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August 18, 2026

Dear Honorable Mayor Duncan and Coronado City Council Members,

The County of San Diego would like to provide clarifying information regarding your August 18, 2026, General Business item 8.b. Quarterly Cross-Border Water Quality Update specifically related to the staff report's "Local Water Quality Testing Methodologies".

Attachment A is a copy of a letter that the County of San Diego sent to City Manager Tina Friend on September 16, 2024, regarding the City of Coronado's request to accept an alternative threshold for the digital droplet polymerase chain reaction (ddPCR) testing method based on a pilot study the City of Coronado conducted June 26, 2023 – September 18, 2023.

ddPCR Testing Method Based on Empirical Data and Rigorous Testing; approved by Federal and State Agencies

The current ddPCR testing method that the County is using was launched on May 5, 2022, after years of thorough research, study, and side-by-side testing by federal, state, regional and local agencies and academic and scientific research partners. After this rigorous evaluation, determinations of Enterococcus bacteria contamination thresholds (health standards), equivalent to the existing culture-based live bacteria growth method, were established and agreed upon by federal, state and local agencies. Findings were published in peer-reviewed journals, evaluated by public and environmental health professionals, and guided by a technical advisory group of academic experts in the field, researchers, and scientists, ultimately resulting in the United States Environmental Protection Agency (U.S. EPA, Attachment B) and California Department of Public Health (CDPH, Attachment C) issuing approvals for the County of San Diego to use the ddPCR method at their established threshold of 1,413 copies/100ml for the ddPCR test. The

County does not have the authority to change that threshold. According to the CDPH to reevaluate the approved threshold, the County's Public Health Officer would need to make a finding that the threshold is not effective to protect public health based on evidence-based data (Attachment D). To date, there is no data or evidence that the ddPCR testing method is not protecting public health; therefore, the County's Public Health Office cannot make a finding to request that CDPH reevaluate the threshold.

Increases in Transboundary Sewage Flows

We recognize that the South County beaches have seen an increase in the number of beach water contact closures due to numerous environmental factors, most notably the increase in untreated sewage from the Tijuana River and direct discharge into the ocean at San Antonio de los Buenos Creek in Punta Bandera, Mexico.

We also recognize that the beach water contact closures in Coronado have increased since 2023. During the 1,293-day period from January 1, 2023, to July 17, 2026, Imperial Beach locations have been impacted by sewage and water contact closures for 1,184 days, Silver Strand closed for 913 days, and the Coronado shoreline closed for 496 days. However, these closures are because transboundary infrastructure failures and sewage flows impacting ocean waters have significantly increased during this same timeframe. The County is not seeing an increase in closures occurring in other communities outside the South County region where there is not a known sewage pollution source even though the same testing methods are used at those beaches. For a comparison, during the same 1,293-day period from January 1, 2023, to July 17, 2026, beaches north of the South County beaches throughout the entire remaining approximately 50 miles of San Diego coastline collectively experienced only 134 days of water contact closure. Furthermore, the County is also not seeing a significant increase in bacterial water contact advisories in areas outside South County.

As documented by the International Boundary and Water Commission (IBWC) recently, approximately 108.16 billion gallons crossed the international border from December 28, 2022, through July 17, 2026. On June 9, 2023, U.S. EPA released a Programmatic Environmental Impact Statement (PEIS) and Record of Decision, which states, "These transboundary flows, which enter the U.S. via the Tijuana River and its tributaries and across the maritime boundary, impact public health and the environment and have been linked to beach closures in southern San Diego County." U.S. EPA's PEIS documents the flows' environmental impact to the region, specifically from water quality and public health at local beaches, and identifies their impacts to the marine and estuarine ecosystems and agricultural resources. The PEIS states that eliminating or dramatically reducing pollution would strongly benefit water quality and public health at beaches in the City of Imperial Beach, Silver Strand State Beach, and the City of Coronado.

According to the PEIS, approximately 28 million gallons per day of untreated wastewater have historically discharged directly into the Pacific Ocean at San Antonio de los Buenos Creek at Punta Bandera. Per the PEIS and a study conducted by Scripps Institution of

Oceanography, transboundary maritime flows of untreated wastewater discharged at Punta Bandera pose a substantial health risk to swimmers at beaches in southern San Diego County during the dry season (Feddersen et al., 2021), which coincides with San Diego's peak tourist season in the summertime.

Health Officer Mandate – Issue Water Contact Closures During Known Sewage Impacts

State law requires the County Public Health Officer to issue a beach water contact closure when there is a sewage or chemical spill that impacts ocean or bay waters, or when odors or discoloration are present in the water due to sewage contamination, such as if flows from the Tijuana River or sewage discharges are taking place south of the border that are reaching the ocean. Water testing is generally not used to issue a closure; therefore, the water quality testing method used by the County is not relevant for issuing a beach water contact closure. Once closures are issued, the County uses ddPCR test results to lift the closure once State health and water quality standards are met.

San Diego County Board of Supervisors Priority

Responding to the Tiuana River Valley sewage emergency is a priority of the San Diego County Board of Supervisors (Board). Since July 19, 2023, the Board has declared a local emergency 24 times. Responding to the Tijuana River Valley Sewage Crisis continues to remain one of the Board's top priorities for advocacy. The County continues to call for immediate, sustained binational action from all levels of government to address the ongoing emergency in the Tijuana River Valley. The County continues to support legislative efforts at the state and federal levels to advance solutions to the Tijuana River Valley crisis. At the federal level, this includes root-cause solutions to the crisis requiring binational cooperation and significant infrastructure projects that only the federal government can negotiate and fund. The County is also advocating for state legislation and funding opportunities that direct resources to clean up the Tijuana River Valley watershed and build out long-term infrastructure that mitigates sewage flow and aerosolization of toxic chemical compounds. Additionally, the County will continue to collaborate with local, state, federal, and binational agencies to support the health and well-being of impacted residents and find solutions to the ongoing crisis. The County has focused fiscal resource investments in direct response to the Tijuana River Valley sewage impacts through supporting indoor air purifier/filter distribution, advancing immediate and long-term solutions at Saturn Blvd to address the immediate public health impacts of hydrogen sulfide emissions, and seed funding for a multiyear epidemiological health study monitoring.

Our priority is protecting public health for all San Diego County residents and we remain committed to transparent communication, scientific integrity, and regional collaboration to address the complex issue of the emergency situation our communities are facing due to the transboundary sewage impacts.

Sincerely,

SAYONE THIHALOLIPAVAN, MD, MPH, Public Health Officer
County of San Diego, Health & Human Services Agency, Public Health Services

AMY HARBERT, MBA, Director of Environmental Health and Quality
County of San Diego

Cc:
June Weintraub, ScD, REHS, Deputy Director for Environmental Health & State Environmental Health Director, California Department of Public Health

Eric Dubinsky, PhD, Life Scientist, United States Environmental Protection Agency

Tara Flint Silva, Life Scientist & Project Officer, Water Division, United States Environmental Protection Agency, Region 9

Enclosures

1. **Attachment A_COSD letter to City of Coronado**
September 16, 2024, letter to Tina Friend, City of Coronado, from the County of San Diego Environmental Health and Public Health.

2. **Attachment B_US EPA ddPCR Approval Letter to County of San Diego**
U.S. Environmental Protection Agency letter to the County of San Diego — Re: Request for Concurrence to Implement Digital Droplet PCR (ddPCR) for Beach Water Quality Rapid Detection at Recreational Beaches in San Diego County.

3. **Attachment C_CDPH ddPCR Approval Letter to County of San Diego**
May 4, 2021, California Department of Public Health letter to the County of San Diego — Re: Digital Droplet Polymerase Chain Reaction use authorization for beach water quality assessment in San Diego County.

4. **Attachment D_COSD letter to US EPA_CDPH**
January 21, 2026, letter from the County of San Diego Environmental Health and Public Health to the California Department of Public Health and the U.S. Environmental Protection Agency — Re: Significant Concerns Regarding “The Relationship Between Enterolert and ddPCR Concentration of Enterococci in Summer Samples from Beaches in Coronado, California,” published in *Journal 240* (2026) 107346.

COUNTY OF SAN DIEGO
1600 PACIFIC HIGHWAY, SAN DIEGO, CALIFORNIA 92101-2422

ANKITA KADAKIA, M.D.
INTERIM PUBLIC HEALTH OFFICER

HEATHER BUONOMO, R.E.H.S.
DIRECTOR OF ENVIRONMENTAL HEALTH

September 16, 2024

Tina Friend
City of Coronado
1825 Strand Way
Coronado, CA 92118

Dear Ms. Friend,

This letter is in response to City of Coronado's (Coronado) request to accept an alternative threshold for the digital droplet polymerase chain reaction (ddPCR) testing based on the pilot study that Coronado conducted during June 26, 2023-September 18, 2023.

- On June 12, 2024, Coronado and County of San Diego (County) met in person to discuss Coronado's 2023 water quality testing Pilot Study. In this meeting, Tina Friend, Tony Winney and Coronado's consultant team presented Pilot Study results and the City's request to for the County to adjust the ddPCR threshold from 1413 copies/100ml to 6,791 copies/100ml. This presentation compared the Enterolert data (a different testing culture-based method) collected by Coronado to the County's ddPCR data collected during the same time period.
- During the June 12th meeting, The County Department of Environmental Health and Quality (DEHQ) and the Public Health Services (PHS) asked to see City's raw culture-based testing data for our review and analysis.
- On August 16, 2024, the County received a technical memorandum that compared Coronado's pilot study data to the Crain et al. (2021) study conducted by the County from 2017-2020.
- On August 22, 2024, the County received the subsequent raw data from the pilot study and responded that our internal expert was off until September 1st and we would review the documentation in early September.

The County has reviewed the presentation, technical memorandum and raw data set and has the following comments:

- State law requires the County to issue a beach water contact closure when there is a sewage spill or chemical spill that impacts ocean or bay waters, when there are odors or discoloration in the water due to sewage contamination, such as if flows from the Tijuana River that are reaching the ocean. Water testing is not used to issue a closure, therefore the water quality testing method used by the County is not relevant for issuing a beach water contact closure. Once closures are issued, the County does use ddPCR test results to lift the closure once State health water quality standards are met.
- The current threshold of 1,413 copies/100ml for the ddPCR test was set by California Department of Public Health (CDPH) and the County does not have the authority to change it.
    - ddPCR was launched on May 5, 2022, after an extensive yearlong study, comparing this method with culture-based methods, in partnership with CDPH, US EPA, UCSD and the Southern California Coastal Water Research Project.
    - Additionally, this study was peer reviewed and published in a scientific journal.

- There is insufficient evidence to that would support reevaluating the current threshold.
    - According to the CDPH the County's Public Health Officer (PHO) would need to make a finding that the threshold is not effective to protect public health based on evidence-based data.
    - If the County's PHO was able to make such a finding, we would then be required to request that CDPH re-evaluate the threshold. It is CDPH's authority to make the decision as to whether there is sufficient evidence to re-visit the threshold and if so, to take appropriate steps. Those steps would involve extensive multi-year study similar to the Crain et al. (2021) study.
- The data and subsequent results and recommendations that Coronado has submitted to the County raises several concerns as to the accuracy of the data and the validity of the study results. Please see the attachment, Coronado Special Study Statistical Response Memo.

As the County shared with Coronado in the June 12, 2024 meeting, the south county beaches have seen an increase in the number of beach water contact closures due to numerous environmental factors, most notably the increase in untreated sewage from the Tijuana River and direct discharge into the ocean at San Antonio de los Buenos Creek at Punta Bandera, that have been flowing for over 1,000 consecutive days. As documented by IBWC on their website at https://waterdata.ibwc.gov/AQWebportal/Data, the transboundary flows in the Tijuana River has substantially increased around January 2023, with IBWC reporting approximately 78 billion gallons of cumulative transboundary flow since then. We recognize that the beach water contact closures in Coronado have increased in 2023, however, this is due to the fact that sewage flows impacting ocean waters have significantly increased during this same timeframe. The County is not seeing an increase in closures occurring in other communities outside of the south county region where there is not a known sewage pollution source. Nor is the County seeing a significant increase in bacterial water contact advisories in areas outside of south county.

In summary, based on the data and analysis found, the County has not found that Coronado's data presented is sufficient to justify the County's PHO request that CDPH reevaluate the threshold. The County is still confident the current threshold of 1,413 copies/100ml is the most protective of public health.

