



RAPID MICROBIOLOGICAL MEASUREMENT METHODS

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Research Project**
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WHAT IS SCCWRP?

- **Joint Powers Agency founded in 1969**
- **Initiated to address regional monitoring and research needs**
 - Cumulative impact assessment
 - Methods development
 - Data integration
- **Member organizations include city, county, state, and federal agencies**
 - Unique combination of regulators and regulated

MEMBER ORGANIZATIONS

City of Los Angeles

City of San Diego

Ventura County Watershed Protection Division

Orange County Watershed and Coastal Resources Department

Los Angeles County Department of Public Works

Los Angeles County Sanitation Districts

Orange County Sanitation District

San Diego Regional Water Quality Board

Santa Ana Regional Water Quality Board

Los Angeles Regional Water Quality Board

State Water Resources Control Board

U.S. Environmental Protection Agency

BACKGROUND

- **Laboratory analysis for beach monitoring requires 24 hours, which compromises effectiveness**
 - Warnings are issued after exposure has occurred
 - Beaches remain closed 24 hours longer than necessary
 - Slow response limits upstream tracking
- **New molecular methods are coming on line**
 - Primarily developed for the food service and health industries
 - SCCWRP has been providing grants and assistance to facilitate application to beaches
- **These new methods require rigorous independent evaluation**
 - Users need to know which methods work
 - Developers need a certification process

APPROACH TO METHOD EVALUATION

- **Process samples using both new and traditional methods**
 - Evaluate equivalency to existing methods
 - Epidemiological testing is a preferred option but equivalency is a more expeditious first step

- **Have conducted three evaluation tests to date**
 - Screening tests
 - Beta tests

