Meadows Report 24-002

Texas Stream Team Final Report: Contract #20-10156 (Amendment No.1)

April 19, 2023 – May 31, 2024

Galveston, Texas near San Luis Pass © Jesse, Adobe Stock

The Meadows Center
for Water and the Environment

Texas State University

Texas Stream Team

EPA United States Environmental Protection Agency

Texas Commission on Environmental Quality

Texas State University
The rising STAR of Texas

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ACKNOWLEDGEMENTS

Prepared in cooperation with the Texas Commission on Environmental Quality and the U.S. Environmental Protection Agency.

The Texas Stream Team encourages life-long learning about the environment and people's relationship to the environment through its multidisciplinary community science programs. We also provide hands-on opportunities for Texas State University students and inspire future careers and studies in natural resource-related fields. Preparation of final reports serves as contract deliverables for granting entities, but they also serve as valuable educational experiences for the students and staff that prepare the reports. Texas Stream Team values the staff contributions and recognizes each individual for their role.

The following staff and student workers assisted in the preparation of this report and are acknowledged for their contributions:

- Madison Mitchell, Student Research Assistant
- Aspen Navarro, Community Science Manager
- Sandra Arismendez, Senior Watershed Scientist
- Desiree Jackson, Science and Stakeholder Engagement Specialist
- Anna Huff, Science Communications Manager

Essential Element Statement using the FY2022-2026 Environmental Protection Agency Strategic Plan:

- Essential Element 1 - Goal 5: Ensure Clean and Safe Water for All Communities
- Essential Element 2 - Objective 5.2: Protect and Restore Waterbodies and Watersheds
- Essential Element 3 - Non-point Source Pollution Control (Clean Water Act Section 319)Project period: 11/27/2019-5/31/2024

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Welder Flats at sunset by San Antonio Bay in Matagorda Island, Texas. © Maresa Pryor/Danita Delimont

Contents

List of Figures

List of Tables

Aerial of Port Aransas, Texas. © Ryan Conine

Project Significance and Background

Texas Stream Team at The Meadows Center for Water and the Environment (the Meadows Center) is dedicated to facilitating environmental stewardship by empowering a statewide network of concerned stakeholders in a collaborative effort to promote water quality education and nonpoint source pollution reduction. Through water quality monitoring, data collection and analysis, and educational programs, Texas Stream Team and our partner organizations work to expand the public's understanding of how human activity impacts water quality in Texas. Participation in the Texas Stream Team community science program influences individuals to adopt activities that positively impact water quality and mitigate the effects of nonpoint source pollution while involving them directly in watershed protection plans and community science initiatives. Throughout the contract, Texas Stream Team has worked to support and enhance the public outreach objectives and priorities identified under the U.S. Environmental Protection Agency's 319(h) Nonpoint Source Pollution Program through the Texas Commission on Environmental Quality, with a special emphasis placed on promoting services to organizations and partners identified as interested in, or actively developing a watershed protection plan. Increasing Texas Stream Team's services to these stakeholders, as well as working to expand community scientist monitoring in these areas, engages communities in the process of watershed stewardship and nonpoint source pollution reduction.

The contract, which commenced in 2019, concluded all work between November 2019 and November 2020, submitting a final report upon completion. In April 2023, the contract underwent an amendment to add funds and include the development of a Bacteria Technique Study. This study aimed to facilitate the establishment of a dependable bacteria method for inland and coastal monitoring. The initiative stemmed from vendor issues with our current Coliscan Easygel method and coastal counties consistently exhibiting high bacteria levels. Furthermore, this inclusion addresses volunteer requests for resources related to Enterococcus bacteria monitoring and training. It aligns with the Texas Stream Team's mission to expand community science initiatives along the Texas coast and address specific coastal water quality concerns, given the importance of waterfront recreational activities and ecotourism. This project will guide the next steps in establishing a standardized volunteer monitoring system for Enterococcus bacteria.

The contents of this report describe the work completed by Texas Stream Team staff between April 2023 – May 2024.

