E. COLI ANALYSIS

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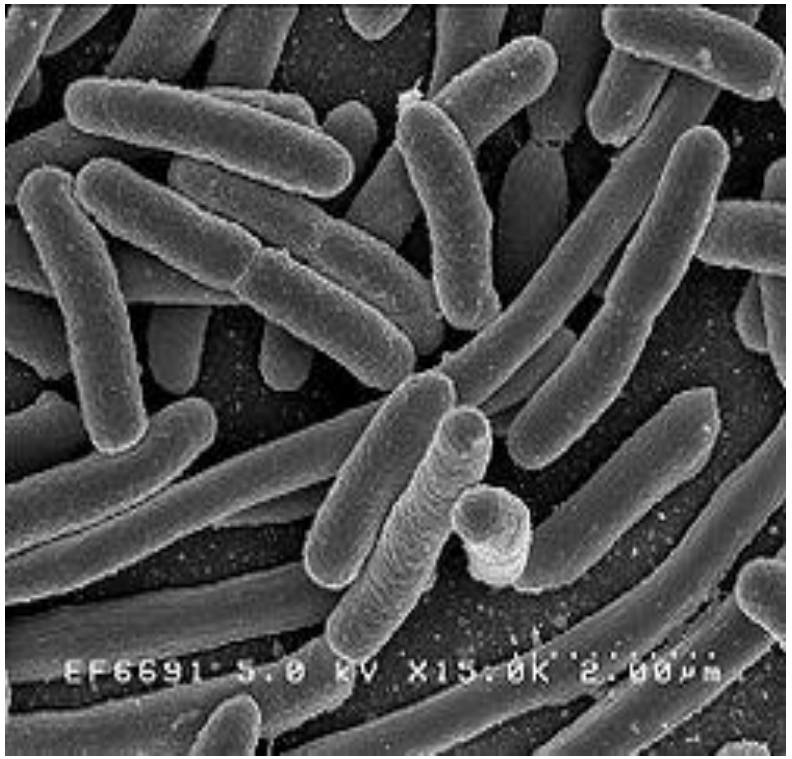
Overview

- Background and Why *E. coli*
- Method options
- Membrane Filtration methods
- *E. coli* verification procedure (MF only)
- Most probable number methods
- Calculation of duplicates

Background

- *E. coli* is an indicator bacterium for fecal contamination
- There are hundreds of different *E. coli* strains
  - Most are harmless and reside in the intestinal track
    - Protecting host organisms from pathogenic bacteria
    - Produce vitamin K_2



- However, *E. coli* 0157:H7 produces a harmful toxin
  - Can cause diarrhea and abdominal cramps
  - In children, elderly, and people with immunocompromised systems it can be life threatening

- Sources of *E. coli*
  - Mainly from human and animal waste
    - Washed in to rivers, lakes and ground water via rainfalls, snow melts, etc.

Why *E. coli* and why now?

- A study by Dufour showed a statistically significant relationship between *E. coli* and enterococci concentrations in freshwater and rates of swimming- related illness
  - There was no relationship between fecal coliform numbers and swimming-related illnesses
- Also, in tropical and subtropical aquatic systems many fecal coliforms can grow and multiply
  - Bad because less helpful as an inicator

Dufour, A.P.,1984, Health effects criteria for fresh recreational waters: Cincinnati, Ohio, U.S. Environmental Protection Agency, EPA-600/1-84-004, 33p.

