The Use of Defined Substrate Technology for the detection of Total Coliforms, Fecal Coliforms and E.coli (Indicator Bacteria) for Reuse Water.

Gil Dichter

World wide Technical Support Manager, Water

gil-dichter@idexx.com

IDEXX

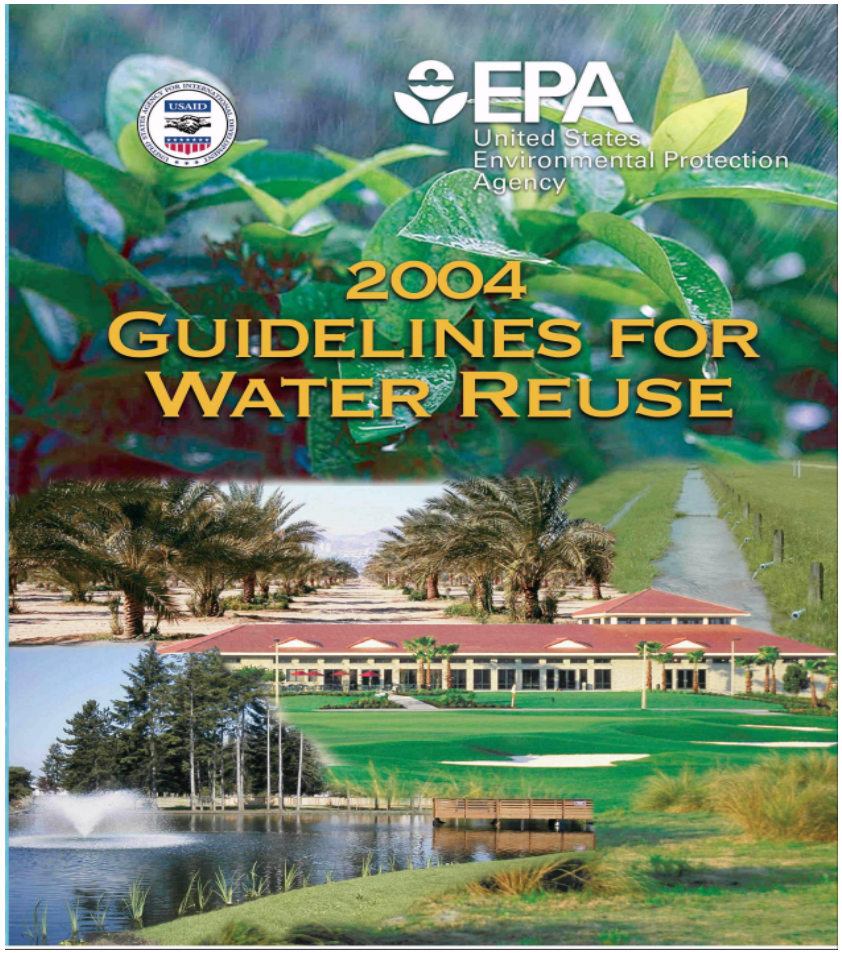
OBJECTIVES

- Introduction to Reuse Water
- Coliform bacteria
- 15 tube MTF/ MPN & MF for TC, FC or *E.coli*
- Colilert and Colilert-18
  - Theory
  - How to test
  - Interpretation of results
- Review of several studies comparing MTF to Colilert
- Q & A

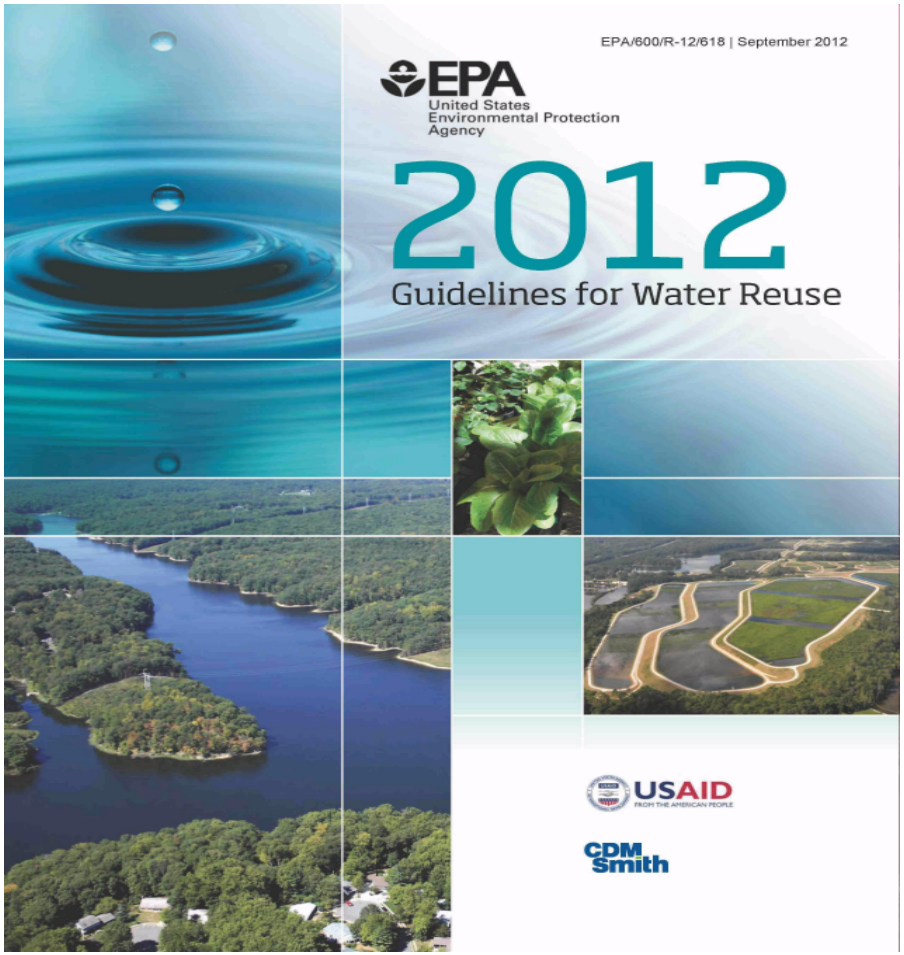
IDEXX

Guidelines for Water Reuse

EPA/625/R-04/108 Sept 2004



EPA 600/R-12/618 Sept 2012



IDEXX

Reclaimed/ Reuse Water

- Wastewater that has been treated to meet specific water quality criteria
- It is used for a range of purposes

