CALUSA WATERKEEPER
FINAL PROJECT REPORT

Community-Based Water Quality Monitoring: Bacteria Testing and Field Sampling Kits

Applicant	Calusa Waterkeeper
Funding Program	Coastal & Heartland National Estuary Partnership (CHNEP) Conservation Grant FY2026
Grant Period	June 1, 2026 - September 1, 2026
Focused Sentinel Sampling	June 19, July 17, and August 14, 2026
Scientific Focus	Five distinct sentinel sites; Enterococcus and *E. coli*; paired certified-laboratory comparison
Primary Decision Question	Should CWK continue Enterococcus across the network, or transition to *E. coli* at freshwater sites?

Prepared September 4, 2026

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Executive Summary

The big picture

CHNEP funding produced far more than a three-month bacteria dataset. Across five distinct sentinel sites sampled during three focused events, CWK and AEL together generated 60 sentinel analytical results for *E. coli* and Enterococcus, creating 30 paired indicator-level comparisons. The project also delivered 30 standardized Ranger sampling kits, two formal field/laboratory documentation tools, a centralized results workflow, and at least 57 documented volunteer and in-kind project hours - before counting field collection and sample-transport time that was not separately logged. The result is both a defensible pilot dataset and durable monitoring infrastructure that remains in service after the grant period.

Volunteer and in-kind effort. The project documents at least 57 hours of hands-on volunteer and in-kind project effort, plus additional field collection and transport time that was not separately timed. Timed volunteer activities account for 51 hours: 30 laboratory volunteer-hours across the three focused events and 21 volunteer-hours assembling the 30 Ranger kits. Field sampling averaged two volunteers per event, creating six field-volunteer deployments that collected the paired samples, transported CWK bottles to the Calusa Nature Center laboratory, and delivered comparison bottles to AEL. Project reporting added 6 in-kind project-lead hours (three monthly reports plus this final report at approximately 1.5 hours each). The 57-hour figure is intentionally conservative because it excludes the unlogged duration of field collection and transport.

Why this mattered. Fecal contamination is spatially patchy and can change rapidly over short distances and through time. Four of the five sentinel sites are separate reaches of Manuels Branch and the fifth is a distinct Mullock Creek location; they are not interchangeable replicates. Treating each site independently revealed persistent hotspots, divergent indicator behavior, and a practical basis for improving future network design.

What we found. Across the 12 Manuels Branch site-month observations, Enterococcus exceeded the Florida TPTV screening reference of 130 MPN/100 mL in all 12, while *E. coli* exceeded its 410 MPN/100 mL TPTV screening reference in 11 of 12; the exception was MB4 in July (253 MPN/100 mL). MBZ remained the clearest recurring priority site. At MC3, Enterococcus exceeded 130 in June and July but not August, while *E. coli* remained at or below 410 in all three months.

What *E. coli* added. The two indicators were related but not interchangeable. Across all 15 site-months, they produced the same above/below Florida TPTV screening interpretation in 12 cases (80.0%), and their ranks were moderately associated (Spearman rho = 0.60). *E. coli* preserved the overall high-priority signal at the Manuels Branch sites while producing different screening interpretations at MC3 in June and July and at MB4 in July.

What the AEL comparison showed. Using uncensored paired co-located observations, CWK *E. coli* tracked Advanced Environmental Laboratories, Inc. (AEL) more closely than CWK Enterococcus during this pilot: Spearman rho = 0.78 for *E. coli* and 0.66 for Enterococcus. Eleven of 14 uncensored *E. coli* pairs were within two-fold of AEL, compared with 6 of 12 uncensored Enterococcus pairs. Because separate bottles were collected, this is an independent real-world comparison rather than a formal split-sample laboratory validation.

Monitoring recommendation

Continue both Enterococcus and *E. coli* at the five CHNEP sentinel sites through at least one full seasonal cycle, with salinity required for every sample. For the broader CWK network, move toward *E. coli* as the primary routine indicator at confirmed freshwater sites, retain Enterococcus at marine/tidal sites, and continue both indicators at brackish or hydrologically transitional sites until classification is supported by measured salinity and seasonal conditions.

30 standardized Ranger sampling kits assembled for continued community monitoring	**60** sentinel analytical results across CWK and AEL (5 sites x 3 events x 2 indicators x 2 laboratories)	**57+** documented volunteer and in-kind project hours, plus unlogged field collection/transport time
30 paired CWK-AEL indicator comparisons (15 site-months x 2 fecal indicators)	**15** repeated site-month comparisons spanning 5 distinct sites across 3 focused events	**12/15** same above/below screening interpretation for *E. coli* and Enterococcus (80.0%)

1. Grant Commitments and What CHNEP Built

The funded project expanded CWK's existing Enterococcus monitoring by adding routine *E. coli* testing, standardizing volunteer field collection, submitting paired samples for certified-laboratory comparison, and documenting results in a final report. Across the focused five-site study, the design produced 15 repeated site-month observations, 30 CWK sentinel FIB results, 30 corresponding AEL results, and therefore 30 paired indicator-level CWK-AEL comparisons. The grant also committed to 30 complete volunteer sampling kits and photographic documentation of equipment and sampling activities.

1.1 Deliverables and funding translated into outcomes

Table 1. CHNEP-funded deliverables and the operational/scientific capacity created by the project. The application requested $9,750 in CHNEP funds and also documented substantial cash-match and in-kind contributions.

Grant deliverable	Requested CHNEP funds	What the project produced
FIB testing supplies	$4,950	30 CWK sentinel FIB results across 15 repeated site-months, with both *E. coli* and Enterococcus measured at each site/event.
Certified laboratory comparison	$1,300	30 paired indicator-level CWK-AEL comparisons (15 site-months x 2 indicators), contributing to 60 total sentinel analytical results across the two laboratories.
30 standardized volunteer sampling kits	$3,500	30 Ranger kits assembled for deployment; 7 volunteers x 3 hours contributed 21 volunteer-hours to kit assembly.
Final project report	$0 requested	Three monthly reports plus this final integrated report; approximately 6 in-kind project-lead hours, in addition to scientific analysis, photographs, forms, QA/QC documentation, and recommendations.

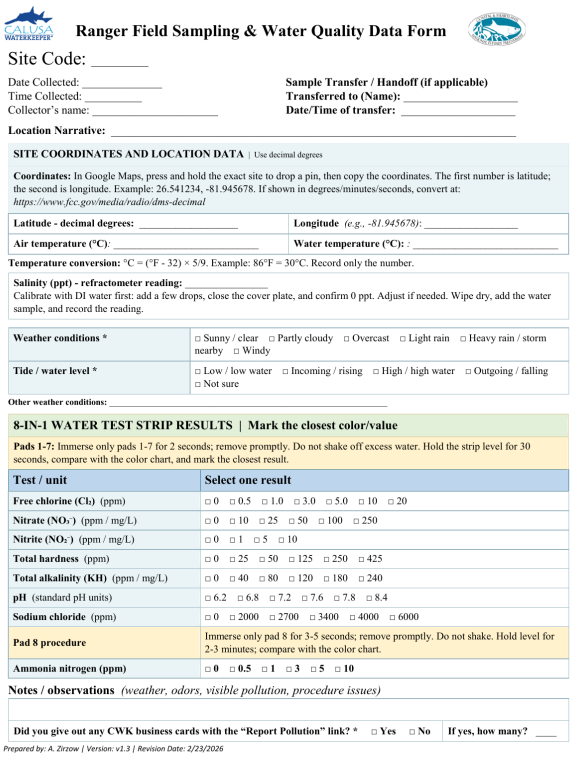
1.2 Ranger kits: durable field capacity

A central grant outcome was the assembly of 30 standardized Ranger sampling kits - durable equipment that remains available for future monitoring after grant close-out. Seven volunteers worked for approximately 3 hours each to assemble and prepare the kits, contributing 21 volunteer-hours in a single capacity-building session. The standardized kits reduce variability in field collection, support safer sample retrieval without direct water contact, and create a consistent equipment package for the Ranger network. The photographs below document the kit itself, volunteer assembly, and field deployment.

Figure 1. CHNEP-supported monitoring capacity in practice: standardized Ranger kit components, volunteers assembling kits for deployment, and volunteers using telescoping sampling equipment during field collection. Photos provided by Calusa Waterkeeper.

1.3 Standardized field and laboratory documentation

The project workflow was supported by two standardized forms. The Ranger Field Sampling & Water Quality Data Form documents site identity, coordinates, sample handoff, air and water temperature, salinity, weather, tide/water level, basic field-screening results, and observations. The Calusa Waterkeeper Lab Cover Sheet documents personnel, processing times, dilution, test/reagent type, reagent and tray lots, incubation conditions, blank/control use, deviations, and final review. Together, these forms create a traceable field-to-laboratory record rather than relying on informal notes.



Ranger Field Sampling & Water Quality Data Form

Site Code: _____

Date Collected: _____ Time Collected: _____ Collector's name: _____

Sample Transfer / Handoff (if applicable)
Transferred to (Name): _____
Date/Time of transfer: _____

Location Narrative: _____

SITE COORDINATES AND LOCATION DATA | Use decimal degrees

Coordinates: In Google Maps, press and hold the exact site to drop a pin, then copy the coordinates. The first number is latitude; the second is longitude. Example: 26.541234, -81.945678. If shown in degrees/minutes/seconds, convert at: https://www.fcc.gov/media/radio/dms-decimal

Latitude - decimal degrees: _____	Longitude (e.g., -81.945678): _____
Air temperature (°C): _____	Water temperature (°C): : _____

Temperature conversion: °C = (°F - 32) × 5/9. Example: 86°F = 30°C. Record only the number.