Study Area

This project's initial phase, which focused primarily on testing methods for monitoring Enterococcus bacteria, specifically includes coastal recreation beaches monitored by the Texas Beach Watch Program in Harris, Galveston, Matagorda, and Aransas counties (refer to Figure A). Texas Beach Watch personnel conduct weekly monitoring of recreational beaches along the Texas Gulf Coast from May to September and biweekly from October to April. Leveraging existing resources, Texas Stream Team sampled alongside Texas Beach Watch at 36 sites in August and September 2023 and compared the results from this project with those from Texas Beach Watch (refer to Table 1).

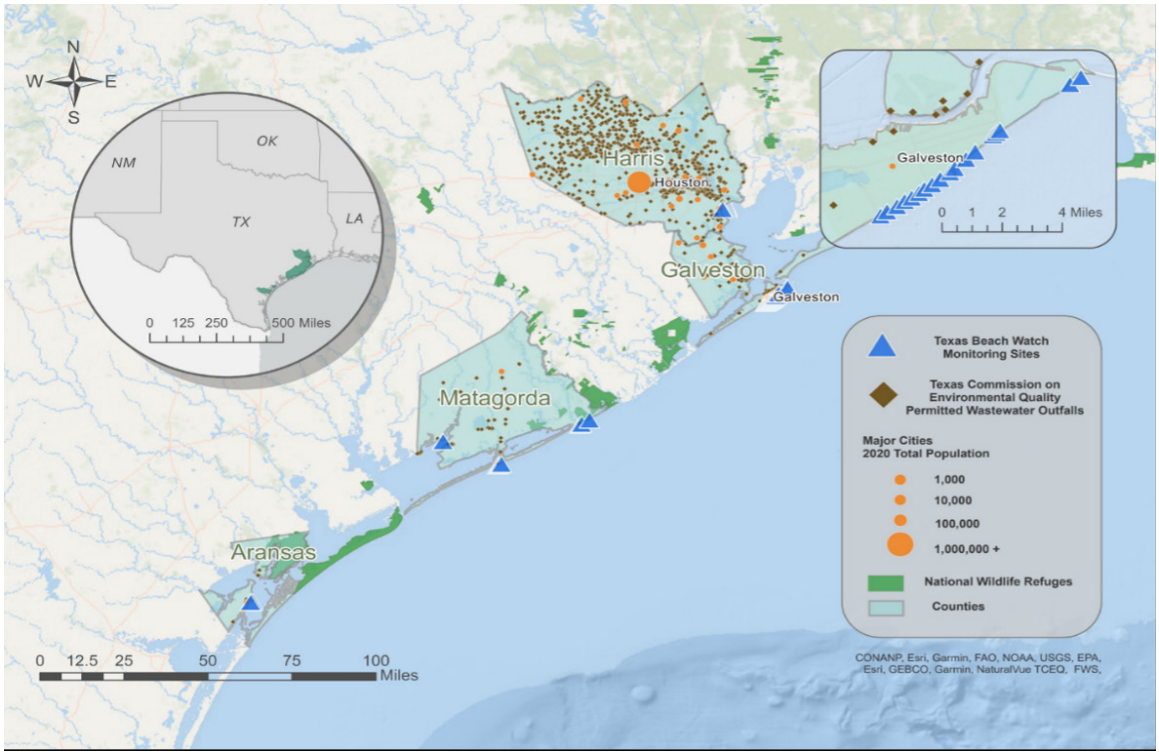

Figure A: Texas Beach Watch monitoring sites at recreational beaches in Harris, Galveston, Matagorda, and Aransas counties.

Table 1. Texas Beach Watch Sites in Harris, Galveston, Matagorda, and Aransas counties, Texas.