- Requirements for microbiological and chemical parameters
  - Varies from state to state (majority of states test for fecal coliforms followed by total coliforms and several for *E.coli*
    - Bacteria - total or fecal coliforms or *E.coli*
    - *Pathogens - Cryptosporidium, Giardia, viruses*
    - *Chemistry: pH, Turbidity, BOD, COD, TSS*

Background Information

- 2009 Data
  - 32 million GPD of municipal wastewater was produced of which 7-8% was used as reclaimed water.
  - 95% of water reuse from 4 states; CA, FL, AZ & TX
  - Recently, several other states are utilizing reuse water- CO, NM, NV, WA, OR & VA

Background Information

- Worldwide - many countries using reclaimed water with goals of using more
  - Singapore: currently 30% and goal is to reduce dependence of water from Malaysia
  - Israel: currently reusing 70% of generated domestic water
  - Australia; currently 8% and by 2016 to 30%
  - Saudi Arabia- reuse is 16% and a goal of 65% by 2016

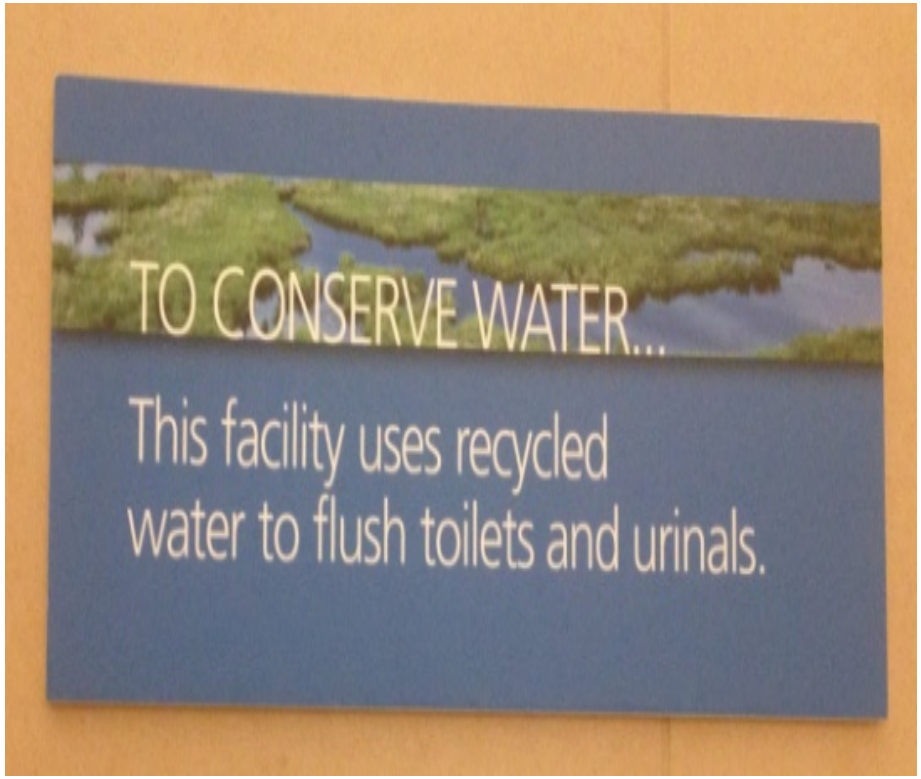
Categories of Water Reuse Applications

- **Many states have rules, regulations or guidelines for the range of reuse water**
- **Urban Reuse** for non-potable applications.
  - Unrestricted: public access - 32 states
  - Restricted: no public access- 40 states
- **Agricultural Reuse**
  - Irrigate food crops for human consumption – 27 states
  - Process food and non food crops: processed before consumption or not consumed - 43 states
- **Impoundments (recreational waters)**
  - Unrestricted: No limitations imposed -13 states
  - Restricted: Body contact is restricted – 17 states