Salinity (ppt) - refractometer reading: _____
Calibrate with DI water first: add a few drops, close the cover plate, and confirm 0 ppt. Adjust if needed. Wipe dry, add the water sample, and record the reading.

Weather conditions *	□ Sunny / clear □ Partly cloudy □ Overcast □ Light rain □ Heavy rain / storm nearby □ Windy
Tide / water level *	□ Low / low water □ Incoming / rising □ High / high water □ Outgoing / falling □ Not sure

Other weather conditions: _____

8-IN-1 WATER TEST STRIP RESULTS | Mark the closest color/value

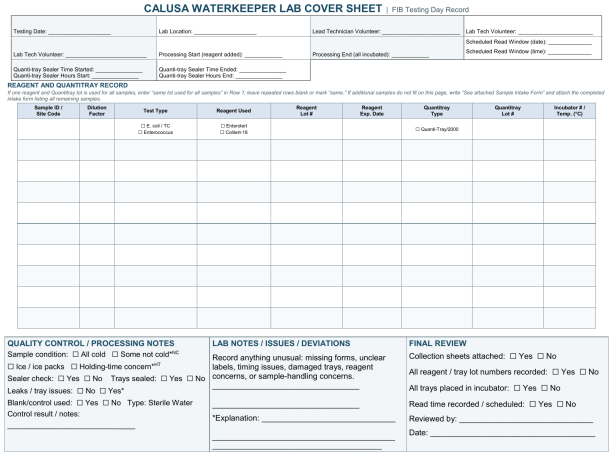
Pads 1-7: Immerse only pads 1-7 for 2 seconds; remove promptly. Do not shake off excess water. Hold the strip level for 30 seconds, compare with the color chart, and mark the closest result.

Test / unit	Select one result
Free chlorine (Cl_2) (ppm)	□ 0 □ 0.5 □ 1.0 □ 3.0 □ 5.0 □ 10 □ 20
Nitrate (NO_3^-) (ppm / mg/L)	□ 0 □ 10 □ 25 □ 50 □ 100 □ 250
Nitrite (NO_2^-) (ppm / mg/L)	□ 0 □ 1 □ 5 □ 10
Total hardness (ppm)	□ 0 □ 25 □ 50 □ 125 □ 250 □ 425
Total alkalinity (KH) (ppm / mg/L)	□ 0 □ 40 □ 80 □ 120 □ 180 □ 240
pH (standard pH units)	□ 6.2 □ 6.8 □ 7.2 □ 7.6 □ 7.8 □ 8.4
Sodium chloride (ppm)	□ 0 □ 2000 □ 2700 □ 3400 □ 4000 □ 6000
Pad 8 procedure	Immerse only pad 8 for 3-5 seconds; remove promptly. Do not shake. Hold level for 2-3 minutes; compare with the color chart.
Ammonia nitrogen (ppm)	□ 0 □ 0.5 □ 1 □ 3 □ 5 □ 10

Notes / observations (weather, odors, visible pollution, procedure issues)

Did you give out any CWK business cards with the "Report Pollution" link? * □ Yes □ No If yes, how many? ____

Prepared by: A. Zirzow | Version: v1.3 | Revision Date: 2/23/2026



Calusa Waterkeeper Lab Cover Sheet

CALUSA WATERKEEPER LAB COVER SHEET | FIB Testing Day Record

Testing Date: _____ Lab Location: _____ Lead Technician Volunteer: _____ Lab Tech Volunteer: _____
Lab Tech Volunteer: _____ Processing Start (reagent added): _____ Processing End (all incubated): _____ Scheduled Read Window (date): _____ Scheduled Read Window (time): _____
Quanti-tray Sealer Time Started: _____ Quanti-tray Sealer Time Ended: _____
Quanti-tray Sealer Hours Start: _____ Quanti-tray Sealer Hours End: _____

REAGENT AND QUANTITRAY RECORD

If one reagent and Quantitray lot is used for all samples, enter "same lot used for all samples" in Row 1; leave repeated rows blank or mark "same." If additional samples do not fit on this page, write "See attached Sample Intake Form" and attach the completed intake form listing all remaining samples.

Sample ID / Site Code	Dilution Factor	Test Type	Reagent Used	Reagent Lot #	Reagent Exp. Date	Quantitray Type	Quantitray Lot #	Incubator # / Temp. (°C)
		□ E. coli / TC □ Enterococcus	□ Enterolert □ Colilert-18			□ Quanti-Tray/2000		

QUALITY CONTROL / PROCESSING NOTES
Sample condition: ☐ All cold ☐ Some not cold[HIC] ☐ Ice / ice packs ☐ Holding-time concern[HIT]
Sealer check: ☐ Yes ☐ No Trays sealed: ☐ Yes ☐ No
Leaks / tray issues: ☐ No ☐ Yes*
Blank/control used: ☐ Yes ☐ No Type: Sterile Water Control result / notes: _____

LAB NOTES / ISSUES / DEVIATIONS
Record anything unusual: missing forms, unclear labels, timing issues, damaged trays, reagent concerns, or sample-handling concerns.
_____
_____
*Explanation: _____
_____
_____

FINAL REVIEW
Collection sheets attached: ☐ Yes ☐ No
All reagent / tray lot numbers recorded: ☐ Yes ☐ No
All trays placed in incubator: ☐ Yes ☐ No
Read time recorded / scheduled: ☐ Yes ☐ No
Reviewed by: _____
Date: _____

Figure 2. Standardized project documentation. Full-size forms are reproduced in Appendices C and D.

A practical improvement identified by the science

The field form already includes salinity, which is directly relevant to choosing between freshwater and marine/tidal fecal indicators. Salinity values were not present in the five-site database export analyzed for this final report. Future sentinel sampling should therefore make salinity completion and database entry a required QC check so indicator selection can be tied to measured site conditions rather than map position alone.

2. Scientific Purpose and Study Design

The scientific component of the project was not simply a question of whether bacteria were present. It was designed to answer four practical monitoring questions:

- What bacterial patterns occur when the same five distinct sentinel sites are sampled repeatedly through the summer?
- Do *E. coli* and Enterococcus identify the same priority locations, or does indicator choice change interpretation?
- How closely do CWK results from paired co-located samples track results produced by AEL?
- What monitoring strategy best balances public-health relevance, continuity, cost, and Southwest Florida's freshwater-to-estuarine setting?

2.1 Five distinct sentinel sites

The five sites are separate environmental locations. Four occur along Manuels Branch at different positions in the urban tributary, and the fifth is on Mullock Creek. Although the Manuels Branch sites are within the same drainage, the mapping and analytical results show that they represent distinct reaches and must not be treated as replicate samples of one location.

Table 2. The five sentinel sites are analyzed independently throughout this report.

Site	Waterbody / location	Role in sentinel network	Three-month interpretation
MB5	Manuels Branch at Cortez Avenue	Western/downstream Manuels Branch sentinel reach near Cortez Avenue.	Persistent *E. coli* elevation; Enterococcus declined across the three events.
MB4	Manuels Branch at Cleveland Avenue	Distinct Manuels Branch sentinel reach at/near Cleveland Avenue (US 41).	Enterococcus exceeded its screening reference in all three events; *E. coli* exceeded its reference in two of three.
MBC	Manuels Branch at Broadway and Canal	Distinct central Manuels Branch sentinel reach at Broadway and Canal.	Persistent *E. coli* elevation; Enterococcus declined by August.
MBZ	Manuels Branch above Evans Avenue	Eastern/upstream Manuels Branch sentinel reach above Evans Avenue.	Highest-priority site; largest and most persistent paired elevations.
MC3	Mullock Creek at San Carlos Park / Constitution	Mullock Creek sentinel reach in San Carlos Park; July documentation describes the reach as above the weir near US 41.	Lower bacterial range; indicator choice changed screening interpretation in June and July, while both were below in August.