COUNTY	SITE ID	DESCRIPTION	LONGITUDE	LATITUDE
Harris	HAR001	Sylvan Beach - North	-95.007969	29.65506
	HAR002	Sylvan Beach - South	-95.009461	29.65244

COUNTY	SITE ID	DESCRIPTION	LONGITUDE	LATITUDE
Galveston	GAL034	60th Street	-94.82532	29.26696
	GAL035	57th Street	-94.82221	29.26888
	GAL036	San Luis Resort	-94.81735	29.27126
	GAL037	Fort Crockett Seawall Park	-94.8137	29.27365
	GAL038	West	-94.81034	29.27519
	GAL039	Fort Crockett Seawall Park	-94.80686	29.27728
	GAL040	Beach Plaza Shopping Center	-94.80427	29.27915
	GAL041	39th St.	-94.8005	29.28128
	GAL042	35th St.	-94.79664	29.28374
	GAL044	Between 31st and 32nd Street	-94.7919	29.28679
	GAL045	Flagship Hotel/27th Street.	-94.78967	29.28874
	GAL046	East of Flagship Fishing Pier	-94.78423	29.29295
	GAL047	18th/19th Streets	-94.77982	29.2964
	GAL048	14th/15th Streets	-94.77123	29.30384
	GAL049	Stewart Beach #1	-94.76959	29.30501
	GAL050	Stewart Beach #2	-94.7679	29.30622
	GAL053	Stewart Beach #3	-94.7342	29.32732
	GAL055	East Beach/Apffel Park #2	-94.72905	29.33056
	GAL083	Princeton Street	-94.84992	29.25306
	GAL084	81st Street	-94.84511	29.25586
	GAL085	69th Street	-94.83383	29.26224
Matagorda	MAT001	Palacios Pavilion West	-96.218026	28.69857
	MAT002	Palacios Pavilion East	-96.214689	28.698337
	MAT003	Jetty Park #1	-95.976111	28.597222
	MAT004	Jetty Park #2	-95.971389	28.599167
	MAT005	Jetty Park #3	-95.965833	28.601389
	MAT006	Jetty Park #4	-95.960556	28.603611
	MAT007	Sargent Beach West	-95.623333	28.765278
	MAT008	Sargent Beach	-95.615556	28.769167
	MAT009	Sargent Co Park	-95.581389	28.786389
Aransas	ARA001	Rockport Beach Park South	-97.04233	28.02859
	ARA002	Rockport Beach Park North	-97.03763	28.0302
	ARA003	Rockport Saltwater Pool	-97.03215	28.03251
	ARA004	Little Bay Ski Basin	-97.03961	28.03064

Summary Of Tasks

As previously indicated, a comprehensive final report detailing the work conducted under contract 10156 has been submitted to the Texas Commission on Environmental Quality. The subsequent report pertains exclusively to the tasks undertaken pursuant to the contract amendment. This amendment concerned the development of both a draft and final Bacteria Technique Study report, the details of which will be expounded upon in subsequent sections of this document. Notably, the project amendment facilitated updates to Task 3, which primarily revolves around community science activities. As a component of this enhancement, Task 3.3 was introduced, specifically dedicated to the drafting and finalization of the Bacteria Technique Study report. The project's scope of work included four main tasks and subsequent deliverables that fell within each task:

- Project Administration
- Quality Assurance
- Focused Citizen Science Activities
- Final Report

The information within this section highlights each specific task and the completed deliverables. Task three specifically presents the technique study conducted by the Texas Stream Team staff.

Project Administration

Project administration involved overseeing and coordinating the work, ensuring technical and financial supervision, and preparing status reports. This included tasks such as project oversight, submitting progress reports, maintaining communications with the Texas Commission on Environmental Quality, and providing a project article upon request. Regular budget updates and discussions with the Texas Commission on Environmental Quality were also included in the project's administrative tasks.

Quality Assurance

The work conducted under this contract was not completed under a Quality Assurance Project Plan, as no monitoring data collection or reporting was implemented within the broader framework of the Texas Stream Team program.

Focused Citizen Science Activities

The task report below presents the outcomes of the Bacteria Technique Study conducted by Texas Stream Team staff. Two distinct approaches were explored to accomplish this objective. The first approach involved integrating trained community scientists into the existing monitoring network operated by the Texas General Land Office's Texas Beach Watch program. The second approach centered on evaluating the viability of the R-CARD method for use by trained community scientists. These methods aim to address a notable gap within Texas Stream Team's current capabilities and meet the demands of volunteers who have expressed a strong interest in receiving training and resources in this regard. The purpose of the study was to investigate

alternative methods to the current Coliscan Easygel *E. coli* Bacteria monitoring method that are more reliable, cost-efficient, accessible, and inclusive of coastal areas.