Categories of Water Reuse Applications

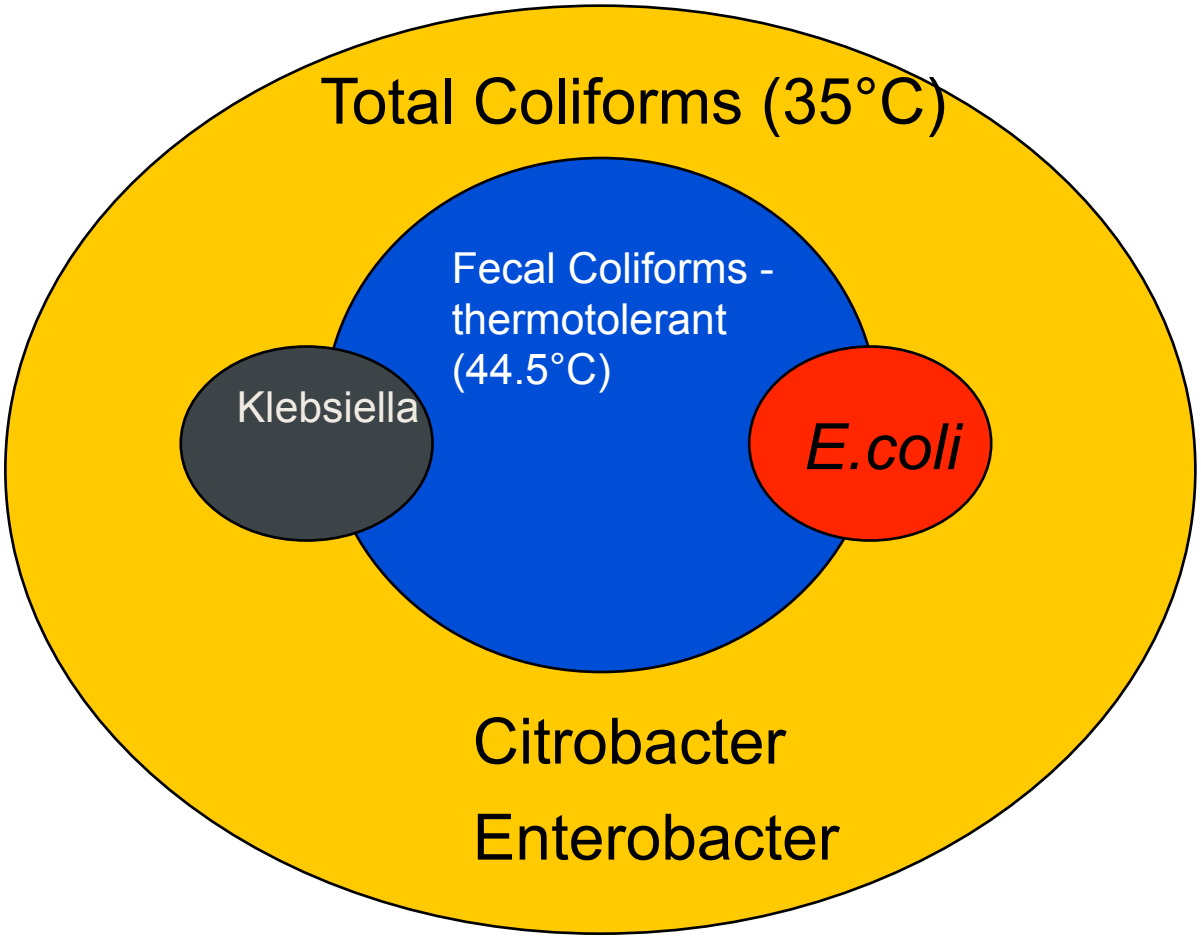
- **Environmental Reuse:** Create, sustain or augment water bodies such as stream flow, wetlands.
- **Industrial Reuse**: for industrial applications such as power production
- **Groundwater Recharge- non-potable reuse** Aquifers that are not used as a potable resource
- **Potable Reuse**
  - **IPR-** augment SW or GW source followed by an environmental buffer that precedes normal DW treatment – 9 states
  - DPR- with or without retention directly into a DWTP- 0 states