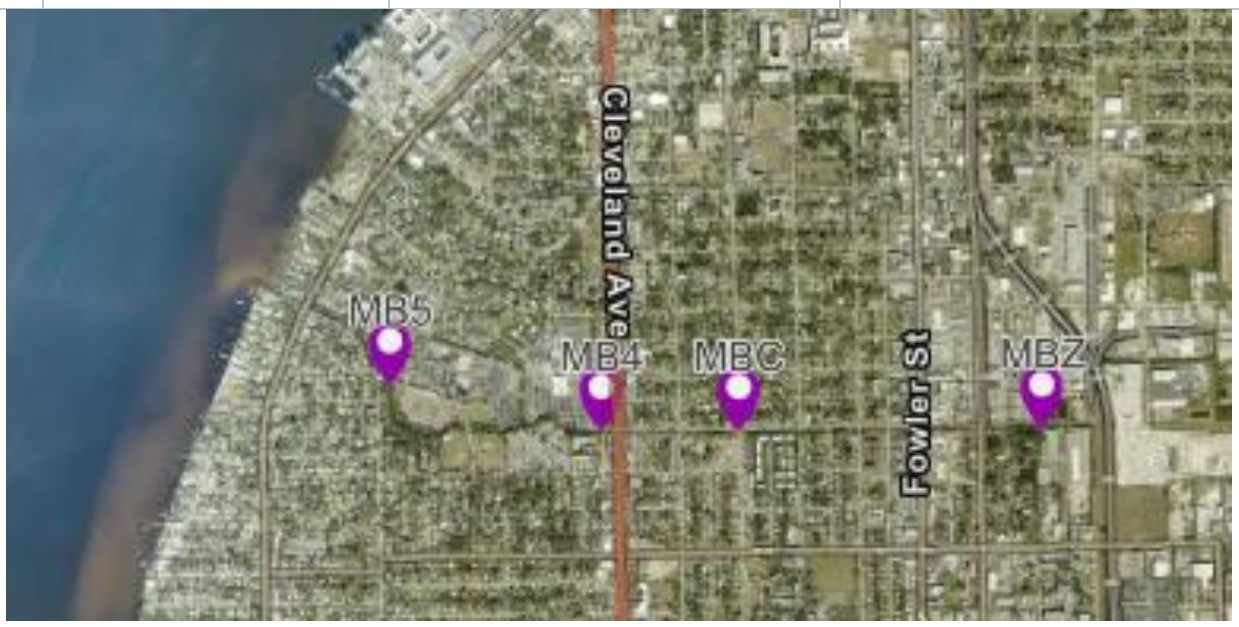


Figure 3. Four distinct Manuels Branch sentinel locations: MB5, MB4, MBC, and MBZ. The map documents spatial separation among the four reaches.

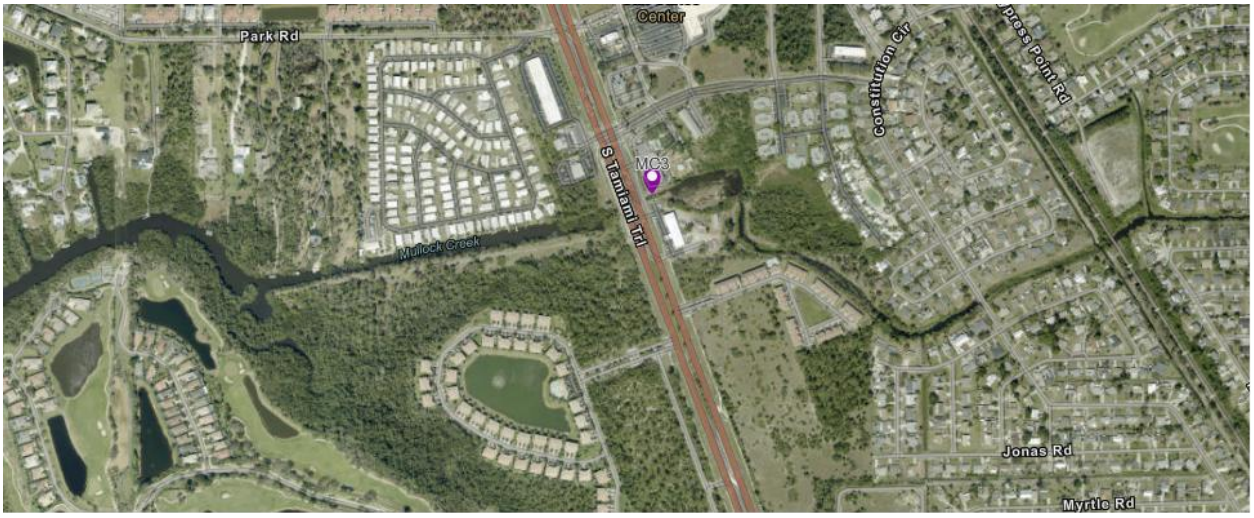


Figure 4. MC3 on Mullock Creek, the distinct fifth sentinel site.

2.2 Paired co-located certified-laboratory design

For each sentinel site during each focused event, two separate bottles were collected at approximately the same location and time. One was processed by CWK and the paired bottle was delivered to AEL. This design evaluates agreement between two complete real-world monitoring pathways, including small-scale field heterogeneity; it is not a split homogeneous sample or formal proficiency test.

2.3 Site-code harmonization

The same five physical sites were preserved even when field/laboratory shorthand changed. In June, CFMKEN corresponds to MB5 at Cortez Avenue and MB5KEN corresponds to the Broadway/Canal site standardized here as MBC. In July, AEL used MBB for the site standardized as MBC; the July field record documents the correction. These changes reconcile naming only and do not combine different physical sites.

3. Field and Laboratory Methods

3.1 Field collection and sample traceability

Across the three focused sampling events, an average of two volunteers per event collected the five sentinel-site samples using standardized Ranger equipment and documentation - six field-volunteer deployments in total. At each event, volunteers collected the paired CWK and AEL bottles, transported the CWK samples to the laboratory at the Calusa Nature Center, and delivered the paired comparison bottles to AEL. The field form captured site, time, coordinates, handoff, temperature, salinity, weather, tide/water level, and observations. Field collection and transport duration was not separately logged, so those hours are not included in the conservative documented-hour total reported in Section 9.

3.2 CWK laboratory methods

CWK analyzed Enterococcus using IDEXX Enterolert and *E. coli*/total coliform using IDEXX Colilert-18 with Quanti-Tray/2000 enumeration. Two laboratory volunteers supported each focused event, spending approximately 3 hours on Day 1 receiving and processing samples and 2 hours on Day 2 completing the read/processing workflow. This represents 10 laboratory volunteer-hours per event and 30 documented laboratory volunteer-hours across the three focused events. Results were stored as MPN/100 mL together with tray counts, dilution information, confidence intervals, lot information, incubation records, and read information in the CWK database. The detailed July report documents a representative batch: both AA negative controls were 0 large / 0 small wells and recorded as <1 MPN/100 mL; Enterolert was incubated at 41 C; Colilert-18 at 35 C; reagent and tray lots were recorded; and July samples were processed within the six-hour benchmark used in the source report for bacteriological grab samples.

3.3 Independent AEL comparison

Advanced Environmental Laboratories, Inc. (AEL), NELAP certificate E84492, analyzed five paired co-located samples each month. AEL reported Enterococcus using Enterolert/Quanti-Tray and *E. coli* using Standard Methods 9223 B/Quanti-Tray. The grant application referred to this activity as certified laboratory validation; because separate bottles were collected, this report uses the more precise term independent paired co-located comparison.

3.4 Technical replicates and data treatment

August included technical replicates at MB4 and MBC. MB4 *E. coli* triplicates were 1,178, 1,106, and 1,187 MPN/100 mL (CV 3.84%); MB4 Enterococcus triplicates were 670, 703, and 691 (CV 2.43%). MBC *E. coli* duplicates were 1,259 and 1,396 MPN/100 mL. Raw replicate values remain in the database; geometric means are used in the three-month trend summaries because FIB concentrations are multiplicative and are plotted on logarithmic scales.

3.5 Florida regulatory screening framework

For decision-oriented comparison, this report uses the Florida Ten Percent Threshold Values (TPTVs) in FAC 62-302.530(6)(b)-(c) as regulatory screening references: 410 MPN/100 mL for *E. coli* and 130 MPN/100 mL for Enterococcus. The same rule also specifies monthly geometric means of 126 MPN/100 mL for *E. coli* and 35 MPN/100 mL for Enterococcus, with monthly geometric means based on at least 10 samples over a 30-day period. Because this pilot collected one focused sample event per month at each sentinel site, TPTV comparisons are used only as screening references for pattern recognition and communication; they are not formal regulatory impairment or compliance determinations.

4. CWK Results at the Five Sentinel Sites

Table 3. CWK site-specific E. coli and Enterococcus results for the five repeated sentinel sites. August MB4 and MBC values use geometric means of technical replicates for trend comparison.

Date	Site	*E. coli* MPN/100 mL	*E. coli* screen	Enterococcus MPN/100 mL	Enterococcus screen	Paired screen
June 19	MB5	1,467	Above 410	1,201	Above 130	Agreement
June 19	MB4	468	Above 410	1,054	Above 130	Agreement
June 19	MBC	1,211	Above 410	1,076	Above 130	Agreement
June 19	MBZ	663	Above 410	6,867	Above 130	Agreement
June 19	MC3	122	At/below 410	259	Above 130	Discordant
July 17	MB5	1,236	Above 410	759	Above 130	Agreement
July 17	MB4	253	At/below 410	644	Above 130	Discordant
July 17	MBC	1,043	Above 410	933	Above 130	Agreement
July 17	MBZ	4,106	Above 410	12,033	Above 130	Agreement
July 17	MC3	241	At/below 410	323	Above 130	Discordant
August 14	MB5	2,481	Above 410	512	Above 130	Agreement
August 14	MB4	1,156.4	Above 410	687.9	Above 130	Agreement
August 14	MBC	1,325.7	Above 410	383	Above 130	Agreement
August 14	MBZ	5,794	Above 410	1,860	Above 130	Agreement
August 14	MC3	233	At/below 410	97	At/below 130	Agreement

4.1 Three-month site summary

Table 4. Three-month site summary. MBZ had the highest median for both indicators. E. coli exceeded the 410 MPN/100 mL TPTV screening reference in all three months at MB5, MBC, and MBZ; in two of three months at MB4; and in none of the three months at MC3.