Sincerely,

Heather Buonomo, R.E.H.S.
Director of Environmental Health

Ankita Kadakia, M.D.
Interim Public Health Officer

Enclosure: Coronado Special Study Statistical Response Memo

CC: Nora Vargas, Chairwoman of San Diego County Board of Supervisors
Amy Harbert, Interim Deputy Chief Administrative Officer
Caroline Smith, Interim Deputy Chief Administrative Officer

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HEATHER BUONOMO
INTERIM DIRECTOR OF
ENVIRONMENTAL HEALTH
AND QUALITY

JOHN_ROSS GLUECK
INTERIM DIRECTOR OF
ENVIRONMENTAL HEALTH

MEMORANDUM

TO: HEATHER BUONOMO

FROM: LYNNE WETMORE

DATE: SEPTEMBER 15, 2024

SUBJECT: CORONADO SPECIAL STUDY STATISTICAL RESPONSE MEMO

Response to "EVALUATION OF THE COUNTY OF SAN DIEGO ddPCR ACTIONABLE THRESHOLD METHODOLOGY BY CRAIN et al. (2021) FOR APPLICATION TO THE CORONADO BEACH WATER QUALITY SPECIAL STUDY" by Verbyla & LaCarra (2024)

Author: Lynne Wetmore, Ph.D. Ecology, M.S. Wildlife & Fisheries Science, B.A. Biology

Summary of major issues identified in the Coronado Special Study (2023): (See pages 2-5 and attached supporting Figures for more detailed explanations)

1) ***Lack of a true "paired sample" study design:*** Unlike the Crain et al. (2021) study where seawater samples were split and analyzed using both Enterolert and ddPCR to directly compare analytical results between the two methods (on the exact same water sample), the Coronado Special Study used two completely different sets of seawater samples to analyze Enterolert and ddPCR, respectively.

2) ***Improper ICE regression model development/validation:*** The researchers conducting the 2023 Coronado Special Study did not use separate "training" and "test" data sets to develop and validate their new "Coronado-specific" ICE regression model, but instead "tested" the predictive effectiveness of their ICE model on the exact same set of 59 samples that they used to create the linear equation.

3) ***Inadequate sampling period/data distribution:*** Unlike the Crain et al. (2021) study where split seawater samples ($n \approx 2000$) were collected year-round over a 20-month period (May 2017-January 2019), the 59 seawater samples included in the Coronado Special Study were limited to a three-month period (June 26 – September 18) entirely within the summer (dry) season in 2023, when Tijuana River flow was consistently low. This, combined with the improperly paired samples in Issue 1 (above) may have led to the observed differences in

data distribution, ICE equation & ddPCR actionable threshold predictions compared to the more-comprehensive Crain et al. study (**see DEHQ Figures 1 & 2 below**).

4) ***Violations of statistical (regression model) assumptions:*** The dataset used to generate the linear regression (ICE) model for the 2023 Coronado Special Study appeared to violate several major statistical assumptions required for linear regression analysis to be valid. Based on descriptions in the text, it seems that the authors may have an incomplete understanding of regression model assumptions and/or interpretation. Furthermore, there are some errors in the charts/figures, and the graphical representation of Fig.3.3 (used in the recalculation of the Coronado-specific ICE equation) is extremely misleading in that it cuts off a major outlier/leverage point that is highly influential on the data (**see DEHQ Figures 2 & 3 below**)

5) ***Statistical (regression model) interpretation:*** Related to the previous issue, no evaluation of regression model significance was evaluated. R^2 is reported, but not a p-value determining whether model is statistically significant. When a single leverage point and/or outlier is removed from the 2023 data, the slope of the best fit line drops substantially and explanatory power of the model drops from 12.7% to 2.9% (**see DEHQ Figure 3 below**), and the model may not be significant for interpretation.

6) ***Statistical analysis/methods:*** In the 2023 Coronado Special Study, authors state that they have replicated methods from Crain et al. 2021, but are missing several important analyses (e.g., ROC, evaluations of sensitivity/specificity) that are recommended by EPA guidelines to evaluate impacts of different cutoff points (e.g., 1413 vs. 30,000) on beach management actions (estimated error rate based on % false negatives and false positives)- this analysis is critical to evaluate potential public health risks that could be associated with altering or raising the enterococcal action threshold values.

Background and Summary Analysis:

Over a three-month period (June -September) in the summer of 2023, the City of Coronado conducted an independent special study comparing enterococcal concentrations between seawater samples quantified by ddPCR (collected by County DEHQ) and "paired" samples quantified by Enterolert (collected at separate times, but on the same date and beach by City of Coronado). The primary goal of this study was to re-evaluate the statistical regression model developed by Crain et al. (2021) to calculate the "cutoff" ddPCR actionable threshold value (1413 copies/100mL) currently in use for beach management decisions along the Coronado shoreline (and elsewhere throughout San Diego County).

The authors of the current report claim to directly replicate statistical and analytical approach(es) from the Crain et al. publication; however, several notable distinctions in methodology are apparent. To summarize the original 2021 study, individual seawater samples were collected year-round by County DEHQ over an extended 20-month period from May 2017 to

January 2019. Immediately prior to processing, each sample was split in half so that both analytical methods (Enterolert, ddPCR) could be run on (ostensibly) identical paired seawater samples by the County Public Health Lab. These directly paired results could then be used to generate a best-fit linear regression model between ddPCR enterococcal concentration (in copies/100mL) and the corresponding Enterolert value (in MPN/100 mL) recorded for the exact same volume of seawater. Ultimately, the resulting linear equation (i.e., intrinsic copy number equation [ICE]) was used to convert the established EPA Enterolert actionable threshold of 104 MPN/100mL into a directly comparable ddPCR concentration of 1413 copies/100mL.

It should be noted that for statistically proper model validation, Crain et al. (2021) developed the original ICE using two completely separate data sets. An initial independent subset of 185 split seawater samples was first used as "training" data to generate the linear regression equation (ICE). The resulting ICE-scaled conversion was then applied to an entirely different (and much larger) "test" data set of 1086 split seawater samples to evaluate goodness-of-fit for the ICE model based on index of agreement (IA) and correlation (Pearson's r).

In comparison, during the 2023 special study, a total of 164 separately-collected-yet "paired" seawater samples were taken and analyzed from Coronado beaches by County DEHQ (for ddPCR) and City of Coronado (for Enterolert), respectively. Of these nominal "paired" samples, only 59 displayed enterococcal concentrations above the respective detection limits for both analytical methods as collected by the two different agencies (and processed by the two different laboratories). Unlike published methods in the Crain et al. study (2021), this single set of (n = 59) data points in the Coronado special study report was used as both the "training" dataset for initial model development to generate the linear regression equation (ICE), and the "testing" dataset to evaluate that same ICE model for goodness-of-fit (although in some analyses including the ICE recalculation – see Figure 1 below, an additional 8 Coronado samples were added from the 2021 Crain et al. study, for a total n = 67).

Consequently, the current report lacks true independent model validation for the proposed "site-specific" Coronado ICE equation; instead, the statistical evaluations of sample agreement presented here between ICE-scaled ddPCR results and Enterolert values using the "new" site-specific ICE (IA = 0.65, Pearson's r = 0.59) were essentially calculated from the exact same 59 (or 67) data points used to create the linear regression model being tested. Given the near complete overlap between "training" and "test" data sets in the Coronado special study, it is actually somewhat surprising that reported model performance was not greatly improved over the original Crain et al. study, which achieved similar positive correlation (r = 0.60) and index of agreement (IA = 0.64) between ICE-scaled ddPCR and Enterolert results overall, and generally higher IA values when the ICE model was applied within specific beach-groupings (range: 0.63-0.93), despite calculating these values from statistically valid independent test datasets over a much broader geographical range.

Regardless of the potential statistical deficiencies identified above (coupled with limited evidence for improved model performance outside of the relatively limited (n=59) pseudo-paired sample training dataset specifically analyzed in the report), the authors of the 2024 special study still present a re-calculated "site-specific" ICE for Coronado that indicates an actionable ddPCR threshold of approximately 30,000 copies/100mL, over 20x higher than the 1,413 cutoff value

currently used for beach management throughout the county (Figure 1 below). If this cutoff value were to be applied to the Crain et al. ICE equation currently used for beach management, it would correspond to an estimated Enterolert concentration of 1871 MPN/100ML. Thus, if actionable threshold values for beach management actions are raised to be less-conservative based on a site-specific ICE equation that has not been developed with sufficient statistical rigor and/or has not subjected to thorough model validation on independent datasets to confirm accuracy of conversion between effective ddPCR and Enterolert results, opening beaches at these enterococcal concentrations could create a serious risk to public health for recreational beachgoers.

Conclusion and response:

After completing a detailed review of the Coronado Special Study report (and accompanying dataset) submitted by Verbyla & Lacarra (2024) here, DEHQ has identified several fundamental areas of concern in the study design (field sample collections), statistical model development and validation, and interpretation of analytical results. Although the authors state that they have completely replicated the methods published and validated in Crain et al. (2021), there are several major alterations and omissions that introduce substantial confounding factors and caveats in interpreting the analyses. Several of the most notable issues (and potential impacts on study conclusions) are listed above.

Although the authors of the Coronado Special Study do include a brief general statement (Main Report Page 1, Key Findings, Item 2) acknowledging that the results and data analysis from the 2023 study are not statistically valid to be actionable in their current form, they also state fairly definitively that the current work is sufficient to justify development of a separate, site-specific actionable ddPCR threshold for Coronado that is different (and ostensibly less conservative) than current recreational beach water quality requirements for the remainder of the county. In one section of the supplemental material, the authors specifically conclude that low observed agreement (IA) between the published Crain et al. (2021) ICE predictive equation and the 59 "paired" sample values from the 2023 dataset is sufficient confirmation that county ddPCR water quality standards should not be applicable to Coronado beaches. (Attachment Page 3, Tab 2, Paragraph 2; "This means that Crain's ICE did not properly adjust the ddPCR data for Coronado, based on the Coronado Special Study data set. The conclusion from this analysis is that a Coronado ICE needed to be developed."; see also Main Document Page 2, Kay Findings, Item 3.a.)

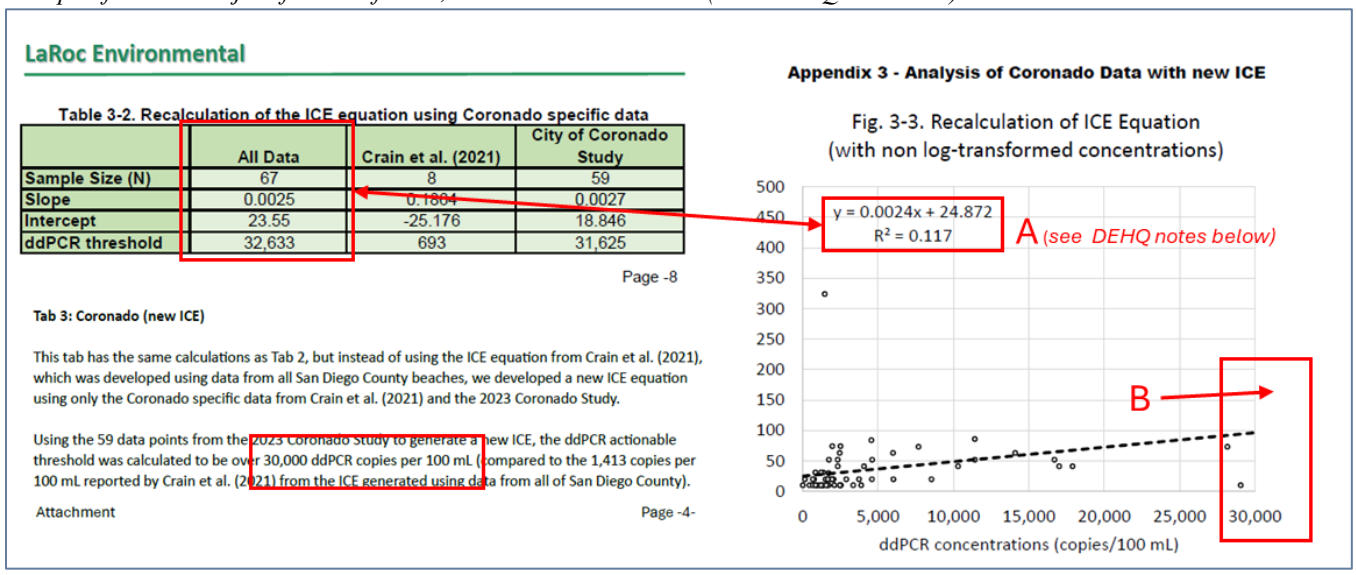
However, based on our thorough review of the data results and analysis in the 2023 report, we feel that differences in the observed linear relationship between Enterolert and ddPCR values observed in the 2023 Coronado Special Study cannot be definitely attributed to inherent site-specific bacterial dynamics that would justify the public health risk associated with raising the allowable enterococcal concentration for beach management thresholds. In particular, because of the lack of true paired/split sampling incorporated into the basic study design for the 2023 Coronado special study, any observed variability in the slope and distribution of "paired" Enterolert/ddPCR results used to generate ICE models in the 2023 report are subject to confounding influence from a number of factors:

- Different sample agency (sampler, collection method, handling time/method, processing, storage)
- Samples taken at different times of day (sometimes up to 4 hrs apart based on publicly available COC forms from the Coronado study) and at different exact GPS locations within the beach- tidal influence, natural variability in vegetation and runoff, animals present during sampling could all influence bacterial levels
- Different laboratory processing samples for each methodology (handling time, temperature, etc.)