Options for analysis

- Membrane Filtration
  - mTEC
  - Modified mTEC
  - m-ColiBlue24®
- Most Probable Number
  - Quanti-Tray®
  - Multiple tube test

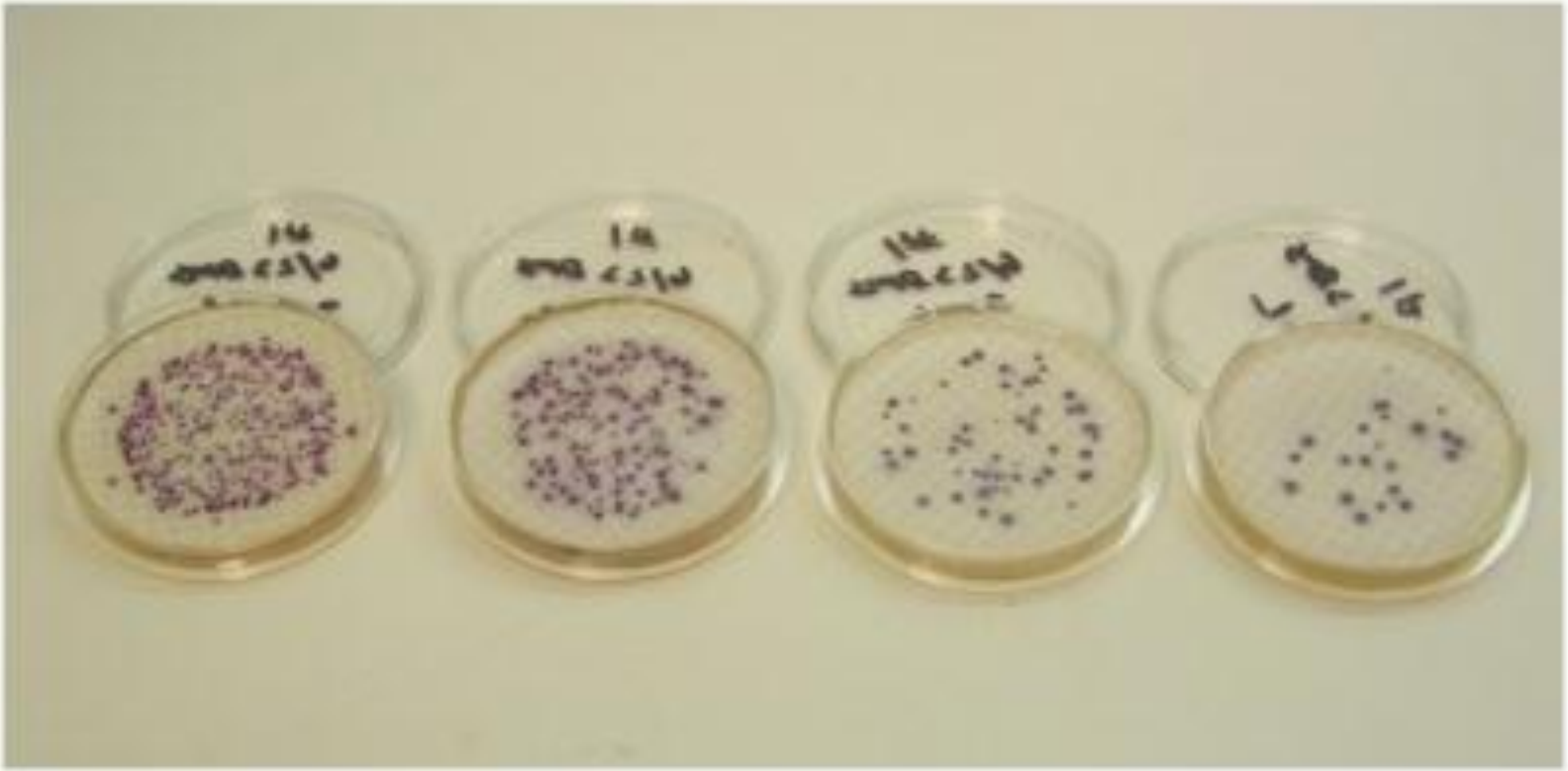
Membrane Filtration

Option One: m-TEC

- Two step procedure
  - Test incubation at 44.5°C
  - Urea confirmation
- Final colony color: yellow
- Intermediate incubation at 35°C