Site	*E. coli* median	*E. coli* range	Months >410	Enterococcus median	Enterococcus range	Months >130
MB5	1,467	1,236-2,481	3/3	759	512-1,201	3/3
MB4	468	253-1,156.4	2/3	687.9	644-1,054	3/3
MBC	1,211	1,043-1,325.7	3/3	933	383-1,076	3/3
MBZ	4,106	663-5,794	3/3	6,867	1,860-12,033	3/3

Site	*E. coli* median	*E. coli* range	Months >410	Enterococcus median	Enterococcus range	Months >130
MC3	233	122-241	0/3	259	97-323	2/3

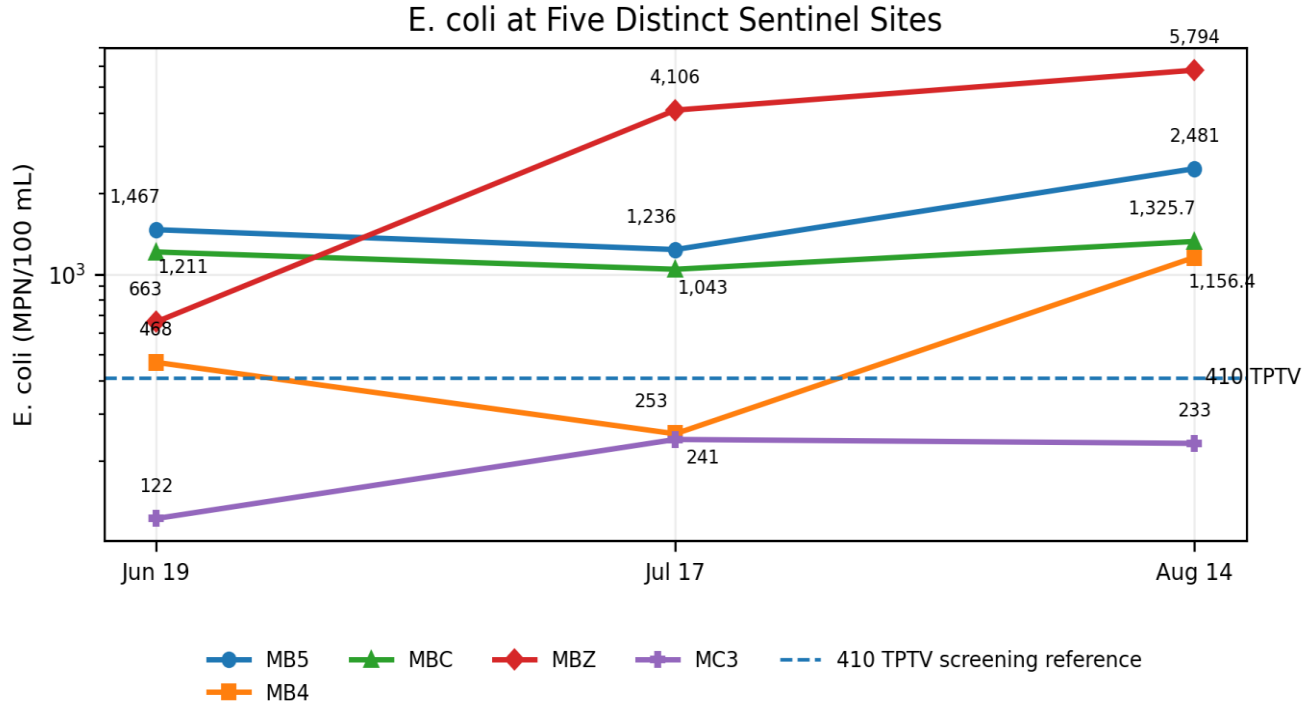


Figure 5. Three-month CWK E. coli trends at the five distinct sentinel sites. The dashed line marks the 410 MPN/100 mL Florida TPTV screening reference. Exact values are labeled at each point.

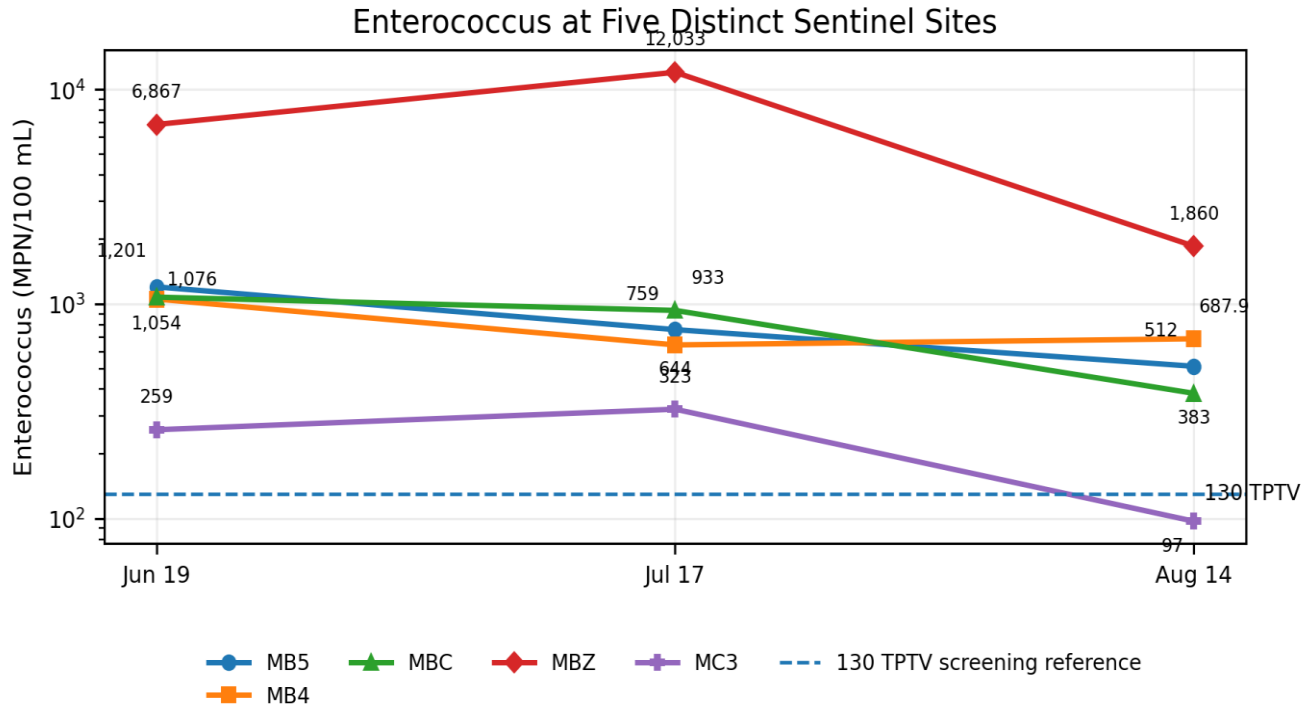


Figure 6. Three-month CWK Enterococcus trends at the same five sites. The dashed line marks the 130 MPN/100 mL Florida TPTV screening reference. MBZ remained the dominant Enterococcus site in all three months.

4.2 Site-by-site interpretation

MBZ - persistent high-priority site. MBZ produced the highest three-month median for both indicators (*E. coli* 4,106; Enterococcus 6,867 MPN/100 mL) and contained the largest CWK Enterococcus peaks in June and July. Even when Enterococcus fell in August, *E. coli* increased to 5,794 MPN/100 mL. MBZ is the strongest candidate for repeat confirmation, spatial bracketing, rainfall review, and future sanitary/source investigation.

MB5 - persistent *E. coli* signal despite declining Enterococcus. *E. coli* remained high in all three months (1,467; 1,236; 2,481 MPN/100 mL), while Enterococcus declined from 1,201 to 759 to 512. This site demonstrates that the indicators can agree on screening status while moving in different directions.

MBC - stable *E. coli* with a late Enterococcus decrease. *E. coli* remained near or above 1,000 MPN/100 mL throughout the study, while Enterococcus decreased to 383 in August. The site remained elevated under both screens, but the magnitude relationship changed.

MB4 - recurrent elevation with one indicator disagreement. Enterococcus exceeded the 130 MPN/100 mL TPTV screening reference in all three months. *E. coli* exceeded 410 MPN/100 mL in June and August but was below the reference in July (253 MPN/100 mL). August technical replicates were tightly grouped, supporting confidence that the higher August *E. coli* concentration was not simply a single-tray anomaly.

MC3 - the indicator-choice test case. Enterococcus exceeded the 130 MPN/100 mL TPTV screening reference in June and July (259 and 323 MPN/100 mL) but was below it in August (97). *E. coli* was 122, 241, and 233 MPN/100 mL and remained at or below 410 in all three months. The indicators were therefore discordant at MC3 in June and July and agreed below the screening references in August.

5. What Did *E. coli* Add to the Program?

The purpose of adding *E. coli* was not to generate a second number for every sample; it was to determine whether a freshwater-oriented indicator changes the information available to CWK. The answer from the pilot is yes. *E. coli* generally confirmed the same high-priority Manuels Branch locations, but it did not merely duplicate Enterococcus.

- Across the 12 Manuels Branch site-months, Enterococcus exceeded 130 MPN/100 mL in all 12, while *E. coli* exceeded 410 MPN/100 mL in 11 of 12; MB4 in July was the single exception.
- Across all 15 sentinel site-months, the two indicators produced the same above/below Florida TPTV screening outcome in 12 cases (80.0%).
- Discordant screening interpretations occurred at MC3 in June and July and at MB4 in July: Enterococcus was above 130 while *E. coli* was at or below 410.
- Across the 15 CWK site-month values, the indicators had a moderate rank association (Spearman rho = 0.60).
- The relative magnitude shifted by site and month; for example, MBZ was strongly Enterococcus-dominant in June/July but *E. coli*-dominant in August.