Beyond the inherent issues with non-paired sample collection and lack of statistical model validation, the 2023 Coronado special study was limited to three months within the summer (dry) season with minimal precipitation and relatively low Tijuana River flow. Because of this, the range of observed values for enterococcal concentrations in the dataset was not sufficient to establish a statistically reliable relationship between Enterolert/ddPCR sample results around the critical cutoff point of 104MPN/100ML, even if the above issues with study execution were not present. Predictive value of regression models is only valid within the observed sample range, and here, only a single sample collected by Coronado and analyzed with Enterolert exceeded the 104 MPN management threshold. Adding the 8 Coronado data points from Crain et al. 2021 adds only one additional data point above this cutoff value (**see Figure 2 below**). Because the Coronado Special Study lacks sufficient seawater sample data within the critical range surrounding the current beach management cutoff value (100 MPN/100ML), this single data point (which is actually excluded from the graphical representation of the data in the Verbyla & Lacarra report, **see Figure 1**) acts as a major leverage point on the ICE equation, and removing this single data point causes the management predictions (and statistical explanatory power) of the model to become highly unstable (**see Figure 3**).

Thus, based on the data presented here, there is minimal justification to alter the current "cutoff" ddPCR actionable threshold value (1413 copies/100mL) currently in use for beach management decisions throughout San Diego County. Further, given the lack of independent model validation and unknown variability associated with ICE results presented here, we feel that adopting less stringent water quality requirements for ddPCR enterococcal concentrations without definitive statistical justification could pose a substantial public health risk.

DEHQ Figure 1. *Excerpts from Coronado Special Study Report (Verbyla & Lacarra 2024) supporting "new" Coronado site-specific ICE and justification for 30,000+ ddPCR threshold (with DEHQ comments)*



LaRoc Environmental

Table 3-2. Recalculation of the ICE equation using Coronado specific data

	All Data	Crain et al. (2021)	City of Coronado Study
Sample Size (N)	67	8	59
Slope	0.0025	0.1864	0.0027
Intercept	23.55	-25.176	18.846
ddPCR threshold	32,633	693	31,625

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Tab 3: Coronado (new ICE)

This tab has the same calculations as Tab 2, but instead of using the ICE equation from Crain et al. (2021), which was developed using data from all San Diego County beaches, we developed a new ICE equation using only the Coronado specific data from Crain et al. (2021) and the 2023 Coronado Study.

Using the 59 data points from the 2023 Coronado Study to generate a new ICE, the ddPCR actionable threshold was calculated to be over 30,000 ddPCR copies per 100 mL (compared to the 1,413 copies per 100 mL reported by Crain et al. (2021) from the ICE generated using data from all of San Diego County).

Attachment Page -4-

Appendix 3 - Analysis of Coronado Data with new ICE

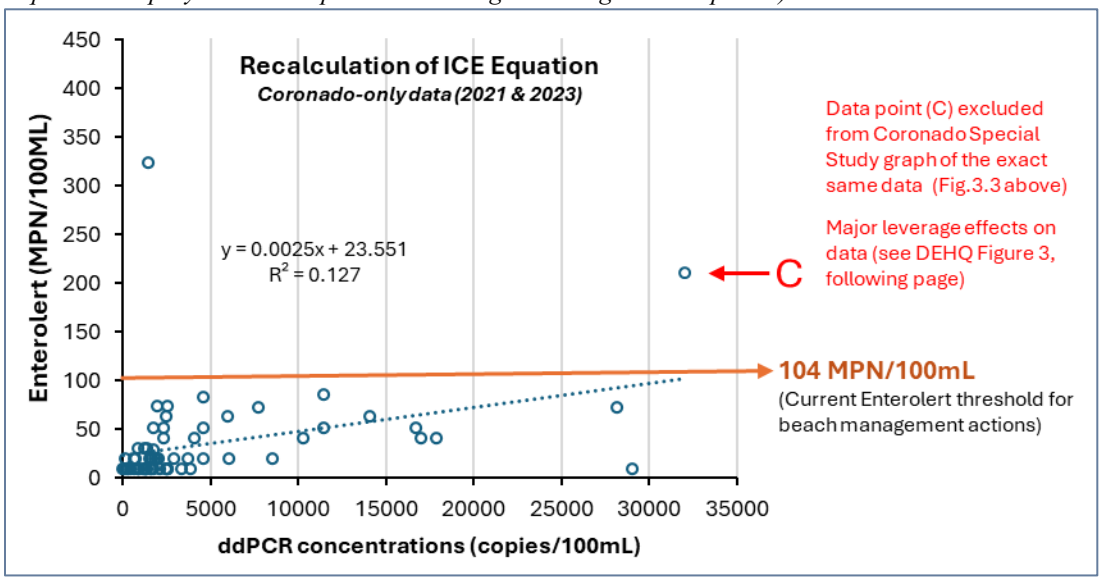
Fig. 3-3. Recalculation of ICE Equation
(with non log-transformed concentrations)

DEHQ Notes:

(A) The ICE equation displayed on Fig 3.3. of the Coronado Special Study Report appears to be incorrect. However, regression parameters on Table 3-2 are correct and match DEHQ re-calculation of the same data (see DEHQ Figure 2 below)

(B) The x-axis chosen for Fig 3.3 of the Coronado Special Study Report is misleading because it cuts the x-axis off at a ddPCR concentration of 30,000 copies/100 mL; this excludes a major outlier (shown in DEHQ Figure 2 below) that was included (and highly influential) in the calculation of the "new" Coronado-specific ICE calculation presented by Verbyla & Lacarra (2024) in their report (see DEHQ Figure 3 below)

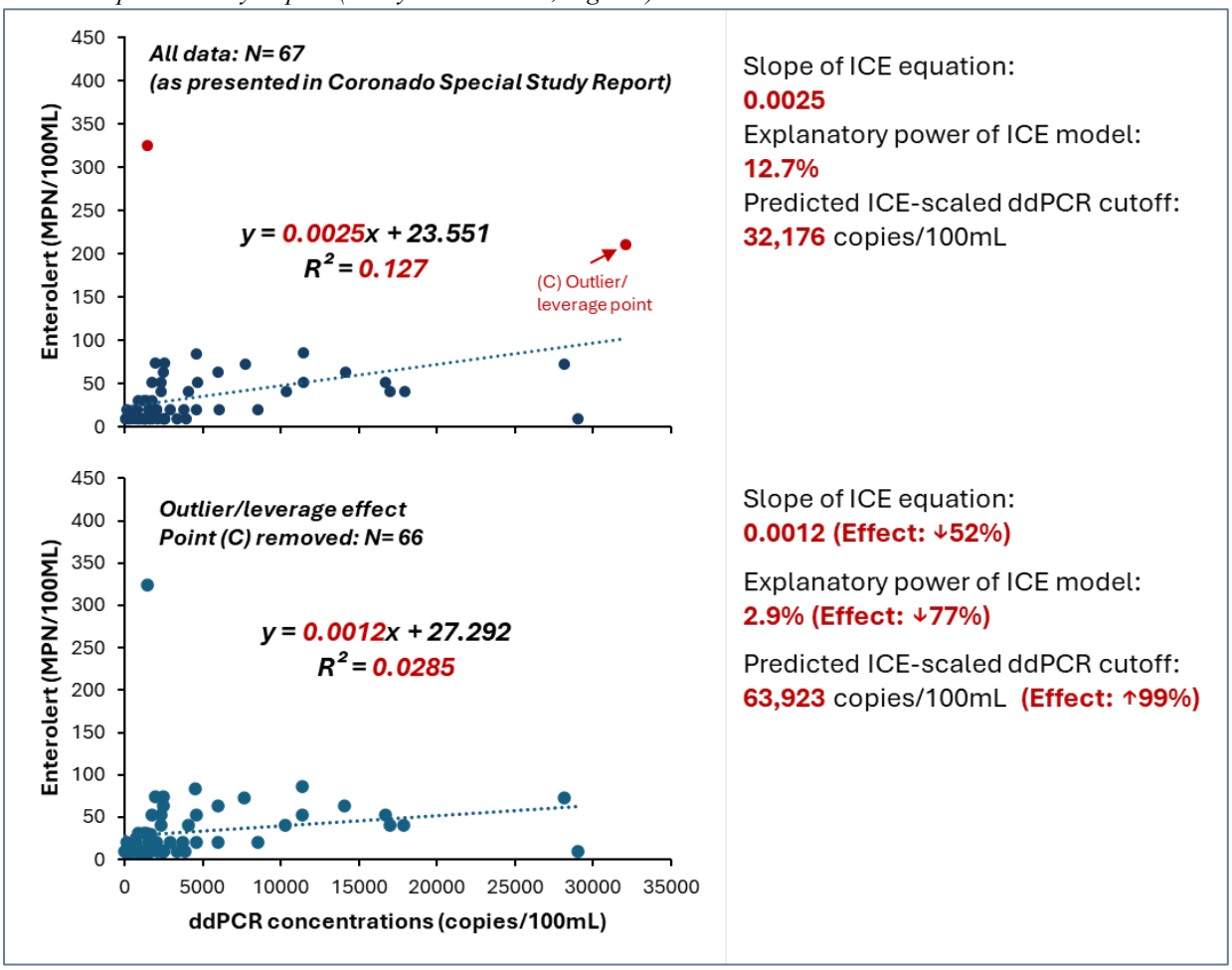
DEHQ Figure 2. *Recalculation of ICE equation and regeneration of Fig.3.3. from the Coronado Special Study Report (with correct ICE equation displayed and complete x-axis range showing all data points)*



DEHQ Notes: Potential issue with data distribution: In the Coronado Special Study combined dataset (2021 & 2023; n = 67), only 2 data points are above the current management threshold, and only one of these is from the 2023 data (C). This is not a sufficient data range for the ICE regression to have predictive power in the critical range around the cutoff point.

(C) Data point is an outlier, and this single data point has major leverage effects on ICE calculation (see analysis in DEHQ Figure 3 below)- despite this, the Coronado Special Study Report presented their data (Fig.3.3 with an x-axis that cut off at 30,000 to exclude this data point which is highly influential on their results

DEHQ Figure 3. *Statistical effects of removing just a single leverage data point (C) from the ICE recalculation presented in the Coronado Special Study Report (Verbyla & Lacarra, Fig.3.3.)*



DEHQ Notes: Removing just a single data point (C, which was the same data point cut off by the x-axis on Figure 3.3.) has major leverage effects on the ICE linear equation and predicted ddPCR cutoff presented in the Coronado Special Study Report. If this data set is not included, the slope of the linear equation decreases by 52% and the predicted ICE-scaled ddPCR cutoff for beach management actions would almost double to 63,923 copies/100mL This suggests that many of the statistical conclusions and recommendations in Verbyla & Lacarra (2024) are driven by a single outlier data point, further emphasizing that the data and analysis presented here are not sufficiently validated or reliable to make major changes in beach management that could have major implications and risks for public health if calculations are not correct.

Author: Lynne Wetmore, Ph.D. Ecology, M.S. Wildlife & Fisheries Science, B.A. Biology

Lynne Wetmore- Biographical information:
Education:

- Ph.D. in Ecology from UC Davis
  (Areas of emphasis: quantitative biology /mathematical population modeling, marine ecology)
- M.S. in Wildlife & Fisheries Sciences- Texas A&M University
- B.A.- Biology- Occidental College

Relevant work experience:

- Graduate lecturer: Biostatistics, San Diego State University
- Graduate lab instructor: Biostatistics, San Diego State University
- Adjunct professor: Biological Statistics (Lecture & Lab), Texas A&M University

4 years – environmental consulting- marine biology
Previously reviewed manuscripts for the following Journals:

- Proceedings of the Royal Society B,
- Ecology
- Journal of Experimental Marine Biology
- Marine Ecology, Marine Biology

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION IX
75 Hawthorne Street
San Francisco, CA 94105-3901

Lars Seifert, Chief
Land & Water Quality Division
San Diego County Department of Environmental Health

Re: REQUEST FOR CONCURRENCE TO IMPLEMENT ddPCR FOR BEACH WATER QUALITY RAPID DETECTION METHOD FOR RECREATIONAL BEACHES IN SAN DIEGO COUNTY

Dear Mr Seifert:

EPA Region 9 is approving the use of the method digital droplet Polymerase Chain Reaction (ddPCR) and beach action value (BAV) for beach water quality monitoring under EPA's BEACH program in San Diego County as a pilot program for California. The ddPCR method will be used by the County of San Diego to provide same-day notice of elevated bacteria at beaches in lieu of the current beach action value using the Enterolert method.

When EPA published revised Recreational Water Quality Criteria in 2012, the agency recognized that technologies were changing rapidly and created a path for the use of new bacterial methods and indicators. In 2014, EPA published a technical support manual[1] (TSM) that described the process the agency would use in approving new bacterial methods or indicators on a site-specific basis for ambient water monitoring and serve as an alternative to the Alternate Testing Procedures (ATP) pursuant to 40 CFR§136. The TSM allows for comparison of different methods and indicators to evaluate health risk.