San Jose, CA airport



Simi Valley, CA WWTP

Coliform Bacteria Group

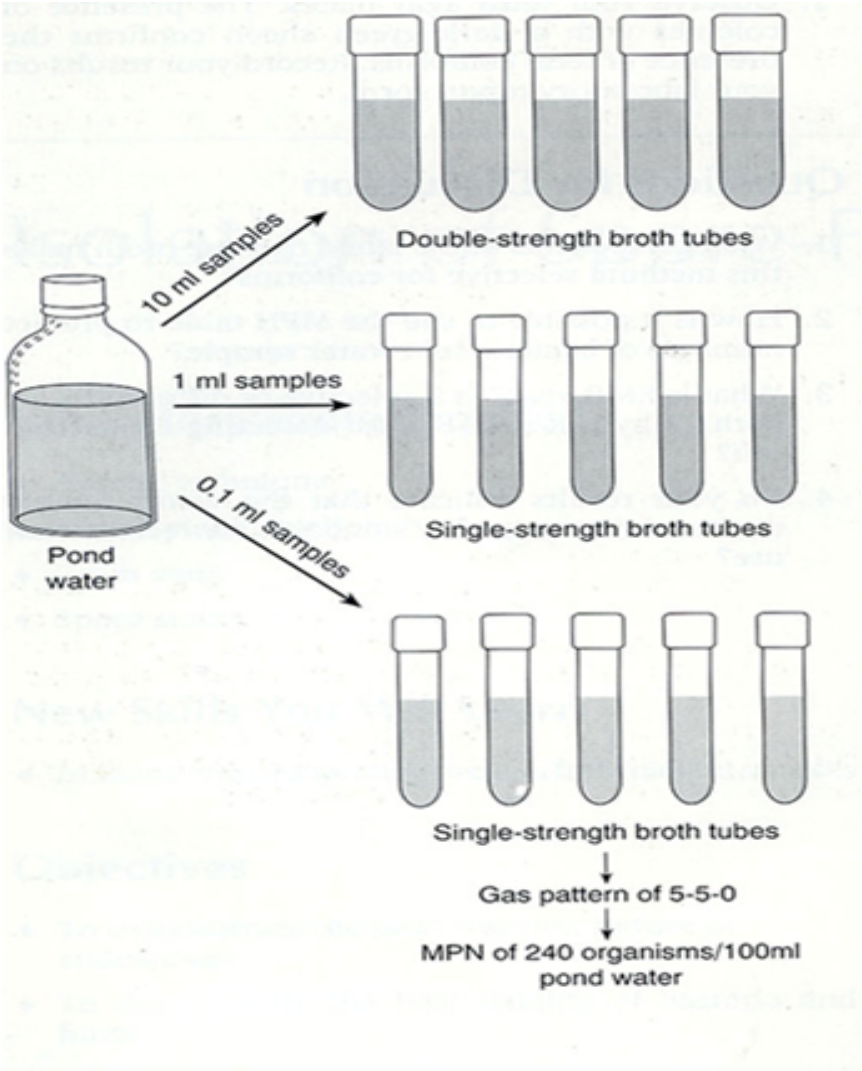


Microbiological Methods

Methods for TC, EC and FC

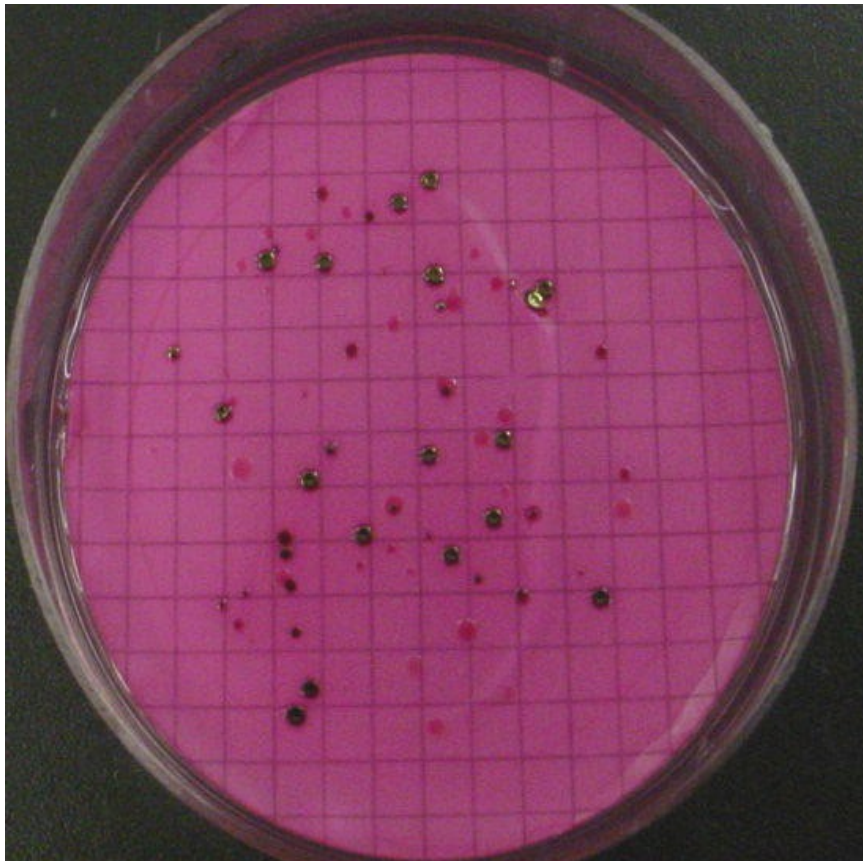
- MTF- 15 tube
  - LTB-presumptive- up to 48 hours at 35°C
  - TC confirmation BGLB - up to 48 hours at 35°C
  - FC or EC confirmation - EC medium or EC-MUG- 24 hours in water bath at 44.5°C
  - Total time for TC results – up to 96 hours and up to 72 hours for FC or EC