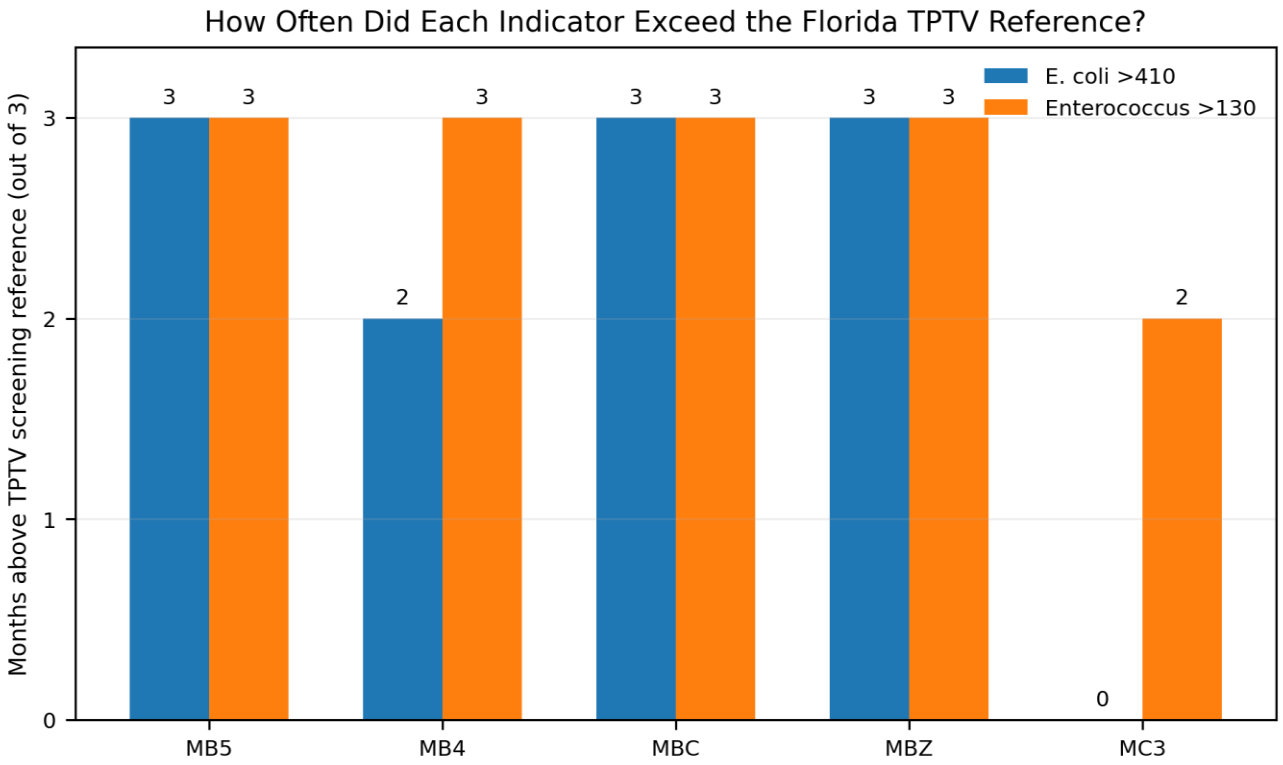


Figure 7. Number of months in which each indicator exceeded its Florida TPTV screening reference. MB5, MBC, and MBZ were above both references in all three months. At MB4, E. coli exceeded 410 in two of three months while Enterococcus exceeded 130 in all three; at MC3, E. coli exceeded 410 in none of the three months while Enterococcus exceeded 130 in two of three.

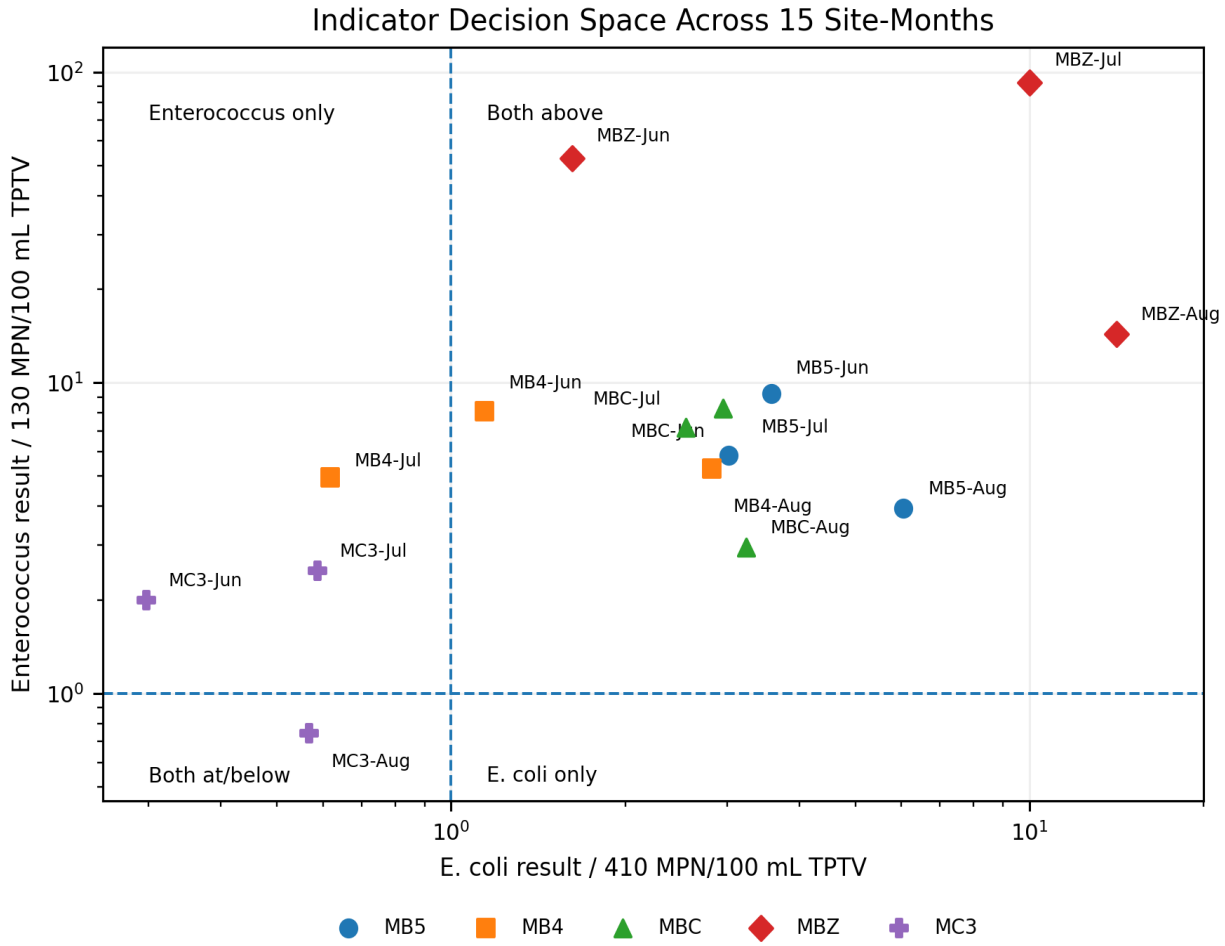


Figure 8. Indicator decision space normalized to each indicator's Florida TPTV screening reference. Points to the upper right were above both references. Enterococcus-only observations were MC3 in June and July and MB4 in July; MC3 in August was at or below both references.

Scientific interpretation

The grant did not identify a universal "winner" between Enterococcus and *E. coli*. Instead, it showed that a one-indicator network can be efficient only after site type is known. The appropriate indicator must be linked to freshwater versus marine/tidal conditions, while transition waters require dual testing until salinity and seasonal hydrology are understood.

6. Independent AEL Comparison

AEL analyzed a paired co-located bottle from each of the five sentinel sites during each monthly event, yielding 15 site-month comparisons for each indicator. Because the two laboratories received separate bottles rather than a split homogeneous sample, differences reflect both analytical variability and natural small-scale heterogeneity. Several July AEL results were reported above the analytical range and are therefore right-censored; those values were excluded from exact numerical agreement statistics.

Comparison result

CWK *E. coli* showed the stronger numerical agreement with AEL during this pilot. Among uncensored pairs, *E. coli* had Spearman rho = 0.78 and 11 of 14 pairs were within two-fold of AEL. Enterococcus had Spearman rho = 0.66 and 6 of 12 uncensored pairs were within two-fold. This is encouraging for the CWK community-laboratory workflow while also identifying Enterococcus as the analyte for which continued external comparison and QA refinement could provide the greatest value.