San Diego Department of Public Health (SD DPH) and collaborators analyzed samples using ddPCR and Enterolert (an EPA approved method for ambient water bacteria) at 51 diverse beaches along the San Diego coastline for over the AB411beach season (April 1 to October 31). Following the procedures in the TSM, SD DPH established an acceptable level of agreement between the two indicators/methods and also showed a 90% agreement in beach notification decisions. The error rates for ddPCR are comparable to error rates associated with other EPA approved bacteria methods such membrane filtration for *Enterococci* (EPA Method 1600). The false positive rate for the application in San Diego County is 6%, making beach notifications protective 96% of the time with increased sensitivity than EPA approved methods.[2]

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[1] EPA 2014. Site-Specific Alternative Recreational Criteria Technical Support Materials For Alternative Indicators and Methods EPA-820-R-14-011 U.S. Environmental Protection Agency Office of Water Office of Science and Technology Health and Ecological Criteria Division December 2014.

[2] C. Crain et al. In Review. Application of ddPCR for the detection of Enterococcus spp. for coastal water quality monitoring. Submitted to Journal of Microbiological Methods

In summary, San Diego County has demonstrated that ddPCR can be used to reliably determine levels of *Enterococci* bacteria as an indication of overall microbiological fecal contamination conditions for San Diego County beaches. This rapid test method can effectively and quickly determine the need for public notification for recreational beach water quality surveillance, thereby reducing the period of time the public is at risk.

If you have any questions about the attached study, please contact me at 415-972-3462 or fleming.terrence@epa.gov.

Sincerely,

TERRENCE FLEMING Digitally signed by TERRENCE FLEMING
Date: 2020.10.06 08:43:48 -07'00'

Terrence Fleming
EPA Region 9 BEACH Coordinator

cc: Dr. Wilma Wooten, Health Officer, County of San Diego
Brett Austin, Director, County of San Diego Public Health Laboratory
William Draper, CDPH, Drinking Water & Radiation Laboratory Branch
Chad Crain, CDPH, Drinking Water & Radiation Laboratory Branch
Dr. John Griffith, Southern California Coastal Water Research Project
Dr. Marva Seifert, University of California, San Diego
Tracy Bone, US EPA, Office of Water, Standards and Health Protection Division

State of California—Health and Human Services Agency
California Department of Public Health

TOMÁS J. ARAGÓN, MD, DrPH
Director and State Public Health Officer

GAVIN NEWSOM
Governor

May 4, 2021

Amy Harbert, Director
Department of Environmental Health and Quality
County of San Diego
5500 Overland Avenue
San Diego, Ca 92123

Re: Digital droplet Polymerase Chain Reaction use authorization for beach water quality assessment in San Diego County

Dear Ms. Harbert,

The California Department of Public Health (CDPH) is authorizing the use of digital droplet Polymerase Chain Reaction (ddPCR) in San Diego County to determine the level of *Enterococci* bacteria in waters adjacent to public beaches, as part of beach water quality assessment. This authorization provided to San Diego County by CDPH is consistent with the provisions in the California Health and Safety Code, Section 115880 (d), and the use of ddPCR is specific to the beach locations in San Diego County included on page two of this letter. The beach locations on page two represent water quality stations included in a side by side comparison study that successfully demonstrated the use of ddPCR as an alternative test method to evaluate microbial conditions for beach water quality assessment in San Diego County. Use of ddPCR can provide water quality results more quickly than conventional test methods, thereby reducing the period the public is at risk while waiting for the presence of bacteria to be confirmed.

Please contact me at Eric.Trevena@CDPH.ca.gov or (916) 210-8548 if you have any questions regarding this approval.

Sincerely,

Eric Trevena

Eric Trevena, R.E.H.S.
Chief, Environmental Health Services Section

cc: Keith Kezer

Group	ID number	LOCATION	LATITUDE	LONGITUDE
(1) South County Beaches	IB-050	Seacoast Drive, south end - Imperial Beach	32.57	-117.13
(Beaches occasionally impacted	EH-010	Cortez Avenue - Imperial Beach	32.57	-117.13
by sewage from Tijuana River)	EH-030	Imperial Beach Pier	32.58	-117.13
	IB-060	Carnation Avenue - Imperial Beach	32.59	-117.13
	IB-080	Avenida Del Sol - Coronado	32.68	-117.18
	IB-070	Silver Strand State Beach - Campground	32.64	-117.14
(2) Tijuana River Valley	IB-010	Border Field State Park - N of border fence	32.54	-117.12
(Beaches routinely impacted by	IB-040	Tijuana Slough - 3/4 mile N of TJ River	32.56	-117.13
sewage from Tijuana River)	IB-030	Tijuana Slough at Tijuana Rivermouth	32.56	-117.13
(3) Lagoons	SE-060	San Elijo Lagoon outlet - Cardiff Beach	33.02	-117.28
(Beaches impacted by lagoon	EH-480	Buena Vista Lagoon - Carlsbad	33.16	-117.36
outfalls)	FM-100	Torrey Pines, Los Penasquitos outlet	32.93	-117.26
	EH-440	Batiquitos Lagoon outlet - Carlsbad Beach	33.09	-117.31
(4) Open Coast (south end)	FM-030	Tourmaline Surf Park - Pacific Beach	32.81	-117.26
(Open coast beaches impacted	EH-255	Pacific Beach Point	32.81	-117.27
by occasional storm drain runoff)	EH-280	Playa Del Norte Windansea - La Jolla	32.83	-117.28
	EH-300	Nicholson Point - La Jolla	32.84	-117.28
	FM-060	Avenida De La Playa - La Jolla Shores	32.85	-117.26
(5) Open Coast (north end)	EH-360	15th Street - Del Mar	32.96	-117.27
(Open coast beaches impacted	EH-390	Seascape Beach Park - Solana Beach	32.99	-117.27
by occasional storm drain runoff)	EH-410	Swami's Beach - Encinitas	33.03	-117.29
	EH-460	Tamarack Ave - Carlsbad State Beach	33.15	-117.35
	EH-455	Warm Water Jetty - Carlsbad State Beach	33.14	-117.34
(6) City of La Jolla	EH-310	Children's Pool	32.85	-117.28
(Beaches impacted by pinnipeds	FM-070	La Jolla Cove	32.85	-117.27
and associated fecal material)	EH-305	South Casa Beach - La Jolla	32.85	-117.28
(7) San Diego Bay	EH-200	Shelter Island - San Diego Bay	32.71	-117.22
(Beaches within large	EH-120	Bayside Park (J Street) - Chula Vista	32.63	-117.11
embayment that receives limited	EH-070	Tidelands Park - Coronado bayside	32.69	-117.16
	EH-080	Glorietta Bay - Coronado bayside	32.68	-117.17
	EH-211	Lawrence Street - San Diego Bay	32.71	-117.24
	EH-160	Spanish Landing Park - San Diego Bay	32.73	-117.21
(8) Mission Bay (west)	MB-090	Wildlife Refuge swim area - Mission Bay	32.79	-117.23
(Beaches within small	MB-100	Crown Point - Mission Bay	32.78	-117.23
embayment that receives limited	MB-120	Fanuel Park - Mission Bay	32.79	-117.24
	MB-160	Bahia Point swim area - Mission Bay	32.78	-117.25
	MB-173	Bonita Cove, northern shore - Mission Bay	32.77	-117.25
	MB-203	Ski Beach, Vacation Isle - Mission Bay	32.77	-117.23
	MB-205	North Cove, Vacation Isle - Mission Bay	32.78	-117.24
(9) Mission Bay (east)	MB-031	Tecolote Playground, watercraft area	32.77	-117.21
(Beaches within small	MB-041	Tecolote Shores, swim area - Mission Bay	32.78	-117.21
embayment that receives very	MB-051	Leisure Lagoon swim area - Mission Bay	32.78	-117.21
	MB-053	Comfort Station at Leisure Lagoon	32.78	-117.21
	MB-060	Visitor's Center shoreline - Mission Bay	32.79	-117.21
	MB-070	De Anza Cove swim area - Mission Bay	32.80	-117.21
	MB-080	Campland - Mission Bay	32.79	-117.22
(10) Creek/River outlets	EH-380	San Dieguito River outlet - Del Mar	32.98	-117.27
(Beaches impacted by routine	OC-100	San Luis Rey River outlet - Oceanside	33.20	-117.39
discharge from creeks or rivers)	FM-010	San Diego River outlet (Dog Beach)	32.76	-117.25
	EH-420	Moonlight Beach (Cottonwood Creek)	33.05	-117.30
	OC-022	Buccaneer Beach - Oceanside	33.18	-117.37

SAYONE THIHALOLIPAVAN, MD, MPH
PUBLIC HEALTH OFFICER

HEATHER BUONOMO, REHS
DIRECTOR OF ENVIRONMENTAL HEALTH

January 21, 2026

June Weintraub, ScD, REHS
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Significant Concerns Regarding "The Relationship Between Enterolert and ddPCR Concentration of Enterococci in Summer Samples from Beaches in Coronado, California" Article Published in Journal 240 (2026) 107346

Dear CDPH and US EPA Colleagues,

The County of San Diego would like to provide additional information and clarifications in response to the request for information regarding an article that was published online in the *Journal of Microbiological Methods* in November 2025*,* "The relationship between Enterolert and ddPCR concentrations of enterococci in summer samples from beaches in Coronado, California," authored by Matthew Verbyla and Rosanna Lacarra.

The County of San Diego (County) has formally requested the Editor in Chief redact the acknowledgment section of the article which cites contributions of "Heather Buonomo, Dr. Ankita Kadakia and Dr. Lynne Wetmore from the San Diego County Department of Environmental Health and Quality, for their helpful feedback and review of an earlier version of this manuscript". The County did not participate in the study, review the manuscript, or have knowledge of the intent to publish their study.

The County also has several concerns regarding the study's methodology, analysis, and resulting conclusions. The study concludes that additional investigation of the use of digital droplet polymerase chain reaction (ddPCR) is warranted due to the discordance in the relationship to Enterolert and questions the validity of the ddPCR to inform beach management actions. The study challenges the analysis of the Crain study (2021), which the State used to approve the use of ddPCR in the County of San Diego.

While the County was not involved in the Coronado study, in September 2024, the City of Coronado requested that the County review preliminary data collected in the study that eventually was published in the November 2025. The data included beach water quality data collected that summer, as part of the City's effort to discontinue ddPCR analysis for beach monitoring. The City of Coronado submitted a presentation, technical memorandum, and raw dataset to the County for review. We noted the following clarifications in a letter to the City of Coronado, which are included in Attachment A:

- *State law requires the County to issue a beach water contact closure when there is a sewage spill or chemical spill that impacts ocean or bay waters, when there are odors or discoloration in the water due to sewage contamination, such as if flows from the Tijuana River that are reaching the ocean. Water testing is not used to issue a closure, therefore the water quality testing method used by the County is not relevant for issuing a beach water contact closure. Once closures are issued, the County does use ddPCR test results to lift the closure once State health water quality standards are met.*

- *The current threshold of 1,413 copies/100ml for the ddPCR test was set by California Department of Public Health (CDPH) and the County does not have the authority to change it.*
  - *ddPCR was launched on May 5, 2022, after an extensive yearlong study, comparing this method with culture-based methods, in partnership with CDPH, US EPA, UCSD and the Southern California Coastal Water Research Project.*
  - *Additionally, this study was peer reviewed and published in a scientific journal*

- *There is insufficient evidence that would support reevaluating the current threshold.*
  - *According to the CDPH the County's Public Health Officer (PHO) would need to make a finding that the threshold is not effective to protect public health based on evidence-based data.*
  - *If the County's PHO was able to make such a finding, we would then be required to request that CDPH re-evaluate the threshold. It is CDPH's authority to make the decision as to whether there is sufficient evidence to re-visit the threshold and if so, to take appropriate steps. Those steps would involve extensive multi-year study similar to the Crain et al. (2021) study.*

- *The data and subsequent results and recommendations that Coronado has submitted to the County raises several concerns as to the accuracy of the data and the validity of the*

study results. Please see the attachment, Coronado Special Study Statistical Response Memo.

Following the County's review in 2024, the County conducted no further review or analysis of the study, never received a manuscript for review, and was never notified that the informal review of preliminary data would be published as a study.

Upon being notified of the published article by the City of Coronado on January 12, 2026, we reviewed the publication and the publicly available supplemental data set, which revealed multiple inaccuracies in the analysis and calculations used to arrive at the conclusions, as well as the conceptual design and execution of the study itself, which are not directly comparable to the methodologies published by Crain in the 2021-EPA reviewed paper. The article notably also fails to provide the context of major sewer treatment pumps, pipelines and processing plant infrastructure failures coinciding with the timing of their study, driving increased bacterial exceedances and beach closures for sewage contamination.