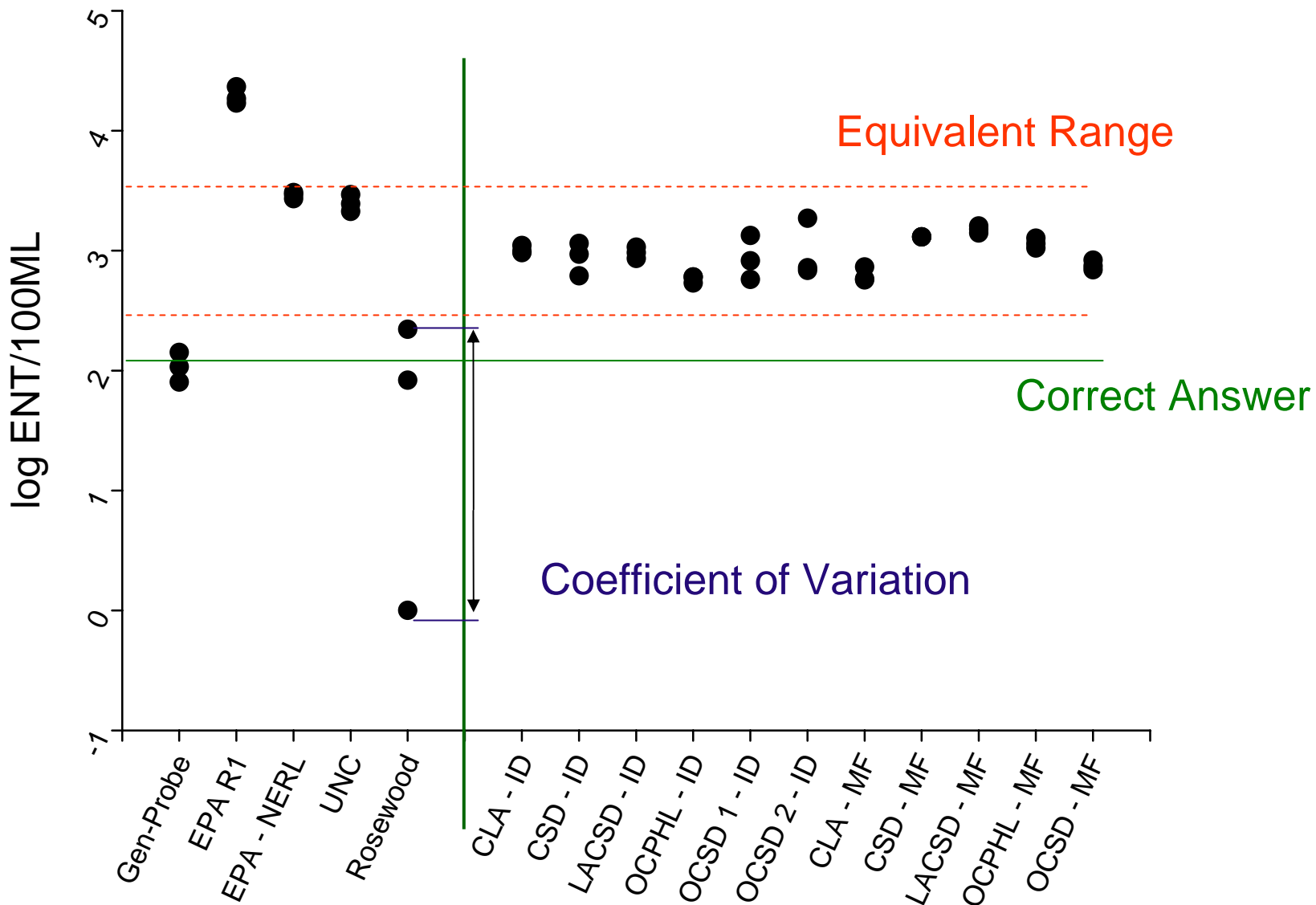
SCREENING EVALUATION

- **Method developers come to a single location**
- **Provided 54 blind samples**
 - Five local laboratories perform traditional methods for reference
 - Both MF and IDEXX used as reference
- **Have tested six methods**
 - Quantitative polymerase chain reaction
 - Transcription mediated amplification
 - Immunomagnetic separation coupled with ATP quantification
 - Flow cytometry
 - Immunological dipstick
 - Dual wave fluorimetry

TEST SAMPLES

- **Seawater inoculated with laboratory culture (3 concentrations)**
- **Seawater inoculated with sewage influent (3 concentrations)**
- **Seawater inoculated with urban runoff (3 concentrations)**
- **Ambient samples (6 samples)**
- **Blanks (3 samples)**
 - Phosphate – buffered saline
 - Offshore seawater
 - Filtered offshore seawater
- **Blind triplicates of each sample**

OCSD INFLUENT INOCULANT



INTEGRATED ANALYSIS

- **Equivalent to current methods**
 - 2 of 3 replicates and the median correct with respect to the California water quality standard
 - 2 of 3 replicates within $\frac{1}{2}$ log unit of reference lab median
 - Smaller variance than worst reference lab
- **Not materially different than current methods**
 - Fails none of the materially different criteria, but does not meet equivalency criteria
- **Materially different than current methods**
 - 2 of three replicates incorrect with respect to standard
 - Median value differs by $>1/2$ log unit from reference lab median
 - Coefficient of variation twice that of the worst reference lab

INTEGRATED EVALUATION FOR ENTEROCOCCUS

	Equivalent to current methods	Not materially different from current methods	Materially different from current methods
Membrane Filtration	88	0	12
Defined Substrate	65	18	18
Transcription Mediated Amplification	59	18	23
QPCR (EPA - R1)	18	0	82
QPCR (EPA - NERL)	47	18	35
QPCR (Univ. of North Carolina)	53	24	23
Dual Wave Fluorimetry	18	12	70