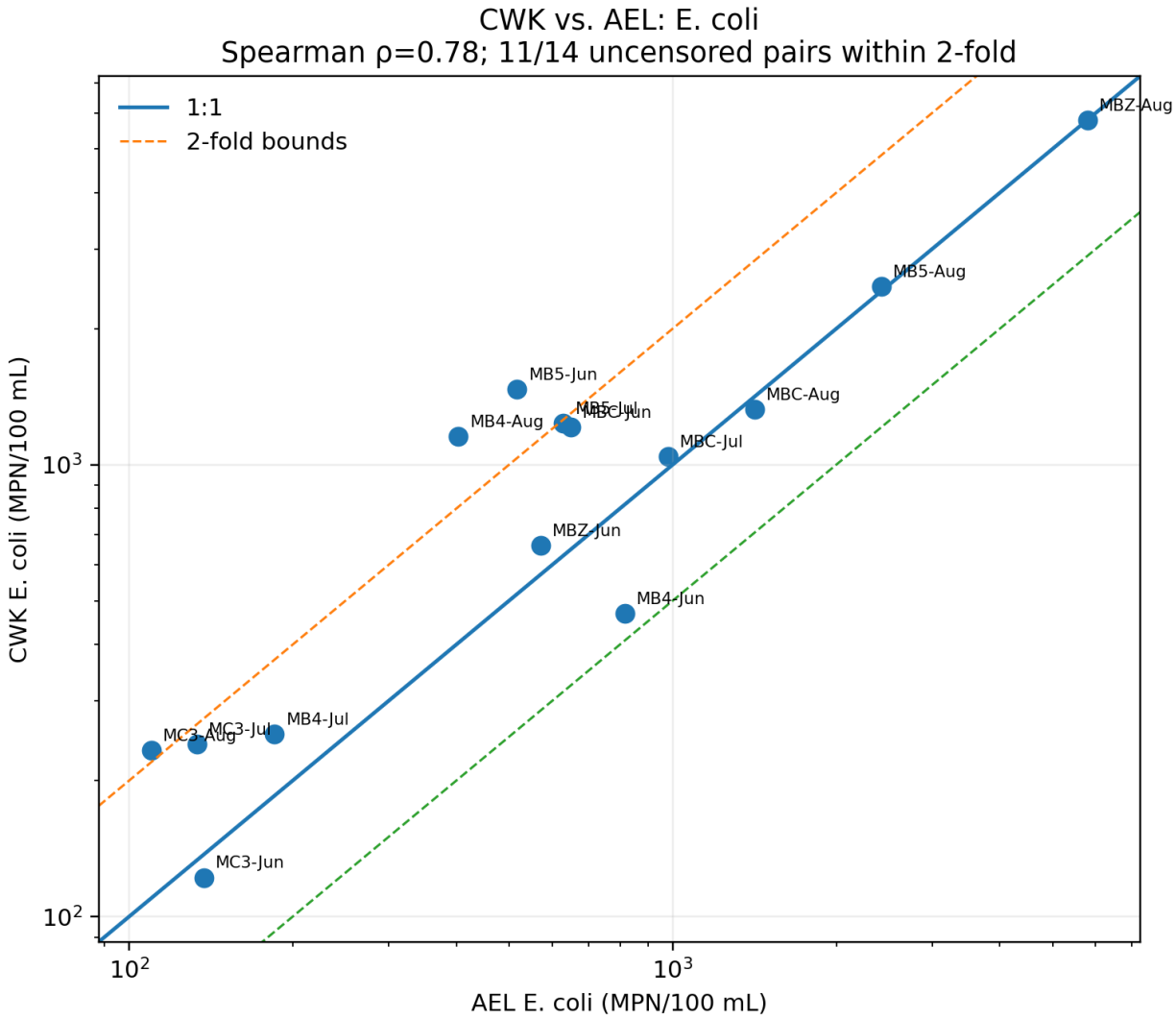


Figure 9. CWK versus AEL E. coli for uncensored paired co-located samples. The solid line is 1:1 agreement and the dashed lines show a two-fold envelope. Point labels identify site and month.

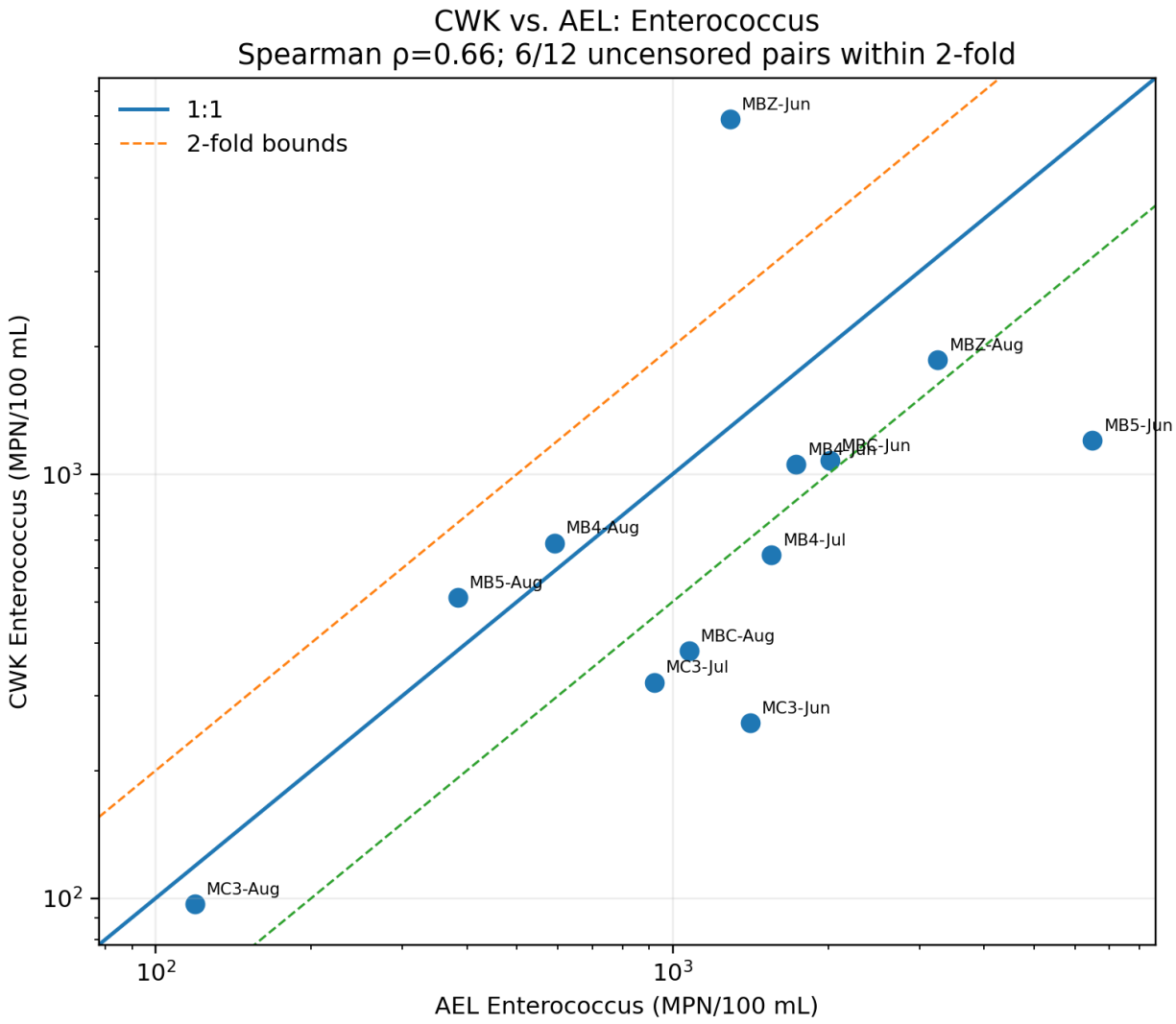


Figure 10. CWK versus AEL Enterococcus for uncensored paired co-located samples. Agreement was positive but more variable than for E. coli during this pilot.

7. Quality Assurance, Documentation, and Reproducibility

The July report provides a detailed representative example of the community laboratory workflow. Both FIB batches included an AA negative control with 0 positive large wells and 0 positive small wells, recorded as <1 MPN/100 mL. Reagent lots, expiration dates, tray lots, processing windows, incubation temperatures, and readers were documented. The July Colilert-18 batch explicitly documented a 1:10 dilution and 35 C incubation; Enterolert was recorded at 41 C.

The standardized Lab Cover Sheet formalizes the same QA/QC elements: processing start/end, scheduled read window, dilution, test type, reagent and tray lots, incubator/temperature, control result, issues/deviations, and final review. This is an important project outcome because defensible community science depends on documenting the process, not only the final MPN value.

August technical replicates added evidence of repeatability. MB4 *E. coli* triplicates had CV 3.84%, and MB4 Enterococcus triplicates had CV 2.43%. MBC *E. coli* duplicates were also reasonably close (1,259 and 1,396 MPN/100 mL). These results support reproducibility of the tested CWK tray-reading and processing workflow.

Total coliform was also measured during Colilert-18 testing and was often extremely high or upper-range-censored in the detailed July report. It is retained as a supporting process/context indicator, but it did not resolve the project's main decision about which fecal indicator should guide freshwater versus tidal monitoring. The final scientific interpretation therefore centers Enterococcus and *E. coli*.

8. Monitoring Strategy: Enterococcus Everywhere or *E. coli* in Freshwater?

The source report notes that EPA's 2012 Recreational Water Quality Criteria recognize Enterococcus for marine and fresh waters and *E. coli* for fresh waters. That framework matters in Southwest Florida because tributaries may be freshwater-dominant, tidal, or transitional depending on location, rainfall, flow, and season.

The CHNEP pilot does not support eliminating Enterococcus from the five sentinel sites now. The paired dataset covers only three summer events, the sites occupy different positions in two tributaries, and the two indicators were not equivalent at MC3 and MB4 on some dates or in month-to-month magnitude. Immediate elimination of Enterococcus would also break continuity with CWK's historical Enterococcus record.

