Marine water and other highly saline samples that contain high levels of salt must be diluted 1:9 as soon as possible with sterile reagent water. Low concentrations of microorganisms in a sample may result in one test being positive and a duplicate sample being negative.

## 7. **Apparatus and Materials**

Enterolert® dry media in “Snap-Packs,” stored in the dark at 4-30°C.  
Incubator at 41 ± 0.5°C  
6 watt, 365 nm UV lamp with UV protective glasses  
10 ml sterile pipettes  
Sterile dilution bottles (use Colilert® bottles)  
Enterolert® Quanti-Tray 2000 MPN trays.  
IDEXX Quanti-Tray Sealer  
Colilert® MPN Tables  
Bromocresol purple dye

## 8. **Quality Control Procedures and Limits**

1. Run positive and negative controls on each new lot of Enterolert®. Record the results on the “Enterolert® Media New Lot Quality Control Check Record” Form. The expected results for various types of bacteria are as follows:

<u>Organism</u>	<u>Expected Result</u>
<i>Enterococcus faecium</i>	fluorescent
<i>Eschericia coli</i> (gram negative)	non-fluorescent

Discard the lot if test results do not agree with the expected results.

2. Check each new lot of Enterolert® by shining the ultraviolet lamp on the media snap-packs. If the lot is fluorescent, discard the lot.

3. Run a positive control and a blank negative control (sterile water) with each set of samples analyzed. Invalidate the samples if control results do not agree with expected results.

## 9. **Procedures**

1. Wash hands before handling samples to ensure aseptic technique.
2. Remove samples from cooler or refrigerator before expiration of the sample holding time, and complete Form “*Enterococci* in Recreational Waters by Fluorogenic Substrate”.
3. Turn on the Quanti-Tray Sealer (IDEXX).
4. Thoroughly clean the lab bench area being used with isopropanol.

5. Using a sterile graduated cylinder, measure out 90 ml of sterile reagent grade fresh water and pour into a sterile Colilert® 120 ml bottle with graduation at 100 ml. **Use sterile water, not buffered water for making dilutions. Enterolert® is already buffered.**
6. Mark the sterile dilution bottle with the Field Name/ID.
7. Carefully separate one Enterolert® Snap Pack from the strip taking care not to accidentally open adjacent pack.
8. Tap the Snap Pack to ensure all of the Enterolert® powder is in the bottom part of the pack.
9. Open one pack being careful not to touch the opening of the pack.
10. Add the entire contents of the Enterolert® Snap Pack to the dilution bottle.
11. Aseptically cap and seal the bottle. Shake the dilution bottle until the reagent is completely dissolved.
12. Shake the sample vigorously about 25 times to distribute the bacteria uniformly.
13. Uncap the sterile dilution bottle and transfer 10 ml of the sample to the dilution bottle by measuring out 10 ml using a sterile pipet. If a sterile pipet is unavailable, pour 10 ml of the sample into the dilution bottle until the 100 ml graduation mark is reached.
14. Aseptically cap and seal the bottle. Shake the diluted sample vigorously at least 25 times.
15. Label a Quanti-Tray 2000 (IDEXX) with Field Name or ID.
16. After foaming in the sample bottles subsides, pour the diluted sample with the reagent into the Quanti-Tray. Avoid sample contact with the foil pull-tab.
17. Fit the Quanti-Tray into the corresponding 59/58 well rubber tray. Lay the vessel in the Sealer loading tray, rubber side down, and gently push until the Sealer grabs the vessel. Remove the newly sealed Quanti-Tray from the Sealer ejection slot.
18. Repeat at step 5 (above) for all samples.
19. When all the samples are sealed, place them into a 41 +/- 0.5°C dry incubator.
20. After 24 hours, remove the samples from the incubator.
21. Before analyzing samples, put on UV-protection glasses. Using a 6 watt, 365 nm wavelength UV lamp, shine light within 5 inches of the vessel in a dark environment and count the number of wells that fluoresce. **Blue fluorescence indicates the presence of enterococci.** For comparison, a water blank can be used when interpreting results.  
**Procedural Note:** If sample is inadvertently incubated over 28 hours without observation, the following guidelines apply: Lack of fluorescence after 28 hours is a valid negative test; fluorescence after 28 hours is an invalid result.
22. Record the number of positive small and large wells onto the “*Enterococci* in Recreational Waters by Fluorogenic Substrate” Form.  
Referring to the MPN table provided with the Quanti-Tray, determine the most probable number (MPN) of *enterococci* grown in each tray. Multiply each MPN table value by 10 to take into account the dilution performed in step 7 (above). The multiplied value is the actual MPN result. Record this actual value on the “*Enterococci* in Recreational Waters by Fluorogenic Substrate” Form. This value will be reported as the instantaneous density.

## 10. Data Acquisition, Reduction , and Documentation

The results for enumeration of *enterococci* using Enterolert® are determined according to the procedures above. A blue fluorescence is confirmed positive for *enterococcus*. Results are entered onto the “*Enterococci* in Recreational Waters by Fluorogenic Substrate” Form. The number of fluorescent wells are counted, and the results are converted to the MPN for *enterococci* using the appropriate IDEXX Quanti-Tray matrices. This MPN table value is multiplied by the dilution factor to calculate the actual MPN. The actual MPN values are then entered into the data sheet.

Laboratory data reports include MPN values for *enterococci*. Results for all QC samples are also similarly reported.

## 11. Waste Disposal and Spill Decontamination

The laboratory must utilize the autoclave to sterilize potentially infectious wastes prior to disposal, or dispose of the wastes at the LBJ Hospital as per agreement. Potentially infectious wastes include all enterococci positive and indeterminate samples, and all media check and performance evaluation samples. In emergency situations, a 10% mixture of household bleach (approximately 1 cup of fresh bleach per gallon of liquid) will be used to ensure disinfection.

Spills of potentially infectious wastes should be wiped up carefully (wear rubber gloves). Paper towels or other absorbants used to wipe up spills should be bagged and disposed of in the same manner as other potentially infectious wastes. After wiping up spills, surfaces should be disinfected with 10% bleach, alcohol, or other suitable disinfectant.

## 12. References

American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992.

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