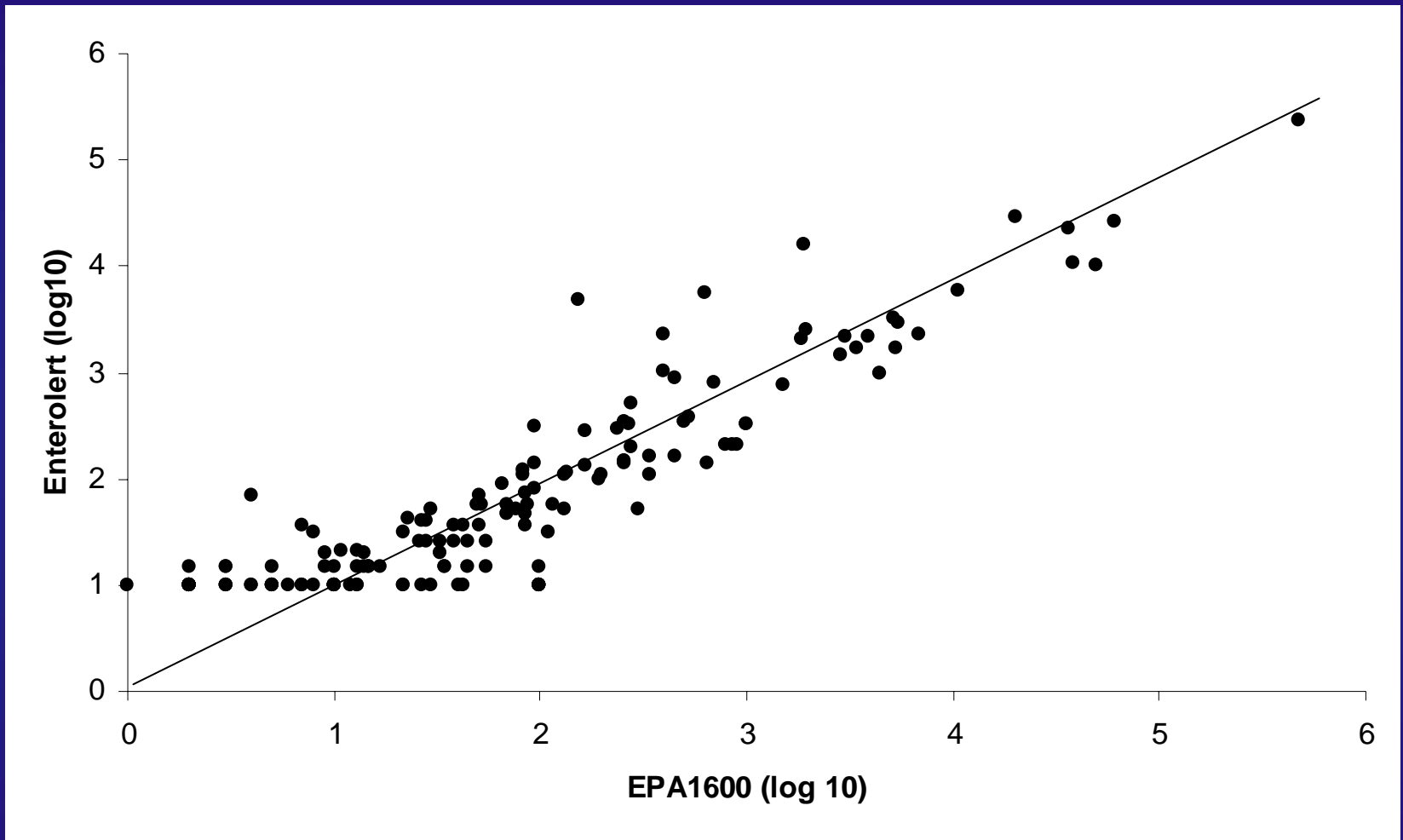
BETA TESTING

- **Screening tells us whether the experts can produce acceptable results using new methods**
 - Also need to know whether the local practitioners can produce acceptable results
- **Screening is based mostly on “created” samples**
 - Need to have a guaranteed range of concentrations when getting folks all in one place
 - Ambient samples may contain additional interferences
- **Beta testing involves local laboratories using both new and traditional methods on routine samples**