Option Two: Modified mTEC

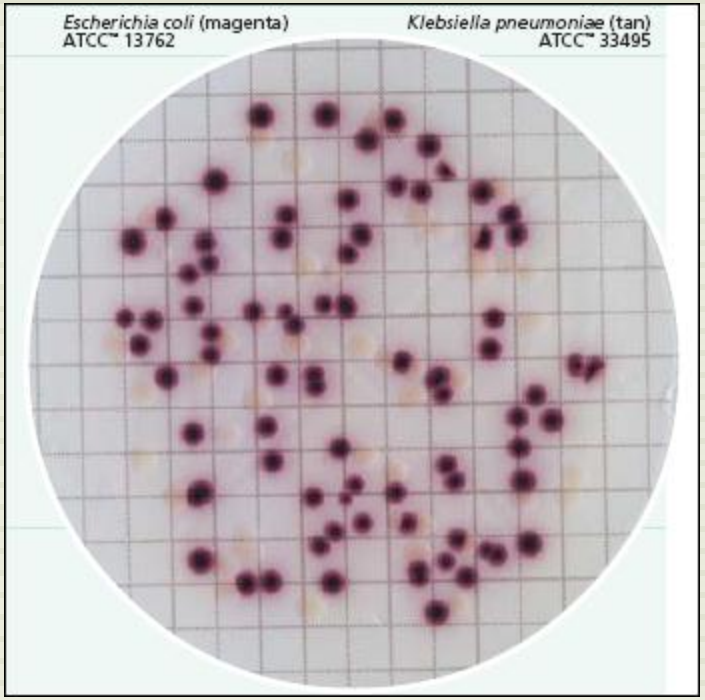
- Single step procedure
  - Test incubation at 44.5°C
- Final colony color: red or magenta
- Intermediate incubation at 35°C
- Utilizes a chromogen which is specific to the detection of *E. coli*



Dilution series of mod mTEC

E. Coli colonies on Modified mTEC agar

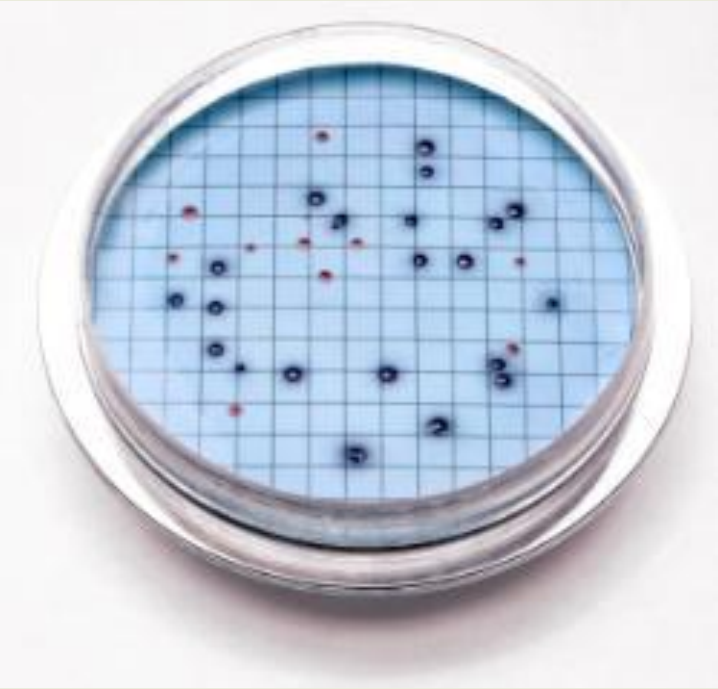
Colonies appear red or magenta



Close up of *E. coli* colonies on Modified mTEC

Option Three: m-ColiBlue24

- Single step procedure
  - Test incubation at 35°C
- *E. coli* colonies, blue
- Total coliform colonies, red
- Simultaneous detection due to
  - Specific non-coliform growth inhibitors
  - Selective enzymatic indicator
- Problem
  - Certain *E. coli* strains respond differently
    - *E. coli* 0157:H7 is beta-glucuronidase negative and will only produce red colonies, no blue