15 Tube MTF Method

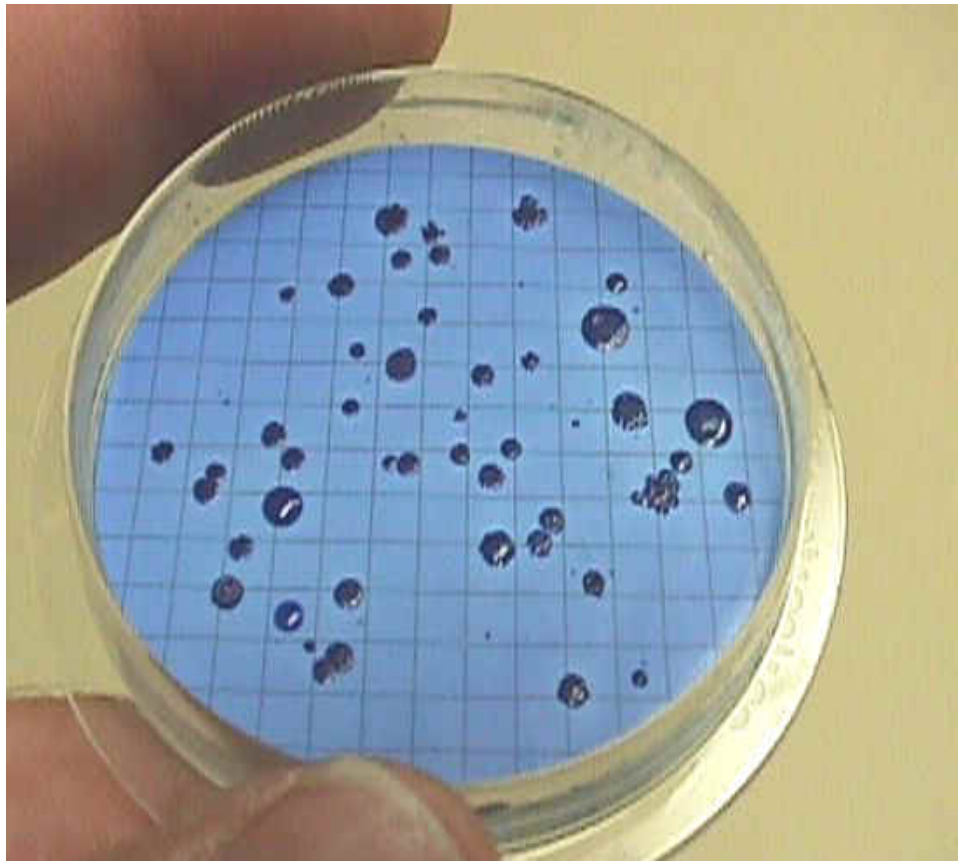


Membrane Filtration

m-Endo

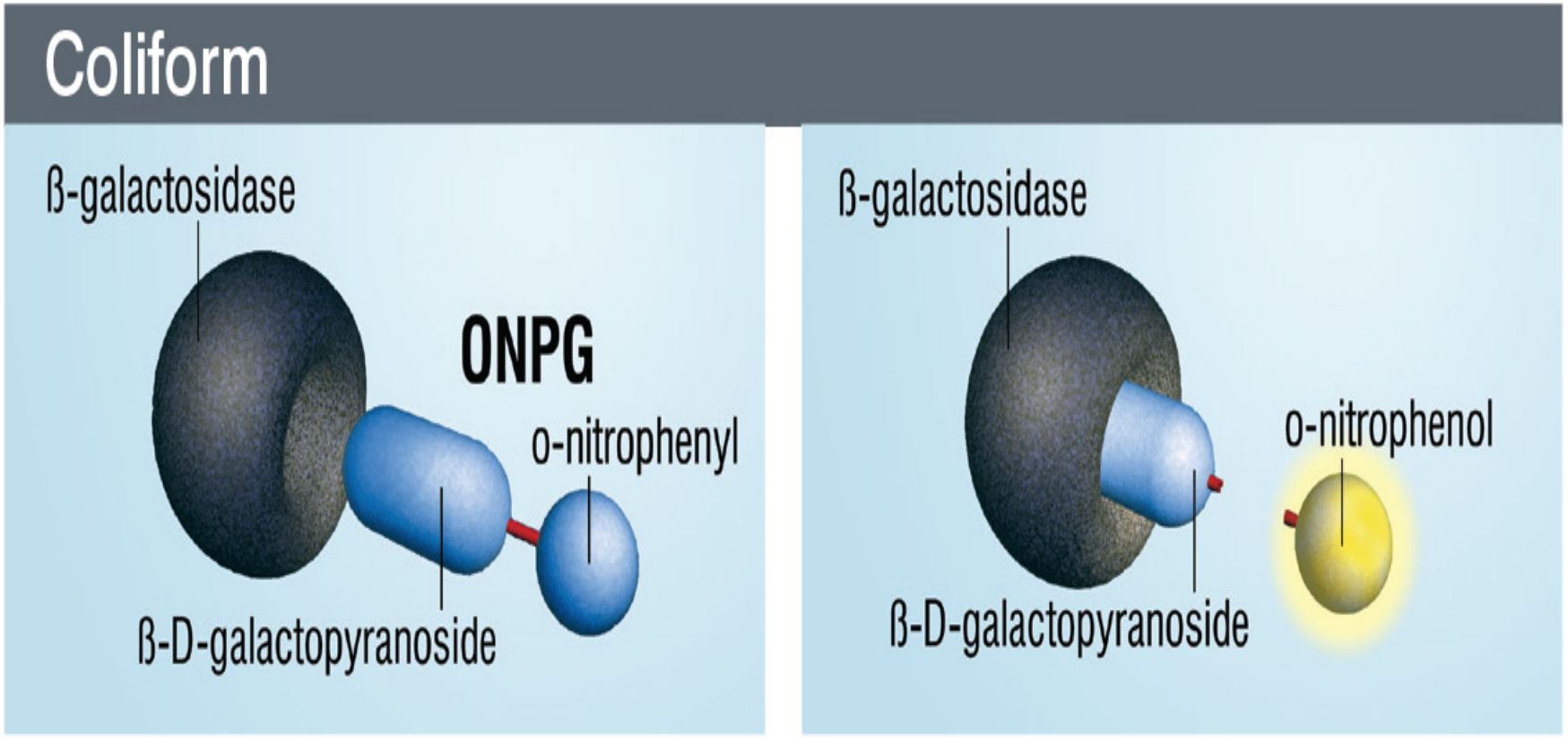


m-FC

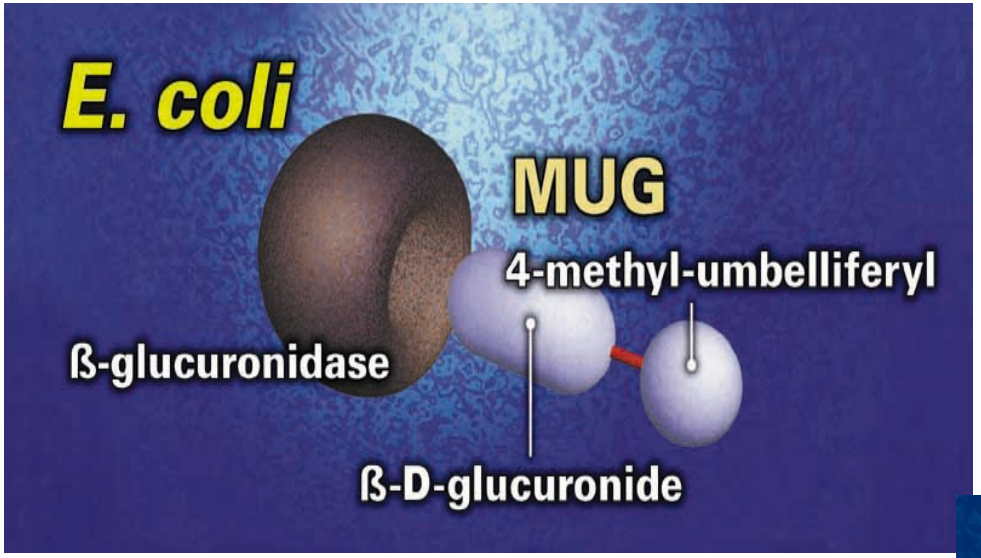


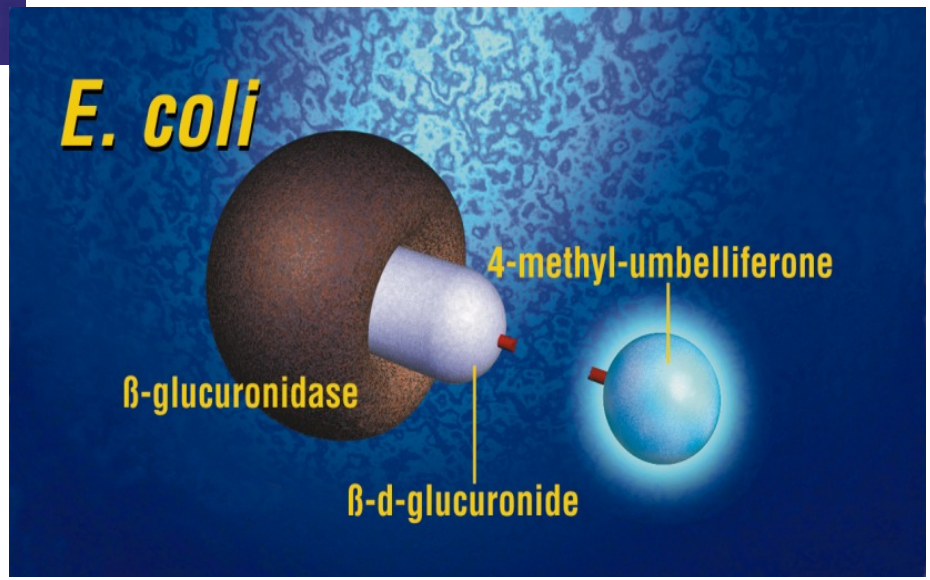
Defined Substrate Technology- Colilert or Colilert-18 and Quanti-Tray for Water and Waste Water