The County's review of the Coronado study is attached to the letter (Attachment B). We are available to answer questions or further discuss the analysis as needed.

Thank you for your time and attention to our concerns.

Sincerely,

Heather Buonomo, REHS
Director of Environmental Health

Sayone Thihalolipavan, MD, MPH
Public Health Officer
County of San Diego

Attachment: A and B

cc: Amy Harbert, Director, Department of Environmental Health and Quality
Dahvia Lynch, Deputy Chief Administrative Officer, Land Use and Environment Group
Elizabeth Hernandez, Interim Deputy Chief Administrative Officer, Health and Human Services

Attachment A

COUNTY OF SAN DIEGO
1600 PACIFIC HIGHWAY, SAN DIEGO, CALIFORNIA 92101-2422

ANKITA KADAKIA, M.D.
INTERIM PUBLIC HEALTH OFFICER

HEATHER BUONOMO, R.E.H.S.
DIRECTOR OF ENVIRONMENTAL HEALTH

September 16, 2024

Tina Friend
City of Coronado
1825 Strand Way
Coronado, CA 92118

Dear Ms. Friend,

This letter is in response to City of Coronado's (Coronado) request to accept an alternative threshold for the digital droplet polymerase chain reaction (ddPCR) testing based on the pilot study that Coronado conducted during June 26, 2023-September 18, 2023.

- On June 12, 2024, Coronado and County of San Diego (County) met in person to discuss Coronado's 2023 water quality testing Pilot Study. In this meeting, Tina Friend, Tony Winney and Coronado's consultant team presented Pilot Study results and the City's request to for the County to adjust the ddPCR threshold from 1413 copies/100ml to 6,791 copies/100ml. This presentation compared the Enterolert data (a different testing culture-based method) collected by Coronado to the County's ddPCR data collected during the same time period.
- During the June 12th meeting, The County Department of Environmental Health and Quality (DEHQ) and the Public Health Services (PHS) asked to see City's raw culture-based testing data for our review and analysis.
- On August 16, 2024, the County received a technical memorandum that compared Coronado's pilot study data to the Crain et al. (2021) study conducted by the County from 2017-2020.
- On August 22, 2024, the County received the subsequent raw data from the pilot study and responded that our internal expert was off until September 1st and we would review the documentation in early September.

The County has reviewed the presentation, technical memorandum and raw data set and has the following comments:

- State law requires the County to issue a beach water contact closure when there is a sewage spill or chemical spill that impacts ocean or bay waters, when there are odors or discoloration in the water due to sewage contamination, such as if flows from the Tijuana River that are reaching the ocean. Water testing is not used to issue a closure, therefore the water quality testing method used by the County is not relevant for issuing a beach water contact closure. Once closures are issued, the County does use ddPCR test results to lift the closure once State health water quality standards are met.
- The current threshold of 1,413 copies/100ml for the ddPCR test was set by California Department of Public Health (CDPH) and the County does not have the authority to change it.
    - ddPCR was launched on May 5, 2022, after an extensive yearlong study, comparing this method with culture-based methods, in partnership with CDPH, US EPA, UCSD and the Southern California Coastal Water Research Project.
    - Additionally, this study was peer reviewed and published in a scientific journal.

- There is insufficient evidence to that would support reevaluating the current threshold.
    - According to the CDPH the County's Public Health Officer (PHO) would need to make a finding that the threshold is not effective to protect public health based on evidence-based data.
    - If the County's PHO was able to make such a finding, we would then be required to request that CDPH re-evaluate the threshold. It is CDPH's authority to make the decision as to whether there is sufficient evidence to re-visit the threshold and if so, to take appropriate steps. Those steps would involve extensive multi-year study similar to the Crain et al. (2021) study.
- The data and subsequent results and recommendations that Coronado has submitted to the County raises several concerns as to the accuracy of the data and the validity of the study results. Please see the attachment, Coronado Special Study Statistical Response Memo.

As the County shared with Coronado in the June 12, 2024 meeting, the south county beaches have seen an increase in the number of beach water contact closures due to numerous environmental factors, most notably the increase in untreated sewage from the Tijuana River and direct discharge into the ocean at San Antonio de los Buenos Creek at Punta Bandera, that have been flowing for over 1,000 consecutive days. As documented by IBWC on their website at https://waterdata.ibwc.gov/AQWebportal/Data, the transboundary flows in the Tijuana River has substantially increased around January 2023, with IBWC reporting approximately 78 billion gallons of cumulative transboundary flow since then. We recognize that the beach water contact closures in Coronado have increased in 2023, however, this is due to the fact that sewage flows impacting ocean waters have significantly increased during this same timeframe. The County is not seeing an increase in closures occurring in other communities outside of the south county region where there is not a known sewage pollution source. Nor is the County seeing a significant increase in bacterial water contact advisories in areas outside of south county.

In summary, based on the data and analysis found, the County has not found that Coronado's data presented is sufficient to justify the County's PHO request that CDPH reevaluate the threshold. The County is still confident the current threshold of 1,413 copies/100ml is the most protective of public health.

Sincerely,

Heather Buonomo, R.E.H.S.
Director of Environmental Health

Ankita Kadakia, M.D.
Interim Public Health Officer

Enclosure: Coronado Special Study Statistical Response Memo

CC: Nora Vargas, Chairwoman of San Diego County Board of Supervisors
Amy Harbert, Interim Deputy Chief Administrative Officer
Caroline Smith, Interim Deputy Chief Administrative Officer

DEPARTMENT OF ENVIRONMENTAL HEALTH AND QUALITY
P.O. Box 129261, San Diego, CA 92112-9261
(858) 505-6700 or (800) 253-9933
www.sdcdehq.org

HEATHER BUONOMO
INTERIM DIRECTOR OF
ENVIRONMENTAL HEALTH
AND QUALITY

JOHN_ROSS GLUECK
INTERIM DIRECTOR OF
ENVIRONMENTAL HEALTH

MEMORANDUM

TO: HEATHER BUONOMO

FROM: LYNNE WETMORE

DATE: SEPTEMBER 15, 2024

SUBJECT: CORONADO SPECIAL STUDY STATISTICAL RESPONSE MEMO

Response to "EVALUATION OF THE COUNTY OF SAN DIEGO ddPCR ACTIONABLE THRESHOLD METHODOLOGY BY CRAIN et al. (2021) FOR APPLICATION TO THE CORONADO BEACH WATER QUALITY SPECIAL STUDY" by Verbyla & LaCarra (2024)

Author: Lynne Wetmore, Ph.D. Ecology, M.S. Wildlife & Fisheries Science, B.A. Biology

Summary of major issues identified in the Coronado Special Study (2023): (See pages 2-5 and attached supporting Figures for more detailed explanations)

1) ***Lack of a true "paired sample" study design:*** Unlike the Crain et al. (2021) study where seawater samples were split and analyzed using both Enterolert and ddPCR to directly compare analytical results between the two methods (on the exact same water sample), the Coronado Special Study used two completely different sets of seawater samples to analyze Enterolert and ddPCR, respectively.

2) ***Improper ICE regression model development/validation:*** The researchers conducting the 2023 Coronado Special Study did not use separate "training" and "test" data sets to develop and validate their new "Coronado-specific" ICE regression model, but instead "tested" the predictive effectiveness of their ICE model on the exact same set of 59 samples that they used to create the linear equation.

3) ***Inadequate sampling period/data distribution:*** Unlike the Crain et al. (2021) study where split seawater samples ($n \approx 2000$) were collected year-round over a 20-month period (May 2017-January 2019), the 59 seawater samples included in the Coronado Special Study were limited to a three-month period (June 26 – September 18) entirely within the summer (dry) season in 2023, when Tijuana River flow was consistently low. This, combined with the improperly paired samples in Issue 1 (above) may have led to the observed differences in

data distribution, ICE equation & ddPCR actionable threshold predictions compared to the more-comprehensive Crain et al. study (**see DEHQ Figures 1 & 2 below**).

4) ***Violations of statistical (regression model) assumptions:*** The dataset used to generate the linear regression (ICE) model for the 2023 Coronado Special Study appeared to violate several major statistical assumptions required for linear regression analysis to be valid. Based on descriptions in the text, it seems that the authors may have an incomplete understanding of regression model assumptions and/or interpretation. Furthermore, there are some errors in the charts/figures, and the graphical representation of Fig.3.3 (used in the recalculation of the Coronado-specific ICE equation) is extremely misleading in that it cuts off a major outlier/leverage point that is highly influential on the data (**see DEHQ Figures 2 & 3 below**)

5) ***Statistical (regression model) interpretation:*** Related to the previous issue, no evaluation of regression model significance was evaluated. R^2 is reported, but not a p-value determining whether model is statistically significant. When a single leverage point and/or outlier is removed from the 2023 data, the slope of the best fit line drops substantially and explanatory power of the model drops from 12.7% to 2.9% (**see DEHQ Figure 3 below**), and the model may not be significant for interpretation.

6) ***Statistical analysis/methods:*** In the 2023 Coronado Special Study, authors state that they have replicated methods from Crain et al. 2021, but are missing several important analyses (e.g., ROC, evaluations of sensitivity/specificity) that are recommended by EPA guidelines to evaluate impacts of different cutoff points (e.g., 1413 vs. 30,000) on beach management actions (estimated error rate based on % false negatives and false positives)- this analysis is critical to evaluate potential public health risks that could be associated with altering or raising the enterococcal action threshold values.

Background and Summary Analysis:

Over a three-month period (June -September) in the summer of 2023, the City of Coronado conducted an independent special study comparing enterococcal concentrations between seawater samples quantified by ddPCR (collected by County DEHQ) and "paired" samples quantified by Enterolert (collected at separate times, but on the same date and beach by City of Coronado). The primary goal of this study was to re-evaluate the statistical regression model developed by Crain et al. (2021) to calculate the "cutoff" ddPCR actionable threshold value (1413 copies/100mL) currently in use for beach management decisions along the Coronado shoreline (and elsewhere throughout San Diego County).

The authors of the current report claim to directly replicate statistical and analytical approach(es) from the Crain et al. publication; however, several notable distinctions in methodology are apparent. To summarize the original 2021 study, individual seawater samples were collected year-round by County DEHQ over an extended 20-month period from May 2017 to

January 2019. Immediately prior to processing, each sample was split in half so that both analytical methods (Enterolert, ddPCR) could be run on (ostensibly) identical paired seawater samples by the County Public Health Lab. These directly paired results could then be used to generate a best-fit linear regression model between ddPCR enterococcal concentration (in copies/100mL) and the corresponding Enterolert value (in MPN/100 mL) recorded for the exact same volume of seawater. Ultimately, the resulting linear equation (i.e., intrinsic copy number equation [ICE]) was used to convert the established EPA Enterolert actionable threshold of 104 MPN/100mL into a directly comparable ddPCR concentration of 1413 copies/100mL.

It should be noted that for statistically proper model validation, Crain et al. (2021) developed the original ICE using two completely separate data sets. An initial independent subset of 185 split seawater samples was first used as "training" data to generate the linear regression equation (ICE). The resulting ICE-scaled conversion was then applied to an entirely different (and much larger) "test" data set of 1086 split seawater samples to evaluate goodness-of-fit for the ICE model based on index of agreement (IA) and correlation (Pearson's r).

In comparison, during the 2023 special study, a total of 164 separately-collected-yet "paired" seawater samples were taken and analyzed from Coronado beaches by County DEHQ (for ddPCR) and City of Coronado (for Enterolert), respectively. Of these nominal "paired" samples, only 59 displayed enterococcal concentrations above the respective detection limits for both analytical methods as collected by the two different agencies (and processed by the two different laboratories). Unlike published methods in the Crain et al. study (2021), this single set of (n = 59) data points in the Coronado special study report was used as both the "training" dataset for initial model development to generate the linear regression equation (ICE), and the "testing" dataset to evaluate that same ICE model for goodness-of-fit (although in some analyses including the ICE recalculation – see Figure 1 below, an additional 8 Coronado samples were added from the 2021 Crain et al. study, for a total n = 67).

Consequently, the current report lacks true independent model validation for the proposed "site-specific" Coronado ICE equation; instead, the statistical evaluations of sample agreement presented here between ICE-scaled ddPCR results and Enterolert values using the "new" site-specific ICE (IA = 0.65, Pearson's r = 0.59) were essentially calculated from the exact same 59 (or 67) data points used to create the linear regression model being tested. Given the near complete overlap between "training" and "test" data sets in the Coronado special study, it is actually somewhat surprising that reported model performance was not greatly improved over the original Crain et al. study, which achieved similar positive correlation (r = 0.60) and index of agreement (IA = 0.64) between ICE-scaled ddPCR and Enterolert results overall, and generally higher IA values when the ICE model was applied within specific beach-groupings (range: 0.63-0.93), despite calculating these values from statistically valid independent test datasets over a much broader geographical range.