Close up of *E. coli* and total coliform colonies on m-ColiBlue24

E. coli colonies are blue and total coliform colonies are red

Membrane Filtration Procedure

Test Preparation

- Label agar filled plates
  - Sample identity
  - Dilution to be used, need 3 per sample
    - Suggested dilutions are 0.1, 1.0 and 10mL
    - Colony range of 20-80cfu
- Quality Control dishes
  - Blank, beginning and end
  - Duplicate of a sample, every 10 samples
  - Positive control at the very end

	Volume of Sample to be Filtered (mL)							
Water Source	**100**	**50**	**10**	**1**	**0.1**	**0.01**	**0.001**	**1E-04**
Drinking water	X							
Swimming pools	X							
Wells, springs	X	X	X					
Lakes, reservoirs	X	X	X					
Water supply intake			X	X	X			
Bathing beaches			X	X	X			
River water				X	X	X	X	
Chlorinated sewage				X	X	X		
Raw sewage					X	X	X	X

Other Recommended Sample Dilutions

Table courtesy of Standard Methods 18th edition, table 9222:III from method 9222D; 1992 pg 9-60

Apparatus Set Up

- Sterilize forceps
- Place sterile membrane on filter base, grid side up
- Attach the funnel to the base, membrane should be between the base and funnel
- Connect all up to a vacuum with a sample waste catch basin

Analysis

- Sample addition
  - Pre wet filters with sterile rinse water, about 20mL
  - Shake sample vigorously, counting 25 full inversions
    - Ensures a uniform distribution of the bacteria
  - Pipette out previously determined dilutions into funnels
    - Do NOT pipette by mouth
- Sample dilutions can be adjusted to minimize interferences of turbidity or for high bacterial densities
- Filter sample, rinse funnel sides with about 20mL of sterile rinse water

Analysis: Post Filtering

- Turn off vacuum and remove funnel from base
- With sterile forceps move the membrane from the filter base to the plate
  - Use a rolling motion when transferring to avoid bubbles, reseat if bubbles occur
  - Move forceps along filter edge to ensure filter is properly seated

Incubation

- Close plates and place in a Whirl-Pak®
- Incubate at 35.0 ± 0.5˚C
  - Modified mTEC for 2hrs
  - m-ColiBlue for 24hrs
- If Modified mTEC move to waterbath at 44.5 ± 0.2˚C for 22-24hrs
- After 22-24hrs, remove the plates from the waterbath
- Count the colonies and report results
  - Plates with colonies exceeding 200 are to identified as Too Numerous To Count (TNTC)

Final Calculations

- *E. coli* results are calculated the same as fecal coliform
  - colonies of bacteria per 100mL

$$\text{E. coli/100mL} = \frac{\text{Number of E. coli colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