Coliform or Fecal Coliform Reaction



MUG Positive Reaction Colilert & Colilert-18





Quantification

Procedure for either P/A or Quantification

Blister Pack

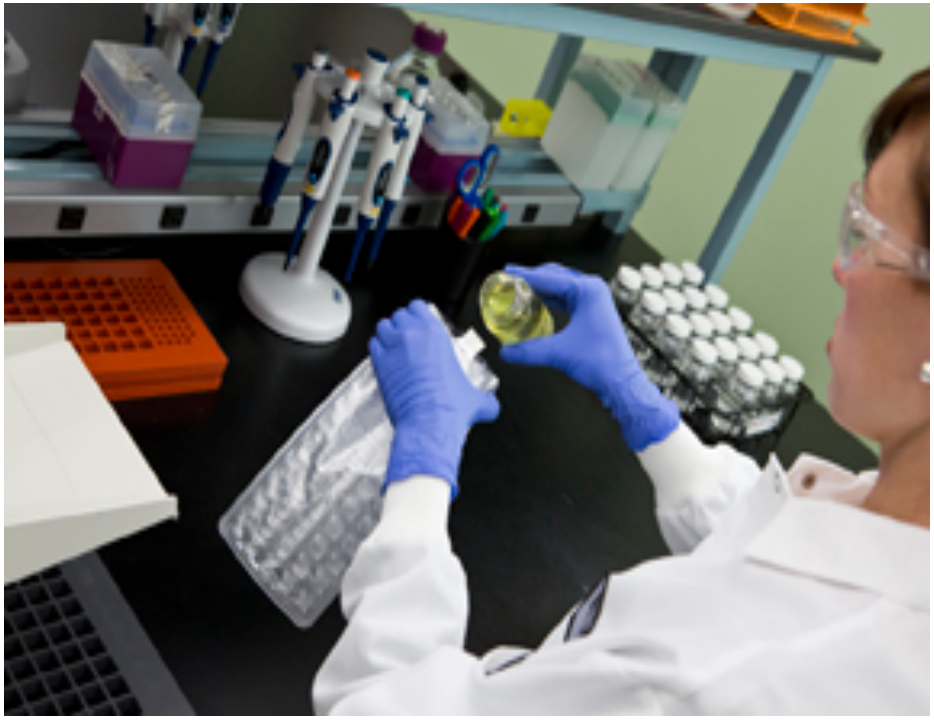
Add Reagent to Sample

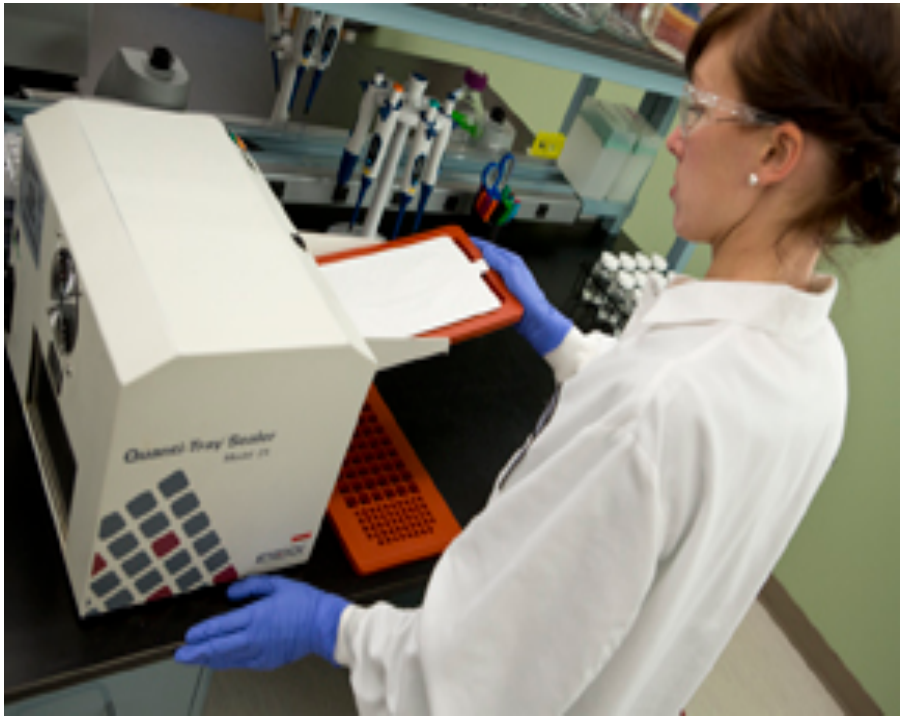
Mix well to Dissolve

Quanti-Trays

Quanti-Tray Sealer and Rubber Insert

Filling and Sealing Quanti-Tray for Quantification



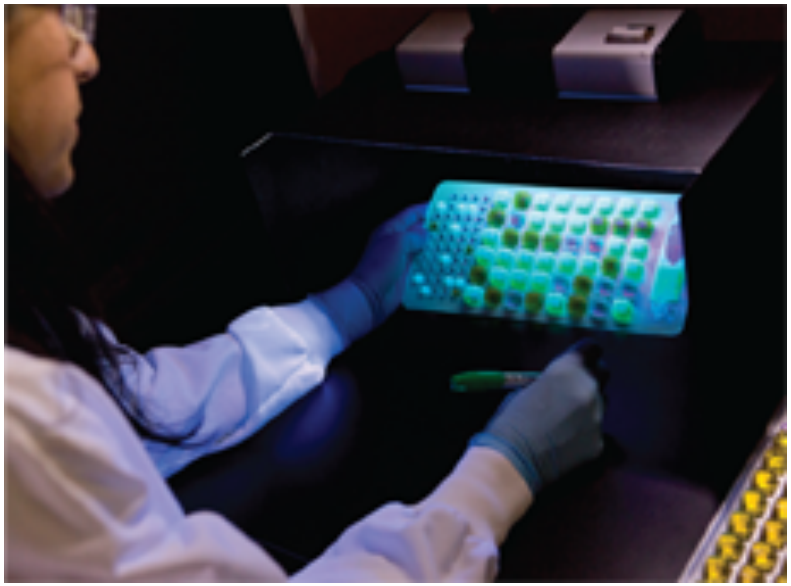


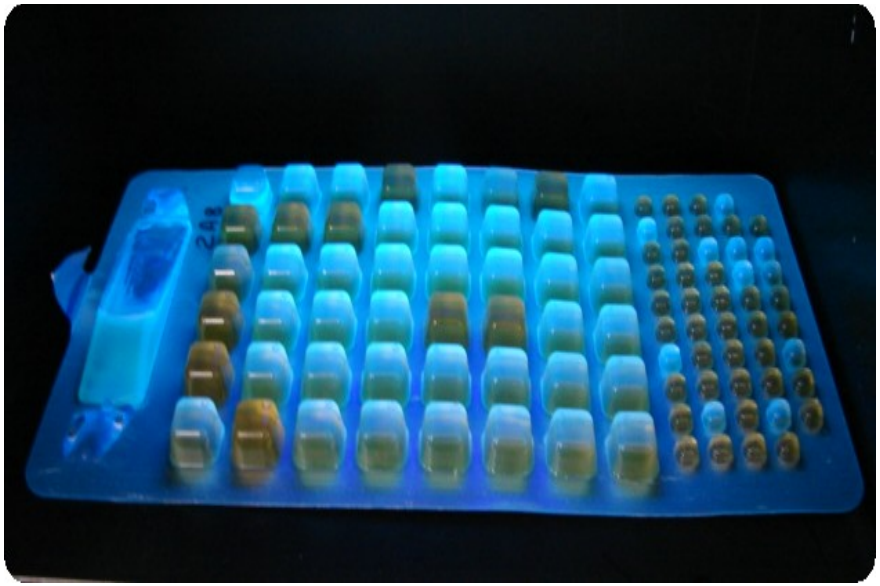