Regardless of the potential statistical deficiencies identified above (coupled with limited evidence for improved model performance outside of the relatively limited (n=59) pseudo-paired sample training dataset specifically analyzed in the report), the authors of the 2024 special study still present a re-calculated "site-specific" ICE for Coronado that indicates an actionable ddPCR threshold of approximately 30,000 copies/100mL, over 20x higher than the 1,413 cutoff value

currently used for beach management throughout the county (Figure 1 below). If this cutoff value were to be applied to the Crain et al. ICE equation currently used for beach management, it would correspond to an estimated Enterolert concentration of 1871 MPN/100ML. Thus, if actionable threshold values for beach management actions are raised to be less-conservative based on a site-specific ICE equation that has not been developed with sufficient statistical rigor and/or has not subjected to thorough model validation on independent datasets to confirm accuracy of conversion between effective ddPCR and Enterolert results, opening beaches at these enterococcal concentrations could create a serious risk to public health for recreational beachgoers.

Conclusion and response:

After completing a detailed review of the Coronado Special Study report (and accompanying dataset) submitted by Verbyla & Lacarra (2024) here, DEHQ has identified several fundamental areas of concern in the study design (field sample collections), statistical model development and validation, and interpretation of analytical results. Although the authors state that they have completely replicated the methods published and validated in Crain et al. (2021), there are several major alterations and omissions that introduce substantial confounding factors and caveats in interpreting the analyses. Several of the most notable issues (and potential impacts on study conclusions) are listed above.

Although the authors of the Coronado Special Study do include a brief general statement (Main Report Page 1, Key Findings, Item 2) acknowledging that the results and data analysis from the 2023 study are not statistically valid to be actionable in their current form, they also state fairly definitively that the current work is sufficient to justify development of a separate, site-specific actionable ddPCR threshold for Coronado that is different (and ostensibly less conservative) than current recreational beach water quality requirements for the remainder of the county. In one section of the supplemental material, the authors specifically conclude that low observed agreement (IA) between the published Crain et al. (2021) ICE predictive equation and the 59 "paired" sample values from the 2023 dataset is sufficient confirmation that county ddPCR water quality standards should not be applicable to Coronado beaches. (Attachment Page 3, Tab 2, Paragraph 2; "This means that Crain's ICE did not properly adjust the ddPCR data for Coronado, based on the Coronado Special Study data set. The conclusion from this analysis is that a Coronado ICE needed to be developed."; see also Main Document Page 2, Kay Findings, Item 3.a.)

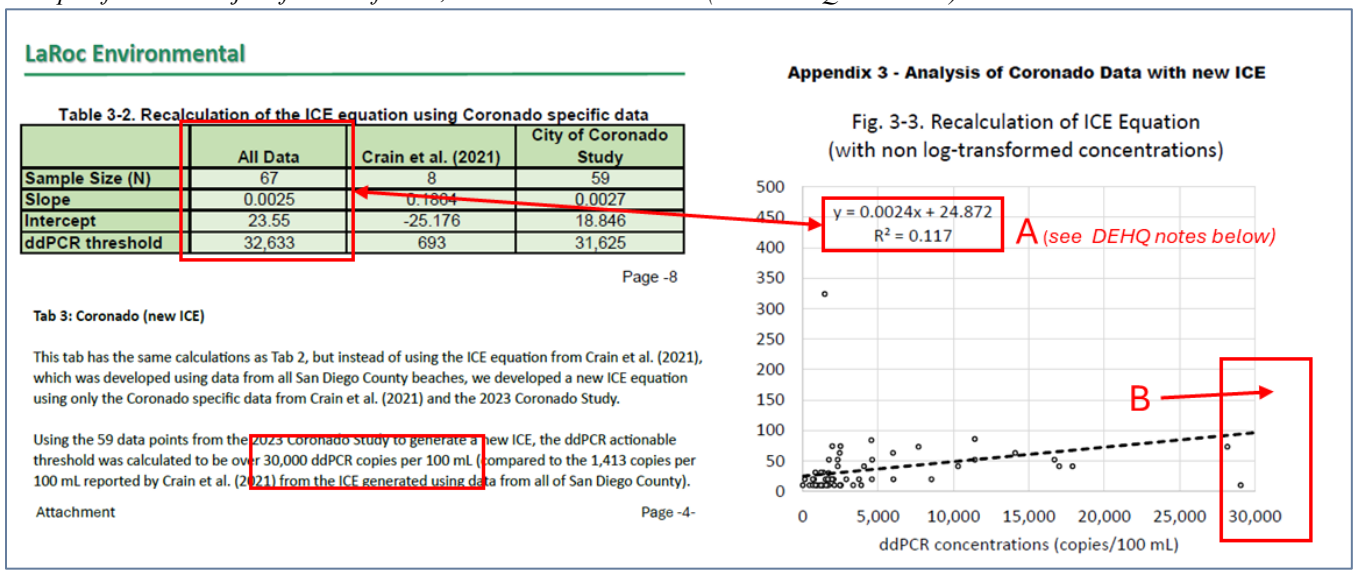
However, based on our thorough review of the data results and analysis in the 2023 report, we feel that differences in the observed linear relationship between Enterolert and ddPCR values observed in the 2023 Coronado Special Study cannot be definitely attributed to inherent site-specific bacterial dynamics that would justify the public health risk associated with raising the allowable enterococcal concentration for beach management thresholds. In particular, because of the lack of true paired/split sampling incorporated into the basic study design for the 2023 Coronado special study, any observed variability in the slope and distribution of "paired" Enterolert/ddPCR results used to generate ICE models in the 2023 report are subject to confounding influence from a number of factors:

- Different sample agency (sampler, collection method, handling time/method, processing, storage)
- Samples taken at different times of day (sometimes up to 4 hrs apart based on publicly available COC forms from the Coronado study) and at different exact GPS locations within the beach- tidal influence, natural variability in vegetation and runoff, animals present during sampling could all influence bacterial levels
- Different laboratory processing samples for each methodology (handling time, temperature, etc.)

Beyond the inherent issues with non-paired sample collection and lack of statistical model validation, the 2023 Coronado special study was limited to three months within the summer (dry) season with minimal precipitation and relatively low Tijuana River flow. Because of this, the range of observed values for enterococcal concentrations in the dataset was not sufficient to establish a statistically reliable relationship between Enterolert/ddPCR sample results around the critical cutoff point of 104MPN/100ML, even if the above issues with study execution were not present. Predictive value of regression models is only valid within the observed sample range, and here, only a single sample collected by Coronado and analyzed with Enterolert exceeded the 104 MPN management threshold. Adding the 8 Coronado data points from Crain et al. 2021 adds only one additional data point above this cutoff value (**see Figure 2 below**). Because the Coronado Special Study lacks sufficient seawater sample data within the critical range surrounding the current beach management cutoff value (100 MPN/100ML), this single data point (which is actually excluded from the graphical representation of the data in the Verbyla & Lacarra report, **see Figure 1**) acts as a major leverage point on the ICE equation, and removing this single data point causes the management predictions (and statistical explanatory power) of the model to become highly unstable (**see Figure 3**).

Thus, based on the data presented here, there is minimal justification to alter the current "cutoff" ddPCR actionable threshold value (1413 copies/100mL) currently in use for beach management decisions throughout San Diego County. Further, given the lack of independent model validation and unknown variability associated with ICE results presented here, we feel that adopting less stringent water quality requirements for ddPCR enterococcal concentrations without definitive statistical justification could pose a substantial public health risk.

DEHQ Figure 1. *Excerpts from Coronado Special Study Report (Verbyla & Lacarra 2024) supporting "new" Coronado site-specific ICE and justification for 30,000+ ddPCR threshold (with DEHQ comments)*



LaRoc Environmental

Table 3-2. Recalculation of the ICE equation using Coronado specific data

	All Data	Crain et al. (2021)	City of Coronado Study
Sample Size (N)	67	8	59
Slope	0.0025	0.1864	0.0027
Intercept	23.55	-25.176	18.846
ddPCR threshold	32,633	693	31,625

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Tab 3: Coronado (new ICE)

This tab has the same calculations as Tab 2, but instead of using the ICE equation from Crain et al. (2021), which was developed using data from all San Diego County beaches, we developed a new ICE equation using only the Coronado specific data from Crain et al. (2021) and the 2023 Coronado Study.

Using the 59 data points from the 2023 Coronado Study to generate a new ICE, the ddPCR actionable threshold was calculated to be over 30,000 ddPCR copies per 100 mL (compared to the 1,413 copies per 100 mL reported by Crain et al. (2021) from the ICE generated using data from all of San Diego County).

Attachment Page -4-

Appendix 3 - Analysis of Coronado Data with new ICE

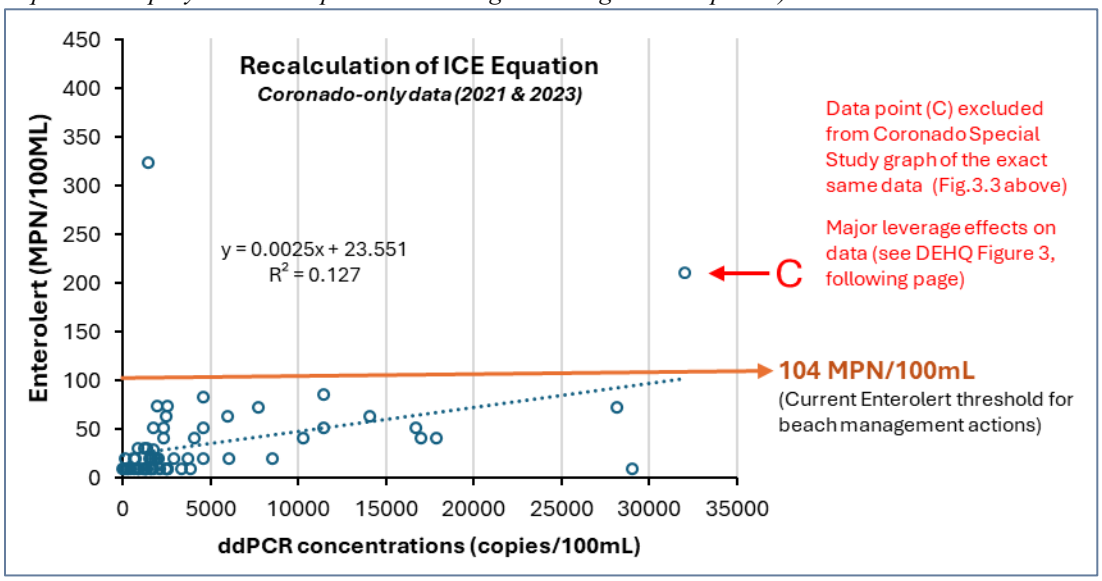
Fig. 3-3. Recalculation of ICE Equation
(with non log-transformed concentrations)

DEHQ Notes:

(A) The ICE equation displayed on Fig 3.3. of the Coronado Special Study Report appears to be incorrect. However, regression parameters on Table 3-2 are correct and match DEHQ re-calculation of the same data (see DEHQ Figure 2 below)

(B) The x-axis chosen for Fig 3.3 of the Coronado Special Study Report is misleading because it cuts the x-axis off at a ddPCR concentration of 30,000 copies/100 mL; this excludes a major outlier (shown in DEHQ Figure 2 below) that was included (and highly influential) in the calculation of the "new" Coronado-specific ICE calculation presented by Verbyla & Lacarra (2024) in their report (see DEHQ Figure 3 below)