APPROACH I. EXISTING NETWORK (TEXAS BEACH WATCH)

The Texas Beach Watch monitors Enterococci bacteria at recreational beaches with a network of partners such as local governments, universities, and commercial laboratories. Water quality samples are collected and tested for Enterococcus bacteria alongside other parameters (field observations, salinity, turbidity, etc.). All water samples are collected by licensed sanitarians, or qualified environmental scientists, trained and certified in accordance with the Texas Beach Watch Quality Assurance Project Plan. Method 1600 and IDEXX Enterolert™ are the U.S. Environmental Protection Agency approved laboratory techniques used by contract laboratories to analyze water samples. Results of the water quality analyses are provided to the public on the Texas Beach Watch Data Hub.

Early on during this project, Texas Stream Team staff proposed development of a monitoring system to enhance ongoing Texas Beach Watch efforts whereby community scientists would be trained in sample collection using Texas Beach Watch monitoring protocols. The sample would then be transported by the volunteer to a NELAP-accredited laboratory for analysis, and the laboratory would submit the results to Texas Beach Watch personnel for upload to the Data Hub for public consumption. The first step in this approach was to develop a GIS tool to co-locate accredited laboratory and monitoring sites to aid with sample collection, transfer, and analysis, then evaluate the tool for its utility and determine its feasibility for this approach.

APPROACH II. RESEARCH METHODS

The second approach to implementing community science Enterococcus bacteria monitoring entails researching new training methods and monitoring protocols. Central to this approach was analyzing potential bacteria testing equipment, prompted by limitations in Texas Stream Team's current equipment, which is designed for freshwater systems and specifically targets Escherichia coli (*E. coli*).

First, a review of Enterococcus bacteria monitoring methods was conducted and revealed the Roth Bioscience LLC R-CARD rapid test method for bacteria and other microorganisms. After researching the R-CARD test procedures and client reviews, Texas Stream Team staff decided the method had potential as a viable option for use by community scientists. The R-CARD rapid test method is used to detect and enumerate a variety of microorganisms including Enterococcus bacteria. The process involves a single test card that gels the water sample between the card and a top layer of clear cellophane-like film. Liquid media, agar, or petri dishes are not needed. The cost for one R-CARD Enterococcus test ranges from $2.12 to 2.65 depending on the number of tests purchased at any given time. When compared to the current cost of the *E. coli* bacteria Coliscan Easygel method for testing bacteria in freshwater ($2.52–3.74 depending on the vendor), the R-CARD is somewhat less expensive.

Second, a monitoring plan was developed to test the R-CARD Enterococcus in saltwater systems. Texas Beach Watch sampling sites were chosen in Harris, Galveston, Matagorda, and Aransas counties. The plan involved collecting a water sample alongside Texas Beach Watch personnel for analysis with the R-CARD

Enterococcus test. Results from the R-CARD test would then be compared to laboratory results from Texas Beach Watch.

Third, Texas Stream Team staff implemented the monitoring plan and conducted sample collections at 36 recreational beaches alongside Texas Beach Watch between August and September 2023. The Texas Beach Watch staff transported their water sample to the contract laboratory, while Texas Stream Team staff transported their water sample to their laboratory setting for analysis using the R-CARD methodology. Water samples were tested in duplicate then the results were averaged. Water samples were plated using undiluted (100% seawater) and diluted (10 times) with deionized water to determine if sodium chloride interference was detected and to mirror the laboratory method used by Texas Beach Watch contract laboratories. The R-CARDs were inoculated then incubated at a temperature of 35°C ± 0.5°C and examined for bacteria colony counts at the 15, 19, 24, and 36-hour marks. The R-CARD detection limit is one colony forming unit per sample. The colony counts from the diluted samples were multiplied by a dilution factor of 10 then by 100 to convert to colony forming units per 100 milliliters (CFU/100ml). Results from R-CARD and Texas Beach Watch laboratory were transformed using Log10 as a multiplier. Then, R-CARD results were compared to the Texas Beach Watch contract laboratory results which were acquired from the Data Hub.