Incubate Samples 35ºC ± 0.5ºC for 18-22 hours for Colilert-18 or 24-28 hours for Colilert to Test for Total Coliforms and/or *E.coli*

Water Bath for Fecal Coliform Testing with Colilert-18 at 44.5±0.2ºC, 18-22 Hours

Positive Yellow Wells for Total Coliforms or Fecal Coliforms

E.coli- Blue Fluorescence- Quanti-Tray under a 365nm UV Light





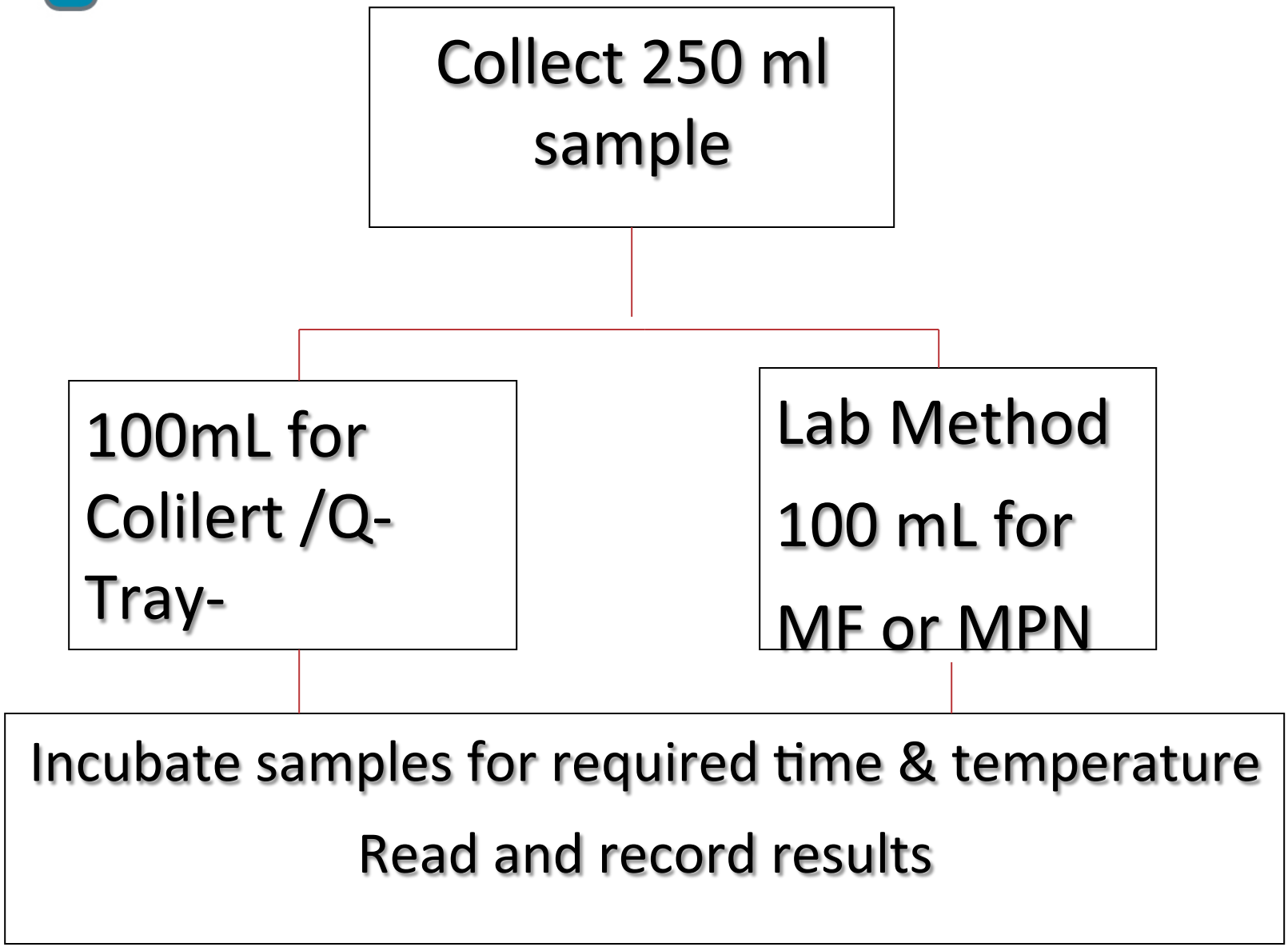
Quanti-Tray 51 MPN Table

No wells giving positive reaction	MPN per 100ml sample	95% Lower Confidence Limit	95% Upper Confidence Limit
0	<1	0	3.7
15	17.8	10.8	29.4
35	59.1	42	84.4
51	>200.5	146.1	infinity

