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
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
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

TO CONSERVE WATER...
This facility uses recycled water to flush toilets and urinals.

Simi Valley, CA WWTP
Coliform Bacteria Group

Total Coliforms (35°C)

Fecal Coliforms - thermotolerant (44.5°C)

- Klebsiella
- E.coli

Citrobacter
Enterobacter
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

Coliform

β-galactosidase

β-D-galactopyranoside

ONPG

o-nitrophenyl

β-galactosidase

β-D-galactopyranoside

o-nitrophenol
MUG Positive Reaction Colilert & Colilert-18

E. coli

β-glucuronidase

MUG

4-methyl-umbelliferyl

β-D-glucuronide

E. coli

β-glucuronidase

4-methyl-umbelliferone

β-D-glucuronide
Quantification
Procedure for either P/A or Quantification

Blister Pack

Add Reagent to Sample
Mix well to Dissolve
Quanti-Trays

51 well Quanti-Tray

Quanti-Tray 2000
Quanti-Tray Sealer and Rubber Insert
Filling and Sealing Quanti-Tray for Quantification
Incubate Samples 35°C ± 0.5°C 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±0.2°C, 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

<table>
<thead>
<tr>
<th>No wells giving positive reaction</th>
<th>MPN per 100ml sample</th>
<th>95% Lower Confidence Limit</th>
<th>95% Upper Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;1</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>15</td>
<td>17.8</td>
<td>10.8</td>
<td>29.4</td>
</tr>
<tr>
<td>35</td>
<td>59.1</td>
<td>42</td>
<td>84.4</td>
</tr>
<tr>
<td>51</td>
<td>&gt;200.5</td>
<td>146.1</td>
<td>infinity</td>
</tr>
</tbody>
</table>
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
Collect 250 ml sample

100mL for Colilert /Q-Tray-

Lab Method
100 mL for MF or MPN

Incubate samples for required time & temperature
Read and record results
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 cont'

<table>
<thead>
<tr>
<th>All results</th>
<th>LTB-BGLB</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert</td>
<td>+</td>
<td>-</td>
<td>Total</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>63</td>
<td>66</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>UV Plant</th>
<th>LTB-BGLB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chlorine Plant</th>
<th>LTB-BGLB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>41</td>
</tr>
</tbody>
</table>
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
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

<table>
<thead>
<tr>
<th>UV</th>
<th>LTB-BGLB</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert</td>
<td>+</td>
<td>-</td>
<td>Total</td>
</tr>
<tr>
<td>+</td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>78</td>
<td>80</td>
</tr>
</tbody>
</table>
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 cont'

<table>
<thead>
<tr>
<th>All results</th>
<th>LTB-BGLB</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colilert</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>
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 in 18 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