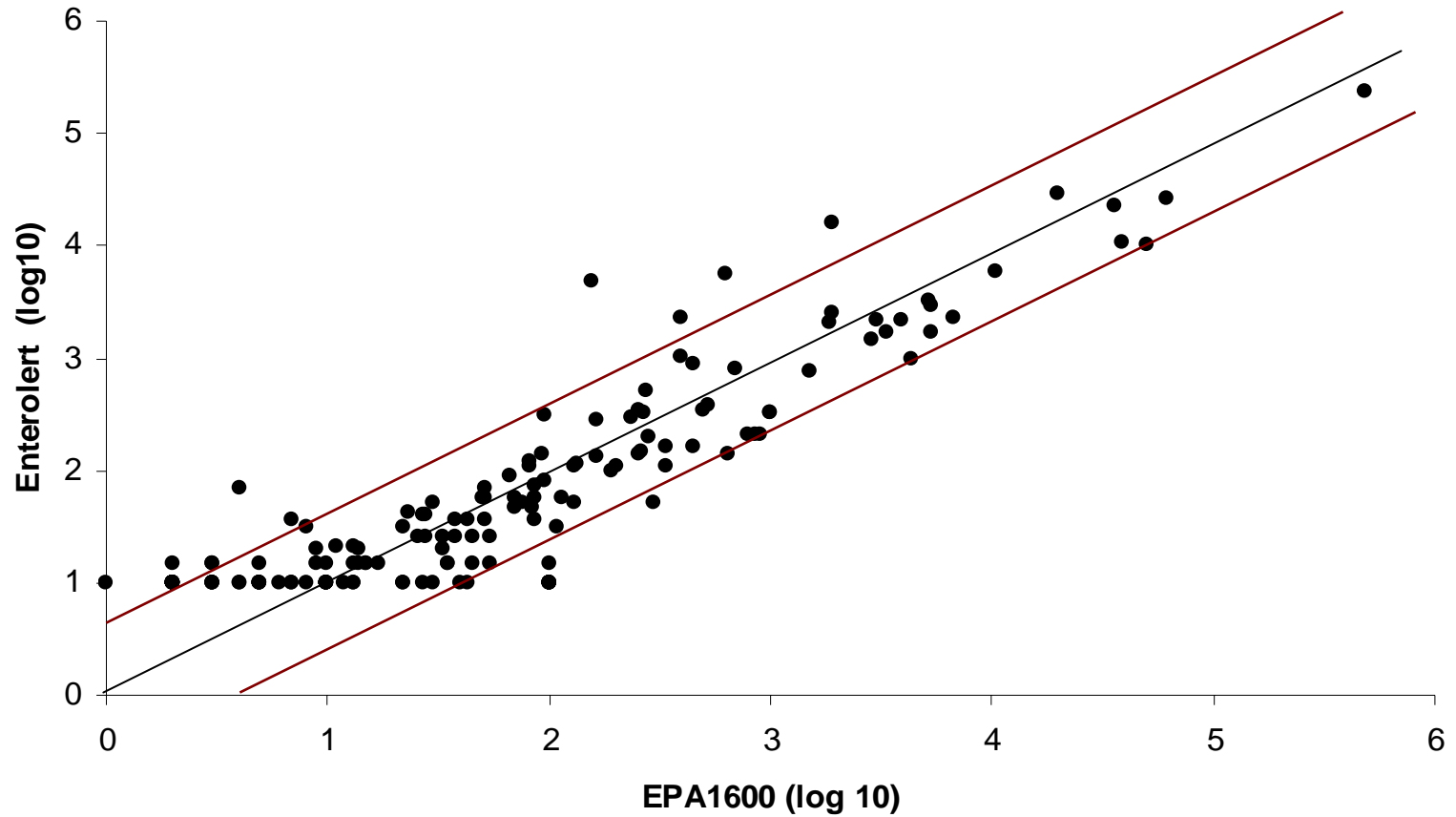
BETA TESTING STUDY DESIGN

- **Simultaneous processing of 163 samples using new and existing methods**
 - QPCR
 - Genprobe Hydrus
 - EPA1600
 - Enterolert
 - Replicates of each method
- **Seven sample types**
 - Open beaches
 - Embayment beaches
 - Sewage influent inoculated into urban runoff
 - Sewage effluent inoculated into seawater
 - Near drain
 - Within drain
 - Wet Weather
- **QPCR method switched from extraction to bead-beaten a quarter of the way through the study**
 - The extraction method was too complex for local laboratory

EPA1600 vs Enterolert



EPA1600 vs Enterolert



REPEATABILITY

	Average Coefficient of Variation
EPA 1600	0.18
Enterolert	0.21
Hydrus	0.26
QPCR Bead Beaten	0.21
QPCR Extracted	0.60

ACCURACY

**% >1/2 log
Above Existing
Methods**

**% >1/2 log
Below Existing
Methods**

EPA1600

0

0

Enterolert

3

8

Hydrus

4

19

**QPCR Bead
Beaten**

4

16

QPCR Extracted

0

41

INTEGRATED ANALYSIS

- **Equivalent to current methods**

- Both replicates and the median correct with respect to the AB411 standard
- Both replicates within $\frac{1}{2}$ log unit of reference lab median
- Variance within a factor of 2 or numeric difference of <25

- **Not materially different than current methods**

- Median correct with respect to AB411 Standard
- Median within $\frac{1}{2}$ log of median
- Variance not greater than a factor of 4 or numeric difference of <25

- **Materially different than current methods**

- Median replicates incorrect with respect to standard, OR
- Median value differs by $>1/2$ log unit from reference lab median, OR
- Coefficient of variance >4 times the worst reference lab and numeric difference of <25

INTEGRATED ANALYSIS

	Equivalent to Current Methods	Not Materially Different	Materially Different
EPA1600	72	10	18
Enterolert	59	16	25
Hydrus	62	7	31
QPCR Bead Beaten	67	6	27
QPCR Extracted	47	3	50

POSSIBLE REASONS FOR DIFFERENCES BETWEEN STUDIES

- **Different types of samples**
 - More emphasis on ambient samples
 - Ambient samples are more likely to contain inhibiting materials
- **Experience of the personnel**
 - They received several days of training
 - Most had limited previous experience with genetic methods
- **Greater species-specificity of the genetic probes**
 - Hydrus is targeted for *E. faecalis* and *E. faecium*

AVERAGE COEFFICIENT OF VARIATION AMONG REPLICATES

	Last Year	This Study
EPA1600	0.32	0.18
Enterolert	0.31	0.21
Hydrus	0.44	0.26
QPCR Bead Beaten	0.27	0.21
QPCR Extracted	0.44	0.60

INHIBITION

- **Some chemicals (e.g., humic acids) are known to inhibit genetic amplification**
- **Internal controls were run on QPCR samples**
 - Internal controls not yet built into the Hydrus method
- **23% of the QPCR false negatives exhibited inhibition in controls**
 - This is probably an underestimate as the controls are not designed to identify inhibition at low cell densities
- **There are remedies (e.g., dilution) when internal controls are not met**

CONCLUSIONS

- **Neither method is ready yet for routine application**
 - False negative rates do not meet BWQWG expectations
 - Methods could be useful right now for tracking applications
- **The false negative problem is more likely due to inhibition than to operator error**
 - More automation to reduce operator error is still desirable
- **Better internal controls need to be incorporated into the methodology**
 - Method developers are working on this as we speak