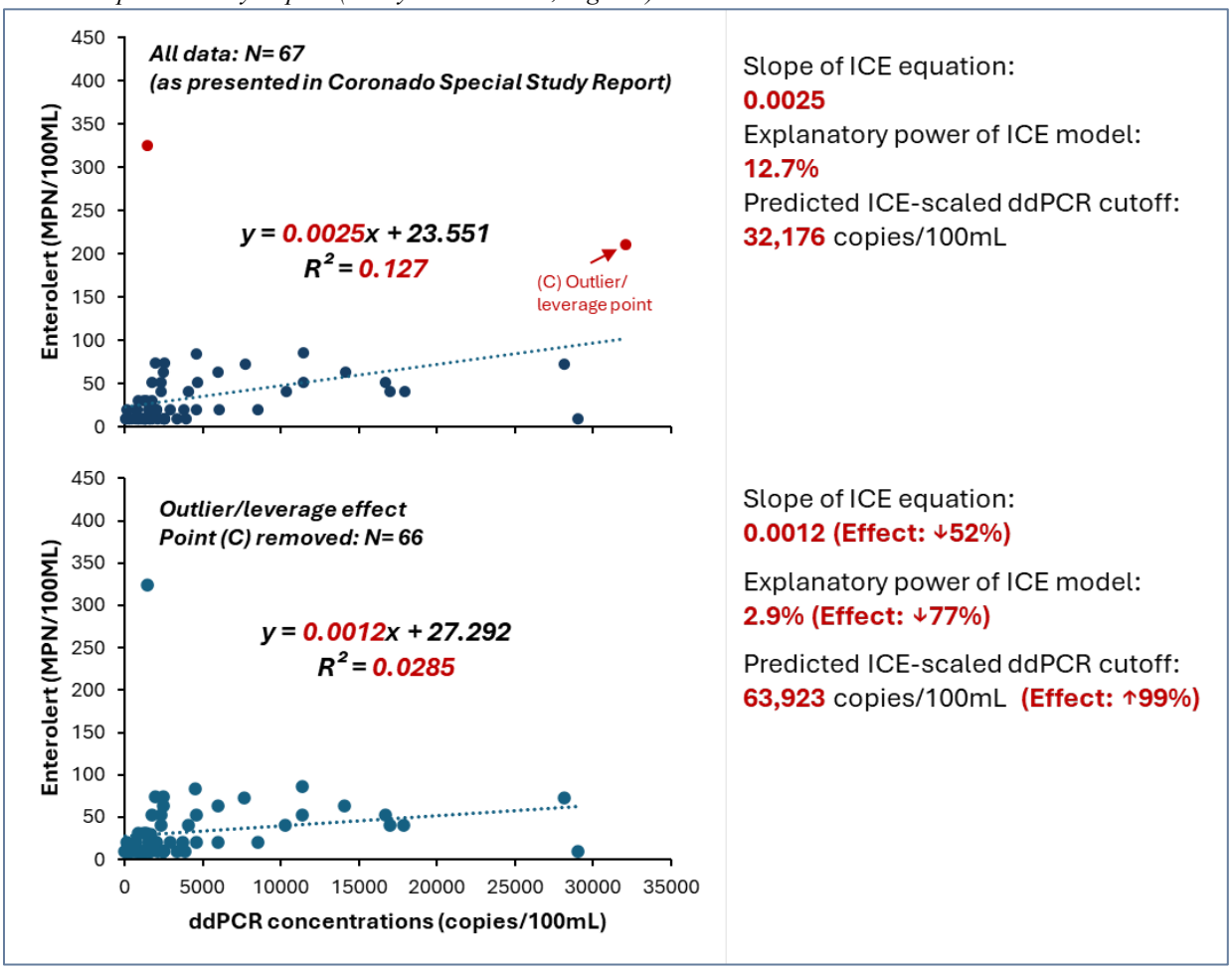
DEHQ Figure 2. *Recalculation of ICE equation and regeneration of Fig.3.3. from the Coronado Special Study Report (with correct ICE equation displayed and complete x-axis range showing all data points)*



DEHQ Notes: Potential issue with data distribution: In the Coronado Special Study combined dataset (2021 & 2023; n = 67), only 2 data points are above the current management threshold, and only one of these is from the 2023 data (C). This is not a sufficient data range for the ICE regression to have predictive power in the critical range around the cutoff point.

(C) Data point is an outlier, and this single data point has major leverage effects on ICE calculation (see analysis in DEHQ Figure 3 below)- despite this, the Coronado Special Study Report presented their data (Fig.3.3 with an x-axis that cut off at 30,000 to exclude this data point which is highly influential on their results

DEHQ Figure 3. *Statistical effects of removing just a single leverage data point (C) from the ICE recalculation presented in the Coronado Special Study Report (Verbyla & Lacarra, Fig.3.3.)*



DEHQ Notes: Removing just a single data point (C, which was the same data point cut off by the x-axis on Figure 3.3.) has major leverage effects on the ICE linear equation and predicted ddPCR cutoff presented in the Coronado Special Study Report. If this data set is not included, the slope of the linear equation decreases by 52% and the predicted ICE-scaled ddPCR cutoff for beach management actions would almost double to 63,923 copies/100mL This suggests that many of the statistical conclusions and recommendations in Verbyla & Lacarra (2024) are driven by a single outlier data point, further emphasizing that the data and analysis presented here are not sufficiently validated or reliable to make major changes in beach management that could have major implications and risks for public health if calculations are not correct.

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- Journal of Experimental Marine Biology
- Marine Ecology, Marine Biology

Technical/statistical review of Verbyla & Lacarra 2026
County of San Diego Department of Environmental Health and Quality

The recent publication by Verbyla and Lacarra (2026) presents (2) major conclusions questioning the validity of ddPCR to inform beach management actions in Coronado, CA. Specifically:

(1) The paper suggests that despite rigorous 2021 EPA review and approval (including a peer-reviewed publication by Crain et al. 2021), ddPCR water quality results used to inform Beach Action Values (BAV) in San Diego County are not statistically comparable to Enterolert, the previous EPA-accepted method for quantifying fecal indicator bacteria in recreational waters.
   a. This is primarily based on index of agreement (IA) values (between ddPCR and Enterolert) that the authors claim fail to meet the EPA requirement (IA = 0.7).
   b. Authors claim to directly replicate all statistical and analytical methodology published in Crain et al. 2021 (i.e., original ddPCR validation study), but report Coronado-specific results (IA = 0.25-0.55) that are substantially lower than Crain's published, EPA-validated results (IA = 0.72; Table 1).

(2) While authors in the paper concede that ddPCR may be acceptable to inform BAV across most other San Diego County beaches, they claim that in Coronado specifically, ddPCR implementation (since May 2022) has led to disproportionate increases in enterococcal detection (and perhaps systematic overestimation of bacterial contamination) in South County waters that are affected by Tijuana River sewage flow (Fig. 5), potentially causing unnecessary beach closures in these sewage-impacted areas.
   a. This is primarily based on the calculated % of "false positive" values in "similar to field duplicates" Enterolert/ddPCR sample pairs collected in Coronado over the two-month summer (i.e., dry season) dataset (n = 55; Figure 4).
   b. Authors claim that the direct relationship between ddPCR bacterial exceedance rates (BAV) and Tijuana River proximity (Figure 5) demonstrates that ddPCR may disproportionately detect enterococcal fecal indicator bacteria (and increase beach closures) at SD County beaches routinely affected by high-discharge sewage flow. Although this seems to be a positive, desired outcome of more sensitive test methods, the authors appear to draw from this an alternative, somewhat baffling conclusion that ddPCR may be overly conservative of public health at sewage-contaminated sites.

Based on these two conclusions, authors of the 2026 paper suggest that ddPCR may not meet the EPA-criteria to inform beach management actions in Coronado and further suggest that based on their findings, the statistical methodology applied by Crain et al (2021) is "not recommended" to evaluated EPA alternative methods.

However, in our review of the 2026 paper (and reanalysis of the publicly available dataset provided by the authors in supplementary material, Appendix 1), we have found several major errors in both the primary data analysis/calculations applied by the authors to arrive at these conclusions and the conceptual design and execution of the study itself, which, despite claims made by the authors, are not directly comparable to methodologies published by Crain in the 2021 EPA-reviewed paper.

Most notably, this includes a major, demonstrable error in the calculation of IA values reported in Table 1. Our recalculation of IA values (correctly following Crain's statistical methodology) from the authors' published dataset results in IA values ranging from 0.71-0.77, which is both highly consistent with previously published

results (i.e., IA =0.72 in Crain et al. 2021) and within the acceptable range for ddPCR to meet EPA alternative method criteria. Our data recalculations are provided in the attached Excel spreadsheet for review.

Detailed responses to author conclusions (1) and (2) are provided in the following sections, as well as a list of detailed line-item concerns noted in our review of various sections of the manuscript (Methods, Results, Discussion, Conclusion).

Response to Verbyla & Lacarra (2026) Conclusion #1

The authors claim that their 2026 publication (using only Coronado beach water quality samples) directly followed all statistical and analytical methodology published by Crain et al. 2021 for validation of ddPCR across all SD County Beaches. Based on this assumption that results are directly comparable, the authors conclude that the lower IA values obtained here for Coronado beaches (in Table 1) indicate that ddPCR is not a statistically reliable indicator of fecal indicator bacteria (or acceptably comparable to Enterolert) in Coronado.

However, in our review of the article and reanalysis of the supplemental data set, we found that Verbyla & Lacarra (2026) utilized several substantial deviations in both statistical and analytical methodology from Crain et al. 2001, meaning that reported results here are not directly comparable. More importantly, we discovered that the lower IA values between ddPCR and Enterolert reported here (in Table 1) are the result of a major, demonstrable error in data analysis/calculation. Correct IA recalculation results in values ranging from 0.71-0.77, which is consistent with Crain et al. 2021 despite major differences in both study scope and methodology.

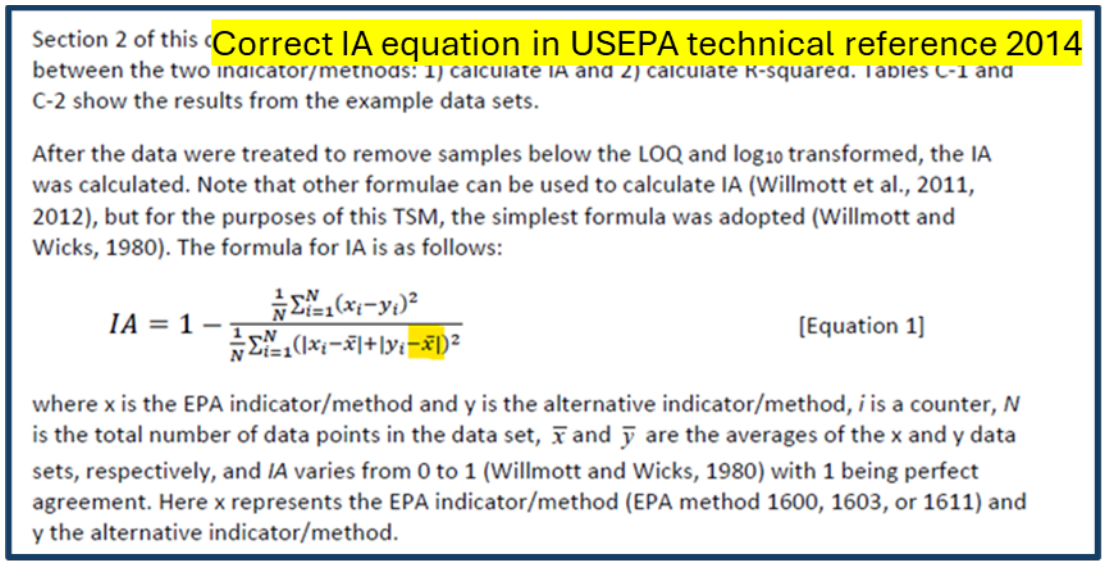
Methodological differences from Crain et al. 2021:

1. **Lack of a true "split-sample" study methodology:**
   Unlike the Crain et al. (2021) study where seawater samples were split and analyzed using both Enterolert and ddPCR to directly compare analytical results between the two methods (on the exact same water sample), the Coronado Special Study used two completely different sets of seawater samples to analyze Enterolert and ddPCR, respectively. In the "Methods" section of the 2026 paper, the authors describe these sample pairs as "similar to field duplicates" even though they were collected by different sampling agencies, processed by different laboratories, and at different times/exact locations on the beach.
   - ddPCR samples were collected by County DEHQ technicians following exact methodology in Crain et al. 2021, then processed by the County Public Health Lab.
   - Enterolert samples were collected on the same day and beach by City of Coronado samplers and "targeted to correspond" to County DEHQ, then processed at an undisclosed ELAP lab.
     - Median difference in collection time was 25 minutes and only 89% of samples were collected within 60 minutes. Based on original dataset provided to DEHQ by City of Coronado prior to this publication, at least three samples were collected by City of Coronado 7-8-h later than sample collection by DEHQ.
     - As authors note in Section 4.2 of the Discussion, differences in sample time as short as 1-10 minutes can result in significantly different *Enterococcal* concentrations for beach water samples (Boehm, 2007)

- Potential sample artifacts affecting direct method comparison: different sampling agency, different laboratory, different handling/processing (collection methods, temperature, holding time, etc.), different location/environmental/tidal conditions on beach)- these are completely glossed over in final paper.
- Presentation of "same-day, same-beach" samples as directly equivalent to true split samples from the same 500mL sample jar (as in Crain et al 2021) is particularly misleading, given that the inferred "coordination"/collaboration with DEHQ for sampling is largely exaggerated, and DEHQ was uninvolved and largely uninformed of City of Coronado research activities (including the current 2026 paper, up until publication this week).

2. **Inadequate seasonal/temporal sample range/sample size:**
   Unlike the Crain et al. (2021) study where split seawater samples (n ≈ 2000) were collected year-round over a 20-month period (May 2017-January 2019), the 56 seawater samples included in the final Coronado Special Study dataset were limited to a < 2 month period (July 6 – August 28) entirely within the summer (dry) season in 2023, when Tijuana River flow was consistently low.
   - As a result, only 1 of the 186 total Enterolert samples in the 2026 study exceeded the EPA MPN BAV threshold of 104, and 0 out of 55 Enterolert values included in the final data set exceeded the 104 closure threshold, resulting in limited predictive value to assess ddPCR at values relevant to management
   - Comparatively in Crain et al (2021) 179 out of 1086 Enterolert samples (16.5%) exceeded 104 MPN, with seasonally higher values observed during the winter-spring rainy season, providing adequate predictive power to estimate the comparable threshold beach closure limit for ddPCR.