At the same time, the results support a more intentional use of *E. coli* in clearly freshwater sites. *E. coli* produced persistent, interpretable signals at the Manuels Branch sites, tracked AEL more closely during this pilot, and directly addressed the freshwater monitoring gap identified in the grant application. The most efficient future design is therefore tiered and site-classified, not all-or-nothing.

Table 5. Recommended tiered indicator strategy arising from the CHNEP pilot.

Site type	Recommended routine indicator	Recommended CWK practice
Clearly freshwater	*E. coli*	Use *E. coli* as the primary routine indicator; retain periodic Enterococcus cross-checks during transition and for continuity.
Marine / clearly tidal	Enterococcus	Continue Enterococcus as the primary recreational indicator.
Brackish / transition / uncertain	Both	Measure both until salinity and seasonal hydrology establish a defensible site classification.
Five CHNEP sentinel sites	Both, temporarily	Continue both through at least a full seasonal cycle so the indicator relationship can be evaluated with salinity and rainfall/flow context.

Operational rule to add now

Make salinity a required value for every sentinel sample and a required database field at close-out. The Ranger Field Sampling form already contains a salinity field. Ensuring that value is actually recorded and transferred to the database is the key next step for defensible freshwater/tidal site classification.

9. Volunteer Contributions and In-Kind Effort

Volunteer participation was a major component of the project and substantially increased the value of CHNEP funding. Across the three focused events, six field-volunteer deployments supported collection and transport; two CWK laboratory volunteers per event contributed 30 documented laboratory volunteer-hours; and seven volunteers contributed 21 hours assembling the 30 Ranger kits. These timed activities alone account for 51 volunteer-hours. Adding 6 documented project-lead reporting hours brings the known volunteer/in-kind project effort to at least 57 hours, while the actual contribution was higher because field collection and transport time was not separately logged.

The volunteer contribution translated directly into scientific output and lasting program capacity: samples from five separate sites were collected on three dates, processed through the CWK laboratory, transported for independent AEL comparison, and converted into a 15-site-month dual-indicator dataset. The kit-assembly effort created 30 reusable monitoring kits, while laboratory volunteers provided the processing effort needed to generate the paired FIB results. Project reporting added approximately 1.5 hours for each of the three monthly reports and 1.5 hours for this final report (6 in-kind project-lead hours).

Activity	Participation	Time basis	Documented project effort
Focused field sampling and transport	Average 2 volunteers/event x 3 events	Field/transport duration not separately logged	6 volunteer deployments; hours not included in minimum
CWK laboratory processing	2 volunteers/event x 3 events	3 h Day 1 + 2 h Day 2	30 volunteer-hours
Ranger kit assembly	7 volunteers	3 h assembly session	21 volunteer-hours
Monthly + final reporting	Project lead	1.5 h x 3 monthly reports + 1.5 h final report	6 in-kind project hours
Minimum documented volunteer total	Timed volunteer activities only	30 lab + 21 kit assembly	**51 volunteer-hours + unlogged field/transport time**
Documented volunteer + in-kind project effort	**Volunteer activities + project reporting**	**51 volunteer h + 6 reporting h**	**57+ documented hours + unlogged field/transport time**

Volunteer accounting note. Counts are reported as volunteer deployments/roles rather than unique individuals because some participants may have served in more than one activity. The 51-hour volunteer minimum includes only timed laboratory processing and Ranger-kit assembly. The 57+ hour volunteer/in-kind total adds documented project reporting time but still excludes field collection and transport duration, making both figures conservative.

10. Community and Public-Health Relevance

The public-health relevance is practical: fecal indicator bacteria are used to screen for conditions associated with increased likelihood of fecal contamination and possible pathogen exposure. These results do not identify a contamination source or prove the presence of a specific pathogen, but repeated elevated results can identify locations where confirmation sampling, sanitary surveys, source tracking, or agency coordination are warranted. This is especially useful in waterways that are not sampled with the same spatial and temporal frequency by regulatory programs.

Figure 11. Volunteers using standardized sampling equipment at an urban tributary monitoring location. Photo provided by Calusa Waterkeeper.

11. Project Outcomes and Value of CHNEP Funding

CHNEP funding created a durable monitoring system rather than a one-time sampling exercise. The focused sentinel component generated 60 analytical results across CWK and AEL, organized into 30 paired indicator-level comparisons across 15 repeated site-months. At the same time, the grant built 30 reusable Ranger kits, formalized two field/laboratory documentation tools, supported a centralized data workflow, and leveraged at least 57 documented volunteer/in-kind hours plus unlogged field collection and transport time.

The five-site results demonstrate why that investment matters. The project identified a recurrent high-priority site (MBZ), showed that nearby reaches of Manuels Branch can have different indicator trajectories, revealed a specific indicator-selection issue at MC3, and produced an external comparison that was especially encouraging for *E. coli*. Those findings can directly improve how CWK allocates volunteer hours, reagents, and external-laboratory funds.

The 30 Ranger kits, standardized forms, trained volunteer workflow, and data system remain available after the grant closes. The project mobilized at least 51 documented volunteer-hours in laboratory processing and kit assembly, six field-volunteer deployments across the three focused events, and 6 additional in-kind project-lead reporting hours. Because field collection and transport duration was not separately logged, the 57+ hour total understates the actual community contribution. CHNEP funding therefore continues to generate value: the same sentinel sites can be followed through a full seasonal cycle, the broader network can adopt a more efficient indicator-by-site strategy, and future source-investigation resources can be directed toward recurring patterns rather than isolated observations.

CHNEP return on investment

The grant converted routine volunteer sampling into a quantified, decision-oriented monitoring program: 30 reusable Ranger kits; 15 repeated site-months; 60 sentinel analytical results across CWK and AEL; 30 paired indicator-level comparisons; 12 of 15 site-months with the same indicator screening interpretation; and at least 57 documented volunteer/in-kind project hours, before counting unlogged field collection and transport time. These assets and workflows remain available beyond the grant.

12. Limitations

- The focused sentinel dataset covers three summer sampling events. It is sufficient for a pilot comparison but not for seasonal or annual characterization.
- Each CWK-AEL comparison used separate co-located bottles rather than a split homogenized sample; field heterogeneity therefore contributes to differences.
- Several AEL July results were reported above the analytical range and are right-censored; they cannot be treated as exact values in agreement statistics.
- Salinity was not present in the five-site database export used for this report, even though the standardized field form includes a salinity field. Site classification should be confirmed before a permanent one-indicator rule is adopted.
- Florida TPTV-based comparisons are used here for screening and communication only. This three-event pilot does not provide the sampling frequency needed for a formal regulatory impairment or compliance determination, which also considers 30-day exceedance frequency and monthly geometric-mean criteria.
- Site-code shorthand changed during the project. Codes were harmonized using location descriptions and documented corrections so that the same five physical sites remained distinct and traceable.
- Total coliform frequently reached the upper range during detailed July testing and is therefore not used as the principal decision metric in this report.

13. Conclusions and Recommended Next Steps

The CHNEP-funded project established a scientifically useful five-site sentinel comparison while also strengthening the infrastructure behind CWK's broader community monitoring network. Across five distinct sites and three focused events, the project produced 15 repeated site-month observations, 30 CWK sentinel FIB results, 30 corresponding AEL analytical results, and 30 paired indicator-level comparisons. The most important scientific result is not a regional average; it is the repeated site-specific pattern. MBZ was the strongest recurring hotspot, MB5/MBC/MB4 showed recurrent elevations with different temporal trajectories, and MC3 - together with the July MB4 result - demonstrated that indicator choice can materially change a screening interpretation on individual dates.

E. coli and Enterococcus are sufficiently related to confirm the same major Manuels Branch concerns, but they are not equivalent measurements. The pilot therefore supports a staged monitoring strategy: preserve dual-indicator testing at the five sentinel sites while adding salinity and extending the record; use *E. coli* as the primary routine indicator at clearly freshwater sites; use Enterococcus at marine/tidal sites; and use both at transition sites until classification is secure.

The independent AEL comparison strengthens the case that the CWK community laboratory can generate useful screening data when standardized collection, IDEXX methods, QA/QC, database traceability, and periodic external comparison are used together. Continued external comparison should be retained as a quality-improvement tool rather than interpreted as a one-time certification exercise.

- Continue monthly dual-indicator testing at MB5, MB4, MBC, MBZ, and MC3 through at least a full 12-month seasonal cycle.
- Make salinity completion and database entry mandatory for every sentinel sample; consistently record rainfall, flow/tide condition, and visible field observations.
- Prioritize MBZ for confirmation sampling, spatial bracketing, and potential sanitary/source investigation.
- Retain negative controls every batch and periodic technical replicates.
- Repeat AEL paired co-located comparison quarterly or seasonally, with deliberate dilutions when high counts are expected.
- After a full seasonal dataset is available, formally evaluate whether clearly freshwater sites can transition to *E. coli*-only routine monitoring without loss of important information.
- Use the 30 standardized Ranger kits and trained volunteer network to sustain and expand the sentinel approach beyond the grant period.
- Track volunteer time by activity in future monitoring events so field collection, transport, laboratory processing, kit maintenance, and reporting can be fully quantified as in-kind project support.
- Retain and audit the standardized field and lab forms as part of routine QA/QC; ensure values collected on paper, especially salinity, are transferred into the database.