KEY FINDINGS AND RESULTS

Upon developing the GIS co-locator tool, several obstacles emerged that needed addressing before proceeding with further study. Firstly, the considerable distance between laboratories and monitoring sites posed logistical challenges for timely sample analysis by an accredited laboratory. Secondly, reliance on volunteers for sample collection raised concerns about the financial burden on Texas Stream Team community scientists of transporting samples to laboratories. Lastly, the turnover rate among volunteer monitors suggested a need for ongoing training and training resources to maintain data consistency. Consequently, we shifted our focus to developing a coastal bacterium monitoring program using the R-CARD methodology (Approach II).

The R-CARD Enterococcus tests were conducted on water samples from 36 recreational beach sites, collected alongside Texas Beach Watch personnel in August and September 2023. Results showed that undiluted sample water yielded fewer colony counts in 100% of the R-CARD tests compared to ten-fold diluted samples. Salinity levels at all sites measured >20 parts per thousand, with 40% of the ten-fold diluted samples producing colony counts after 36 hours of incubation (refer to Table 2).

Bacteria colony counts at 15, 19, 24, and 36-hour incubation periods were examined for each county, revealing that all counties displayed higher counts after 36 hours compared to earlier time points, with the most significant increase occurring between 24 and 36 hours. Comparing the R-CARD analysis to the Texas Beach Watch laboratory results revealed a difference of two orders of magnitude, with R-CARD counts consistently larger for all sites and samples (refer to Figure B).

While none of the Enterococcus results from the Texas Beach Watch laboratory exceeded the BAV (beach action value) (104 CFU/100ml), the R-CARD analysis produced drastically different results. R-CARD colony counts were extrapolated and

converted to CFU/100 ml, resulting in all values exceeding the BAV. This discrepancy underscores the significant difference between R-CARD and laboratory results in assessing Enterococcus levels, indicating potential limitations in the BAV thresholds.

Table 2. Sample counts by county and incubation period for ten-fold diluted water samples collected alongside Texas Beach Watch personnel (August-September 2023).

COUNTY	NO. OF SAMPLES - 10X DILUTION	NO. OF SAMPLES WITH COUNTS @15 HRS	NO. OF SAMPLES WITH COUNTS @19 HRS	NO. OF SAMPLES WITH COUNTS @24 HRS	NO. OF SAMPLES WITH COUNTS @36 HRS
Harris	4	0	0	0	0
Galveston	42	4 (10%)	8 (19%)	17 (40%)	20 (48%)
Matagorda	18	0	0	0	4 (100%)
Aransas	8	0	3 (38%)	3 (38%)	5 (63%)
Total	**72**	**4 (6%)**	**11 (15%)**	**20 (28%)**	**29 (40%)**