Studies

- California - 5 WWTP
  - Site A
  - Site B (2 separate plants)
  - Site C
  - Site D
- Other States
  - Florida
  - Oregon
  - Idaho

Protocol for Comparing Methods and Review of Several Studies



California- Title 22 Requirement for Total Coliforms

- Total coliforms is the indicator bacteria required for testing reuse water
  - MTF 15 tube for total coliforms
  - 2.2/100 mL 7 day median (if 4 consecutive days exceed this, then 4th day is in violation)
  - 23/100 mL not to exceed in more than 1 sample in a 30 day period
  - 240/100 mL maximum for any one sample

Site A

- Secondary treatment for final effluent using chlorination
- Samples tested over a 2 month period with n = 33
- All samples were tested in duplicate
- Positive & negative QC controls tested weekly
- All positive controls were within assigned range
- Negative controls were negative

Site A 2X2 Table con't

All results	LTB-BGLB		
Colilert	+	-	Total
+	3	11	14
-	0	52	52
Total	3	63	66

Site A Tests Results Summary

LTB-BGLB

- 3 positives at 2/100 mL

Colilert

- 11 positives at 1/100 mL
- 3 positives at 2/100 mL

Site B

- Tertiary treatment for final effluent using UV at one plant and chlorination at the other plant
- Samples tested over a 2 month period at UV plant, n = 36
- Samples tested over a 3 month period at chlorine plant, n =51
- Positive QC controls tested weekly
- All positive controls were within assigned range

Site B 2X2 Table

UV Plant	LTB-BGLB		
Colilert	+	-	Total
+	10	1	11
-	0	25	25
Total	10	26	36

Chlorine Plant	LTB-BGLB		
Colilert	+	-	Total
+	10	7	17
-	0	34	34
Total	10	41	51

Site B Tests Results Summary UV Plant

LTB-BGLB

- 10 positives at 2/100 mL

Colilert

- 7 positives at 1/100 ML
- 3 positives at 2/100 mL
- 1 positive at 4.2/100 mL

Site B Tests Results Summary Chlorine Plant

LTB-BGLB

- 8 positives at 2/100 mL
- 2 positives at 8/100 mL

Colilert

- 10 positives at 1/100 mL
- 1 positive at 3.1, 5.2, 7.5/100 mL
- 4 positive at 4.2/100 mL

Site C

- Tertiary treatment for final effluent using UV
- Samples tested over a 3.5 month period with n = 45
- Positive QC controls tested weekly
- All positive controls were within assigned range

Site C 2X2 Table con't

UV	LTB-BGLB		
Colilert	+	-	Total
+	2	3	5
-	0	75	75
Total	2	78	80

Site C Tests Results Summary

LTB-BGLB

- 1 positive at 2/100 mL

Colilert

- 6 positives at 1/100 mL
- 1 positive at 6.4 mL
- 1 positive at 9.9/100 mL

Site D 2X2 Table con't

All results	LTB-BGLB		
Colilert	+	-	Total
+	0	0	0
-	0	25	25
Total	0	25	25

Site D

- Tertiary treatment for final effluent using chlorination
- Samples tested over a 2 month period with n = 25
- Positive spiked control diluted in reuse water tested weekly
- All positive controls were within assigned range

California Regulatory Update

- March 26,2014: Letter sent from DoH to ELAP requesting that:
  - "Consider recycled water as WW or SW for total coliforms sample methods
    - Disinfected secondary & tertiary recycled water, that meets title 22 standards are of a much higher quality than WW. Request ELAP to allow it to be tested for compliance for TC using test methods found in
      - 40 CFR 136
      - 40 CFR 141
    - Request was confirmed by ELAP
    - Approval was given to the facility by ELAP to use Colilert
    - Regional Water Board notified and granted approval

Evaluations – Other States

Florida- Aquifer Storage & Recovery- ASR

- Shallow sand aquifer (sand & gravel)
- Used as storage zone that contains fresh water but contains high levels of iron
- Used for irrigation by both residential and commercial users & golf courses
- Total coliforms is the indicator bacteria required for testing
  - Cannot have any TC present, <1/100 mL
  - No more than one positive/month, 2nd positive shut down
  - If 1 sample ≥4/100 mL, shut down

Florida- Aquifer Storage & Recovery- ASR

- Initially test method was MF, m-Endo
  - Problems with variability in method, sheen colonies not confirmed as coliforms in a number of cases
  - Results 24-72 hours with confirmation
- About 2 years ago; parallel testing with Colilert was performed.
  - NELAC state; required to do PT and parallels along with an audit
- State granted approval to use Colilert based on DW standard for total coliforms.
- Additional aquifers now on line and in future may be used for supplementing DW

Oregon & Idaho

- Parallel studies are in progress to obtain state approval
- Indicator is total coliforms
- Requirements equivalent to Title 22
- Results to date indicate no significant difference between methods
- Studies will conclude in August

Conclusions

- Parallel testing at these sites indicates no significant difference between the methods
- Results can be obtained in18 or 24 hours compared to 48-96 hours
- Data from the sites suggest that Colilert can be used for testing reuse water
- Florida site using it for the past 2 years
- Testing is ongoing at several sites

Thank You

Questions