Final project message

CHNEP funding moved CWK beyond simply collecting water samples. It produced 30 reusable Ranger kits, 60 sentinel analytical results across two laboratories, 30 paired indicator comparisons, a documented field-to-laboratory QA/QC workflow, and at least 57 known volunteer/in-kind project hours plus additional unlogged field effort. Scientifically, the project identified a persistent priority site, demonstrated where *E. coli* and Enterococcus agree and diverge, and created a practical pathway to use the right indicator at the right waterbody while preserving continuity, community participation, and scientific quality.

Appendices and Supporting Documentation

The appendices preserve the project records supporting the final report and provide traceable documentation for future review and program continuity.

- Appendix A - Complete paired CWK-AEL sentinel-site results (15 site-months; 30 paired indicator comparisons)
- Appendix B - Representative July QA/QC details for Enterolert and Colilert-18 processing
- Appendix C - Ranger Field Sampling & Water Quality Data Form
- Appendix D - Calusa Waterkeeper Lab Cover Sheet
- Digital supplement - CHNEP Final Project Data Tables and Graphs workbook with source tables and editable figures

Appendix A. Paired CWK-AEL Sentinel-Site Results

The table below preserves all 15 paired site-month comparisons. AEL values prefixed with > were above the analytical range and were excluded from exact numerical correlation/fold-agreement calculations. The AEL-code column preserves site-code harmonization for traceability.

Date	Site	AEL code	CWK *E. coli*	AEL *E. coli*	CWK Enterococcus	AEL Enterococcus	Censor note
June 19	MB5	CFMM	1467	517.2	1201	6,488	
June 19	MB4	MB4	468	816.4	1054	1,732.9	
June 19	MBC	MB5	1211	648.8	1076	2,014	
June 19	MBZ	MBZ	663	571.7	6867	1,291	
June 19	MC3	MC3	122	137.2	259	1,413.6	
July 17	MB5	MB5	1236	629.4	759	>2,419.6	Enterococcus censored
July 17	MB4	MB4	253	185.0	644	1,553.1	
July 17	MBC	MBB	1043	980.4	933	>2,419.8	Enterococcus censored
July 17	MBZ	MBZ	4106	>2,419.6	12033	>2,419.8	*E. coli* and Enterococcus censored
July 17	MC3	MC3	241	133.3	323	920.8	
August 14	MB5	MB5	2481	2,419.6	512	384	
August 14	MB4	MB4	1156.4	403.4	687.9	591	
August 14	MBC	MBC	1325.7	1,413.6	383	1,076	
August 14	MBZ	MBZ	5794	5,794	1860	3,255	
August 14	MC3	MC3	233	109.8	97	119	

Appendix B. Representative July QA/QC Details

Batch element	Enterolert	Colilert-18
Analyte	Enterococcus	*E. coli* / total coliform
Format	IDEXX Quanti-Tray/2000	IDEXX Quanti-Tray/2000
Reagent lot	L2744	H0084
Reagent expiration	December 13, 2026	August 10, 2027
Tray lot	E2029J	E2029J
Incubation	41 C	35 C
Negative control	AA: 0/0; <1 MPN/100 mL	AA: 0/0; <1 MPN/100 mL
July dilution documentation	Tenfold factor reconstructs results; raw dilution fields incomplete	1:10 explicitly documented

Table B1. Representative July QA/QC information carried forward from the detailed July FIB Results Report.

The July source report also documented complete field forms for the eight July sites, laboratory cover sheets, processing/read windows, and site maps. This final report uses those records as representative documentation while focusing the statistical analysis on the five repeated sentinel sites.

Appendix C. Ranger Field Sampling & Water Quality Data Form

This standardized field form documents sample identity and handoff, coordinates, water/air temperature, salinity, weather, tide/water level, basic water-quality screening, and field observations. Its salinity field should be treated as required for future sentinel-site indicator classification.

Ranger Field Sampling & Water Quality Data Form

Site Code: _____

Date Collected: _____
Time Collected: _____
Collector's name: _____

Sample Transfer / Handoff (if applicable)
Transferred to (Name): _____
Date/Time of transfer: _____

Location Narrative: _____

SITE COORDINATES AND LOCATION DATA | Use decimal degrees

Coordinates: In Google Maps, press and hold the exact site to drop a pin, then copy the coordinates. The first number is latitude; the second is longitude. Example: 26.541234, -81.945678. If shown in degrees/minutes/seconds, convert at: *https://www.fcc.gov/media/radio/dms-decimal*

Latitude - decimal degrees: _____	**Longitude** *(e.g., -81.945678)*: _____
Air temperature (°C): _____	**Water temperature (°C):** : _____

Temperature conversion: °C = (°F - 32) × 5/9. Example: 86°F = 30°C. Record only the number.

Salinity (ppt) - refractometer reading: _____
Calibrate with DI water first: add a few drops, close the cover plate, and confirm 0 ppt. Adjust if needed. Wipe dry, add the water sample, and record the reading.

Weather conditions *	□ Sunny / clear □ Partly cloudy □ Overcast □ Light rain □ Heavy rain / storm nearby □ Windy
Tide / water level *	□ Low / low water □ Incoming / rising □ High / high water □ Outgoing / falling □ Not sure

Other weather conditions: _____

8-IN-1 WATER TEST STRIP RESULTS | Mark the closest color/value

Pads 1-7: Immerse only pads 1-7 for 2 seconds; remove promptly. Do not shake off excess water. Hold the strip level for 30 seconds, compare with the color chart, and mark the closest result.

Test / unit	Select one result
Free chlorine (Cl_2) (ppm)	□ 0 □ 0.5 □ 1.0 □ 3.0 □ 5.0 □ 10 □ 20
Nitrate (NO_3^-) (ppm / mg/L)	□ 0 □ 10 □ 25 □ 50 □ 100 □ 250
Nitrite (NO_2^-) (ppm / mg/L)	□ 0 □ 1 □ 5 □ 10
Total hardness (ppm)	□ 0 □ 25 □ 50 □ 125 □ 250 □ 425
Total alkalinity (KH) (ppm / mg/L)	□ 0 □ 40 □ 80 □ 120 □ 180 □ 240
pH (standard pH units)	□ 6.2 □ 6.8 □ 7.2 □ 7.6 □ 7.8 □ 8.4
Sodium chloride (ppm)	□ 0 □ 2000 □ 2700 □ 3400 □ 4000 □ 6000
Pad 8 procedure	Immerse only pad 8 for 3-5 seconds; remove promptly. Do not shake. Hold level for 2-3 minutes; compare with the color chart.
Ammonia nitrogen (ppm)	□ **0** □ **0.5** □ **1** □ **3** □ **5** □ **10**

Notes / observations *(weather, odors, visible pollution, procedure issues)*

Did you give out any CWK business cards with the "Report Pollution" link? * □ **Yes** □ **No**	**If yes, how many?** ____

Prepared by: A. Zirzow | Version: v1.3 | Revision Date: 2/23/2026

Appendix D. Calusa Waterkeeper Lab Cover Sheet

This laboratory cover sheet documents testing personnel, processing timing, reagent/tray traceability, test type, dilution, incubation, controls, issues/deviations, and final review. It formalizes the QA/QC record needed to support reproducible community-laboratory results.

CALUSA WATERKEEPER LAB COVER SHEET | FIB Testing Day Record

Testing Date: _____	Lab Location: _____	Lead Technician Volunteer: _____	Lab Tech Volunteer: _____
Lab Tech Volunteer: _____	Processing Start (reagent added): _____	Processing End (all incubated): _____	Scheduled Read Window (date): _____ Scheduled Read Window (time): _____
Quanti-tray Sealer Time Started: _____ Quanti-tray Sealer Hours Start: _____	Quanti-tray Sealer Time Ended: _____ Quanti-tray Sealer Hours End: _____		

REAGENT AND QUANTITRAY RECORD

If one reagent and Quantitray lot is used for all samples, enter "same lot used for all samples" in Row 1; leave repeated rows blank or mark "same." If additional samples do not fit on this page, write "See attached Sample Intake Form" and attach the completed intake form listing all remaining samples.

Sample ID / Site Code	Dilution Factor	Test Type	Reagent Used	Reagent Lot #	Reagent Exp. Date	Quantitray Type	Quantitray Lot #	Incubator # / Temp. (°C)
		☐ E. coli / TC ☐ Enterococcus	☐ Enterolert ☐ Colilert-18			☐ Quanti-Tray/2000		

QUALITY CONTROL / PROCESSING NOTES	LAB NOTES / ISSUES / DEVIATIONS	FINAL REVIEW
Sample condition: ☐ All cold ☐ Some not cold*NC ☐ Ice / ice packs ☐ Holding-time concern*HT Sealer check: ☐ Yes ☐ No Trays sealed: ☐ Yes ☐ No Leaks / tray issues: ☐ No ☐ Yes* Blank/control used: ☐ Yes ☐ No Type: Sterile Water Control result / notes: _____	Record anything unusual: missing forms, unclear labels, timing issues, damaged trays, reagent concerns, or sample-handling concerns. _____ _____ *Explanation: _____ _____ _____	Collection sheets attached: ☐ Yes ☐ No All reagent / tray lot numbers recorded: ☐ Yes ☐ No All trays placed in incubator: ☐ Yes ☐ No Read time recorded / scheduled: ☐ Yes ☐ No Reviewed by: _____ Date: _____