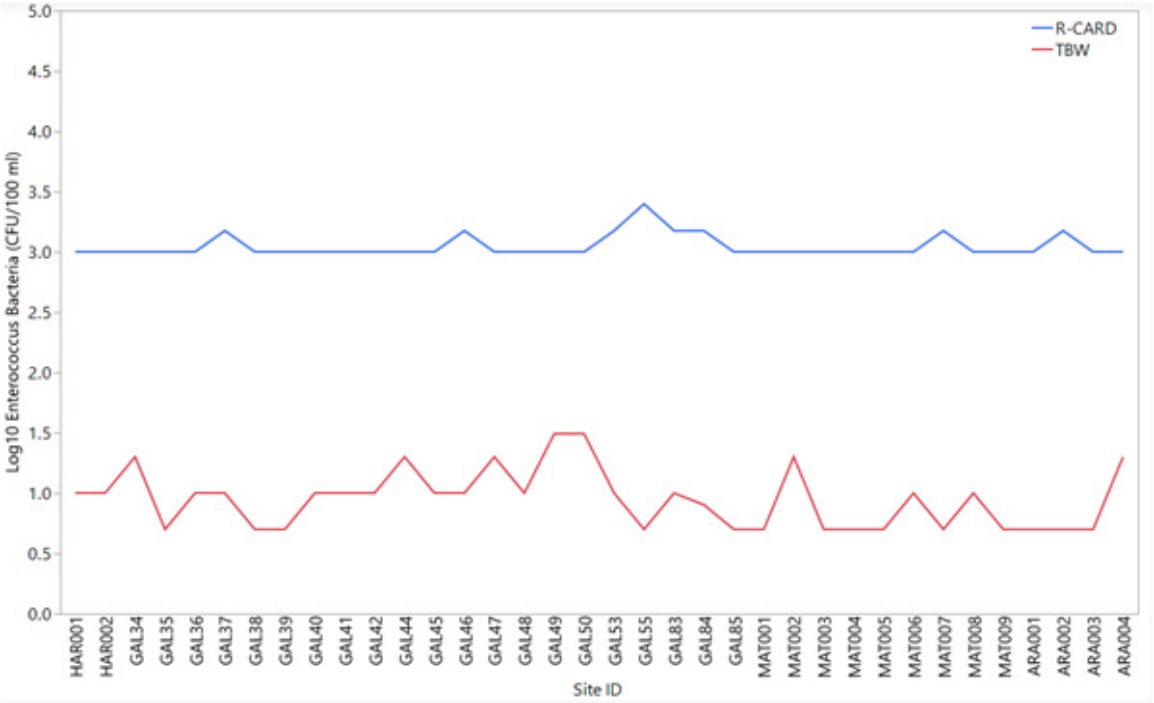

Figure B: Transformed (Log 10) Texas Beach Watch (TBW) and R-CARD Enterococcus bacteria test results by site ID and county (August - September 2023).

Tables Amount of Project Funding and Amount Spent

The total amount of funding awarded for this contract was $110,973. Project costs for this contract were allocated for staff time, supplies, and travel for bacteria monitoring efforts.

The total cost share (40%) requirement for this contract totaled to $73,982. Cost share for this contract has been obtained from Texas Stream Team community scientist monitoring activities that are calculated using the Independent Sector's estimated national value of each volunteer hour, and the International Revenue Service standard mileage rate. In-kind matches are also obtained from Texas State University's waived indirect costs, which are calculated using Texas State University's indirect cost rate, to cover this contract's cost share requirement.

All contract dollars will be spent down by the end of the contract. Table 3 below includes a breakdown of the contract budget.

Table 3. Contract #10156 (Amendment 1) Budget Breakdown.

BUDGET CATEGORY	TOTAL PROJECT COSTS
Salary/Wages	$63, 998.46
Fringe Benefits	$23, 477.48
Travel	$2,216.50
Supplies	$6,806.00
Equipment	$0.00
Contractual	$0.00
Construction	$0.00
Other	$0.00
Total Direct Costs	$96,498.44
Indirect Costs	$47,766.73
Other In-Kind Contributions	$40,689.83
Total Contract Cost	$184,955.00
Cost Share (40%)	$73,982.00
TCEQ Reimbursement Amount (60%)	$110,973.00

Discussion

Challenges

The study examined two approaches, each yielding distinct outcomes. Initially deemed promising, the first approach encountered several obstacles upon completion of the laboratory locator tool. These challenges included high transportation costs due to long distances between monitoring sites and laboratories, frequent turnover among volunteer monitors, and the substantial staff resources required to meet training demands for the program's sustainability.

Upon discovering the R-CARD Enterococcus rapid test method, the second approach was pursued. Preliminary R-CARD testing revealed certain limitations that should be considered for future studies:

1. Results presented in this report are from a single sampling event at 36 sites collected during August and September 2023. Temporal and spatial variability was not captured in the monitoring data.
2. All Texas Beach Watch laboratory results were below the BAV (i.e., <104 CFU/100 ml) and representative of low bacteria colony counts. It is not clear how results from the two methods tested would compare after rainfall events when bacteria counts would likely be higher, and salinities would be lower.
3. The difference in Enterococcus bacteria results between the two methods revealed a difference of two orders of magnitude. This was likely due to the calculations associated with the dilution extrapolations and detection limits (#4 below).
4. Extrapolation of bacteria colony counts for the two methods tested should be reevaluated to ensure conformance with protocols. Unit conversions and detection limits may be calculated differently by the two methods compared in this study resulting in extremely different results. For example, R-CARD bacteria colony counts are multiplied by 10 to account for the dilution then by 100 to convert to CFU/100 ml. Texas Beach Watch counts are multiplied by 10 to account for the dilution and are reported as CFU/100 ml. Both methods have a detection limit of 1 CFU per sample, however the difference in results is exacerbated by the conversion to a comparable unit.
5. Although water samples were collected simultaneously in duplicate by Texas Beach Watch contract personnel, sample analyses should take place with samples from the same container and preferably in the same laboratory setting.

Lessons Learned

Despite the limitations listed above, there were many lessons learned from this coastal Bacteria Technique Study. The lessons learned include:

1. A ten-fold dilution is necessary for seawater; undiluted samples tested with R-CARD Enterococcus produced fewer colony counts in 100% of the tests.
2. A 36-hour incubation period is recommended for the R-CARD test; the largest increase in bacteria counts occurred between 24- and 36-hour incubation periods.

The manufacturer's standard operating procedure states to incubate for 15-36 hours.

3. The use of an incubator with a digital temperature display and a circulating fan is preferred to maintain a constant temperature (35±5°C) during incubation.
4. A cost analysis revealed the R-CARD Enterococcus analysis is less expensive ($2.12 to 2.65 per test) than the Coliscan Easygel for *E. coli* analysis ($2.52 to3.74 per test) and the accredited laboratory analysis for Enterococcus (~$25 per test).
5. The R-CARD, being a newer and more comprehensive bacteria testing equipment currently under research, presents a significant advancement over Coliscan Easygel, the incumbent equipment requiring replacement. The R-CARD is easier to use than the Coliscan Easygel for the following reasons:
    - Differences in storage; R-CARDs have a longer shelf life in ambient conditions, whereas the Coliscan Easygel must be kept frozen. The R-CARD shelf life can be extended when stored in the refrigerator or freezer.
    - Differences in shipping; R-CARDs do not have to be shipped frozen and there's no concern for the media to thaw out like there is for the Coliscan Easygel.
    - R-CARDs are more compact than Coliscan Easygel; R-CARDs require less storage space and do not include bottles and petri dishes.
    - R-CARD plating involves a one-step process as compared to Coliscan Easygel; with the R-CARD a 1 ml sample is placed on the card and the sample is covered with clear film. With the Coliscan Easygel, a 1-, 3- or 5-ml sample is placed in the bottle of media, it is mixed, then poured onto the petri dish prior to incubation. The latter involves more steps than the former.
    - After R-CARDs have been set, they can be stacked on top of each other in the incubator and therefore take up less space.
    - Bacteria colonies are easier to count on the R-CARDs because they are all one color (teal); the colonies on the Coliscan Easygel petri dishes are different colors making it difficult to distinguish color differences and bacteria colony types.

The outcomes of this study have prompted further evaluation of the R-CARD method for rapid testing of Enterococcus bacteria in saltwater during subsequent phases of the work. Phase I identified several limitations and challenges with this method, requiring a thorough analysis to address them effectively. Moving forward, efforts will focus on refining the R-CARD method through extensive research and development to ensure its suitability as the primary tool for Texas Stream Team coastal bacterial training and monitoring. As the piloting phase of the coastal bacterial monitoring network advances, the focus will shift to creating robust training resources.

Once the R-CARD method is confirmed as the primary testing tool, attention will turn to crafting comprehensive training materials and moving away from the Coliscan Easygel method. These resources, modeled after existing Texas Stream Team training materials, will include monitoring forms, field guides, and training manuals to establish official volunteer training procedures. The objective is to ensure that the training and monitoring resources seamlessly align with the established standards of the Texas Stream Team program, maintaining organizational coherence and compliance with

Quality Assurance Project Plan requirements throughout development. By providing comprehensive and standardized training materials, the aim is to equip volunteers with the necessary knowledge and skills to effectively monitor coastal bacteria levels, enhancing the overall effectiveness and reliability of the monitoring network.

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