3. **Differences in data analysis methodology:**
   A central topic of the 2026 paper is a comparison of two statistical methods accepted by the EPA to validate alternative water quality methods. Method (1) is the standard log-transformed linear regression method (USEPA 2014) and Method (2) is the ICE-scaled linear regression method used by Crain et al. (2021). However, the authors here fail to completely/correctly replicate the statistical methodology for Method 2 in particular. Most notably:
   - In the original Crain et al. 2021 paper, all regression analyses used to develop ICE-scaled ddPCR equations (Method 2) were run with both log-transformed and untransformed data. Due to the larger sample size (more robust to regression assumptions) in that paper, untransformed values/equations were ultimately presented because IA values were comparable to log-transformed results. In the 2026 study, the authors fail to apply log-transformations (Table 1) even though normality tests and evaluations of regression assumptions suggest that data are log-linear with multiple violations of regression assumptions in this much-smaller data set (Supplemental material, Appendix A).
   - **In the 2026 paper, there are errors in both IA equations (1,2) under Statistical Methods section (2.2.1, Pg. 3), where ybar (in the denominator of Eq 1) and ymedian (in the denominator of Eq 2) should be xbar and xmedian, respectively, to correspond with the IA equation published in the *USA EPA Technical Support Materials for Alternative Indicators and Methods* (2014). (See highlighted excerpts below).**



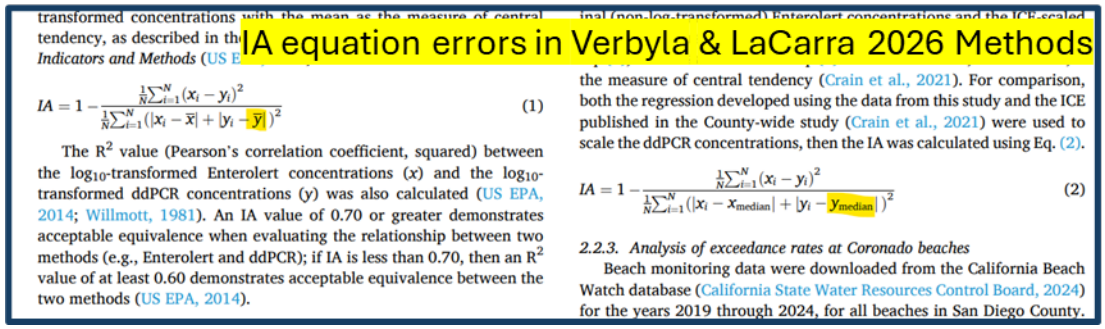
Correct IA equation in USEPA technical reference 2014

Section 2 of this c
between the two indicator/methods: 1) calculate IA and 2) calculate R-squared. Tables C-1 and C-2 show the results from the example data sets.

After the data were treated to remove samples below the LOQ and $\log_{10}$ transformed, the IA was calculated. Note that other formulae can be used to calculate IA (Willmott et al., 2011, 2012), but for the purposes of this TSM, the simplest formula was adopted (Willmott and Wicks, 1980). The formula for IA is as follows:

$$IA = 1 - \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - y_i)^2}{\frac{1}{N}\sum_{i=1}^{N}(|x_i - \bar{x}| + |y_i - \bar{x}|)^2} \qquad \text{[Equation 1]}$$

where x is the EPA indicator/method and y is the alternative indicator/method, *i* is a counter, *N* is the total number of data points in the data set, $\bar{x}$ and $\bar{y}$ are the averages of the x and y data sets, respectively, and *IA* varies from 0 to 1 (Willmott and Wicks, 1980) with 1 being perfect agreement. Here x represents the EPA indicator/method (EPA method 1600, 1603, or 1611) and y the alternative indicator/method.



IA equation errors in Verbyla & LaCarra 2026 Methods

transformed concentrations with the mean as the measure of central tendency, as described in th
Indicators and Methods (US E

$$IA = 1 - \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - y_i)^2}{\frac{1}{N}\sum_{i=1}^{N}(|x_i - \bar{x}| + |y_i - \bar{y}|)^2} \qquad (1)$$

The R^2 value (Pearson's correlation coefficient, squared) between the $\log_{10}$-transformed Enterolert concentrations (x) and the $\log_{10}$-transformed ddPCR concentrations (y) was also calculated (US EPA, 2014; Willmott, 1981). An IA value of 0.70 or greater demonstrates acceptable equivalence when evaluating the relationship between two methods (e.g., Enterolert and ddPCR); if IA is less than 0.70, then an R^2 value of at least 0.60 demonstrates acceptable equivalence between the two methods (US EPA, 2014).

inal (non-log-transformed) Enterolert concentrations and the ICE-scaled the measure of central tendency (Crain et al., 2021). For comparison, both the regression developed using the data from this study and the ICE published in the County-wide study (Crain et al., 2021) were used to scale the ddPCR concentrations, then the IA was calculated using Eq. (2).

$$IA = 1 - \frac{\frac{1}{N}\sum_{i=1}^{N}(x_i - y_i)^2}{\frac{1}{N}\sum_{i=1}^{N}(|x_i - x_{median}| + |y_i - y_{median}|)^2} \qquad (2)$$

2.2.3. Analysis of exceedance rates at Coronado beaches

Beach monitoring data were downloaded from the California Beach Watch database (California State Water Resources Control Board, 2024) for the years 2019 through 2024, for all beaches in San Diego County.

Major statistical error in IA calculation for ICE-scaled ddPCR results in Verbyla & LaCarra (2026) :

One of the major stated findings of the 2026 paper is that IA values for Coronado are apparently substantially lower (0.55 to 0.43) than previous values published for SD County by Crain et al. 2021, and also below the EPA minimum criterion of 0.7 required to validate an alternative analytical method for water quality samples (i.e., ddPCR).

However, based on the published dataset in supplemental material (Appendix A) of the 2026 paper, we re-calculated IA for ICE-scaled ddPCR values using both the full training data set (n = 29) and the outliers-excluded training data set (n = 27), using both un-transformed (raw) and log10-transformed values. We also calculated IA based on both xmean (Eq. 1) and xmedian (Eq2). For all variations, our recalculations of IA based on the author-provided dataset resulted in values greater than 0.7. All calculations are shown in the accompanying Excel workbook. A summary of ICE-scaled equations and IA values recalculated from the Verbyla & Lacarra 2026 publication data is shown in the table below.

Rebuttal Table 1: Recalculations of Index of Agreement (IA) based on supplemental data (Appendix A) published in Verbyla & Lacarra 2026. See Excel spreadsheet for full calculations and regression graphs.

				R^2 Values		IA Values	
	Training data	Transform	Test data	R^2 (training)	R^2 (test)	IA (median)	IA (mean)
ICE- Crain et al. 2021	n =185	None	n=1086	0.38	0.36	0.72	
ICE- Verbyla & Lacarra 2026	**n = 27**	**None**	**n=26**	**0.28**	**0.47**	**0.55**	
Our recalculations of 2026 data		Transform	Test data	R^2 (training)	R^2 (test)	IA (median)	IA (mean)
Recalc-ICE Eq1	All (n=29)	None	n=26	0.63	0.22	0.75	0.71
Recalc-ICE Eq2	All (n=29)	log10	n=26	0.40	0.33	0.72	0.74
Recalc-ICE Eq3 **	**Outliers removed (n=27)**	**None**	**n=26**	**0.28**	**0.47**	**0.77**	**0.73**
Recalc-ICE Eq4	Outliers removed (n=27)	log10	n=26	0.29	0.53	0.71	0.74
** Direct recalculation of method used by Verbyla & Lacarra 2026							

Although the 2026 study took care to remove apparent outliers from the training data set and evaluate a modified IA equation using median instead of mean to account for data skewness, our recalculations of IA suggest that the relationship/agreement between Enterolert and ddPCR is relatively robust to slight variations in both calculation method (mean vs. median) and statistical treatment of training data used to develop ICE equations (removal of outliers, log10-transformation).

We would suggest that the authors of the 2026 paper review their statistical analysis in regard to Method 2 (ICE-scaled ddPCR). In our ICE/IA reanalysis, Eq 3 is ostensibly a direct recalculation of the method/parameters in the 2026 paper, and it is notable that R^2 values and scatterplot graph/equation for our Recalc-ICE Equation 3 (see also Excel workbook) are identical to the published values, despite the observed discrepancy in IA calculations. It is possible that IA errors here may be partially due to the published IA equation errors (Methods, 2.2.1, 2.2.2) noted above.

Review of Verbyla & Lacarra (2026) Conclusion #2

The authors in this 2026 publication suggest that although ddPCR-based BAV may be generally acceptable for most other areas of SD County, ddPCR-based management in Coronado disproportionately overestimates fecal bacteria presence compared to other locations.

To support this, the authors suggest that increased closure rates observed at Coronado/adjacent beaches (but not elsewhere in SD County) since the implementation of ddPCR (in May 2022; Figure 5) have been largely driven by the methodological change from Enterolert to ddPCR resulting in high rates of false positives in Coronado-specific data (ROC curve, Figure 4). The authors further support this by comparing geomean/median values of ddPCR samples in the study (which are both higher than the threshold management value of 1413 copies/100mL) to geomean/median values of Enterolert samples in the study (which are both under MPN management threshold of 104).

However, in our review of the article and data analyses used to support this conclusion, we found that Verbyla & Lacarra (2026) fail to consider critical context in the timing of the Tijuana cross-border sewage crisis when implicating ddPCR methodological artifacts as a driver of increased Coronado/South County Beach closure rates. Additionally, we identified several major flaws and/or areas of concern in the data analyses used to support this conclusion (i.e., ddPCR leading to disproportionate enterococcal overestimation/false exceedance rates in Coronado compared to other areas of SD County).

Concerns/potential issues over data analysis:

1. Figure 4- The primary statistical "evidence" for inflated ddPCR false positives in Coronado is presented in the high % false positive (56.3%) and low % agreement (43.7%) for the current threshold ddPCR cutoff value (1413 copies, corresponding to 104 MPN; see Figure 4). These calculations are based on the Receiver Operating Characteristic (ROC) curve, which was also used by Crain et al. 2021 to calculate a much higher % agreement value of 90% at the management threshold.
    - In the 2026 paper, we have major concerns whether ROC analysis can be validly applied due to the limited range of Enterolert MPN results in the empirical data (see previous discussion on inadequate 2026 dry-season sample design)
    - In the 2021 paper, ROC was generated by splitting all (n=1993) ddPCR samples as "above" or "below" the designated threshold value (i.e., 1413 copies), then comparing the result to the paired Enterolert sample (i.e., above or below 104 MPN). This is then used to calculate % false positive, % false negative, and agreement rate between methodologies based on the area under the curve.
    - In the 2026 study, only a single Enterolert sample (1 out of n = 185) exceeded the threshold cutoff for MPN (i.e., corresponding to ddPCR value of 1413). This would unavoidably result in inflated and/or unstable estimated % false positive rates due to limited predictive range around the critical threshold value, calling into question the validity of the observed 43.7% agreement at the management threshold here. Despite this, the ROC analysis (Figure 4) extrapolates % agreement out to ddPCR values > 30,000 copies.

2. One additional piece of evidence cited in the paper is that median sample values for Enterolert (7.8 MPN) are below the BAV cutoff (104 MPN) while median sample values for ddPCR (1669 copies) are above the BAV cutoff (1413 copies). However, it is notable that the authors' statistically predicted

median value for Enterolert (7.8) is also below the minimum detection limit for the methodology (< 10 MPN), further suggesting that limited sample range (and high levels of Enterolert non-detects during dry-season sampling) may be driving data artifacts here, and predictive values for beach management threshold past the sample range should be interpreted with caution (Results Section 3.3.1).

Misleading omission of timeline for TJ river flow:

1. Fig 5- Authors present increased Coronado/South County exceedances and beach closures following ddPCR implementation (compared to the rest of SD County) as evidence that Tijuana River flow leads to bacterial decay that may alter the relationship between ddPCR and Enterolert, potentially necessitating a different BAV or ICE-equation slope for this area.
    - Attributed to bacterial decay- temperature, light travel time- increased sewage impact compared to North County sites never mentioned
2. Figure 6- "Historical" (2019-2024) data from San Diego beaches shows the relationship between Tijuana River flow, ddPCR results, and Enterolert results, and authors cite this as a direct "linear relationship between the distance to the mouth of the Tijuana River" and the increased "exceedance rates under the new ddPCR-based BAV"
    - Authors suggest that increased exceedance rates following ddPCR implementation in May 2022 are related to systematic overestimation of Coronado enterococcal concentrations using ddPCR methods

Authors do not mention that the timing of ddPCR implementation in 2022 largely coincided with major sewage treatment pump/pipeline failures across the international border, and a substantial increase in sewage release/transboundary flow to South County beaches