Counting Rules

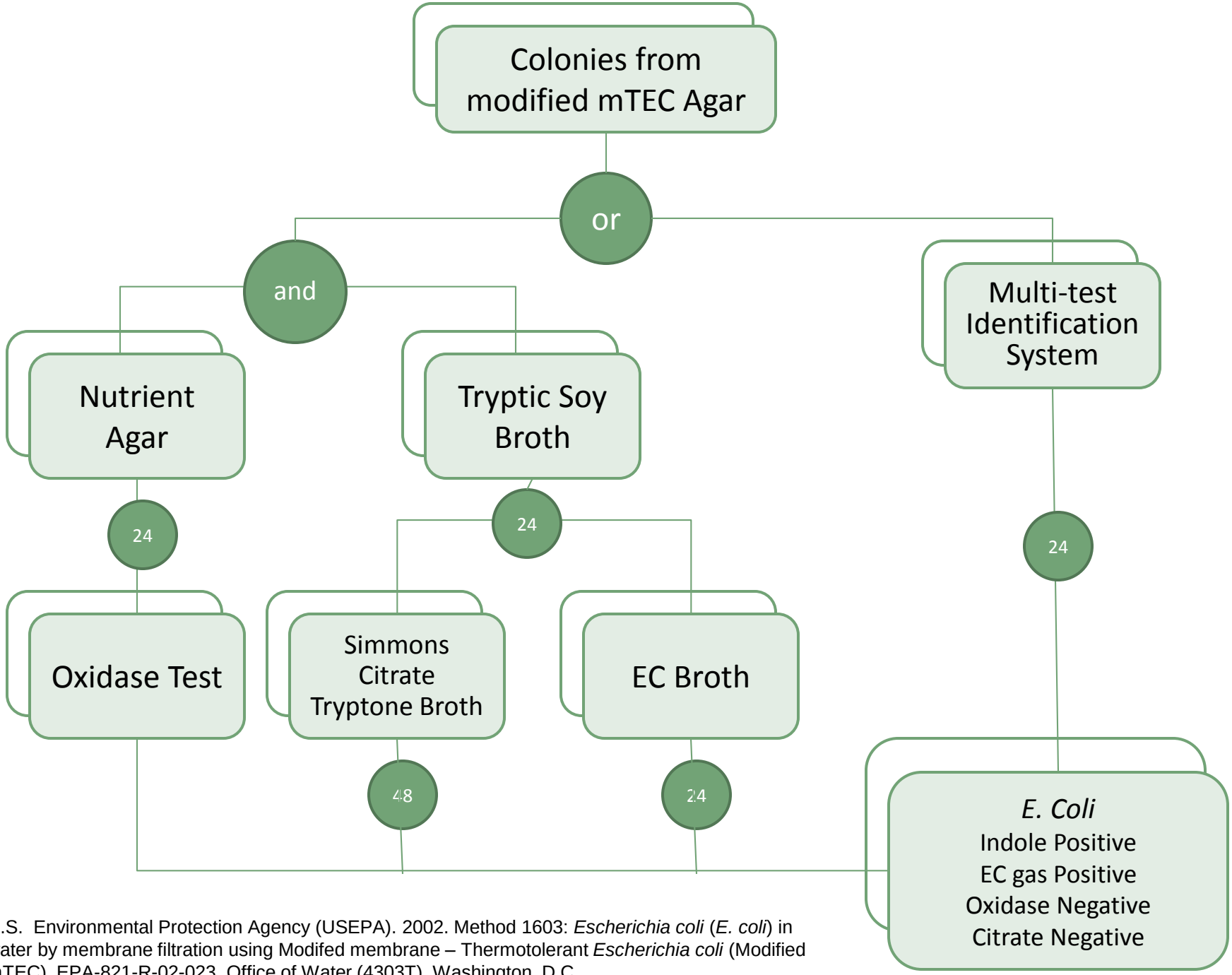
- If all counts are 0: Use a count of 1 for the plate with the largest volume of sample and calculate and report at <
- If all counts are TNTC (too numerous to count): Use the upper ideal limit of the bacteria being tested as the count for the plate with the smallest volume of sample filtered. Calculate and report as >

- If more than one plate has a count in the acceptable range: Calculate CFU/100 ml for each and average the results of each plate in the acceptable range
- If all counts are below the acceptable range: Use the plate with the most nearly acceptable count and calculate
- If all counts are above the acceptable range: Use the plate with the smallest volume of sample and calculate

- If some counts are above and some counts are below but none are in the acceptable range: Add the number of colonies on the plates with the counts below the acceptable range and those on plates with counts above the range (except TNTC) and add the total volume of sample for the plates below and the plates above (except TNTC) and calculate

Verification Procedure

US EPA recommended as a means of quality control for the initial use



U.S. Environmental Protection Agency (USEPA). 2002. Method 1603: *Escherichia coli* (*E. coli*) in water by membrane filtration using Modifed membrane – Thermotolerant *Escherichia coli* (Modified mTEC). EPA-821-R-02-023. Office of Water (4303T), Washington, D.C.

Positive for *E. coli*

- Oxidase: No color change
- Simmon Citrate: Media remains green
- Indole: Alcohol layer turns deep red
- EC: Gas bubble present in smaller vial

Most Probable Number

Quanti-Tray® by IDEXX

- Quanti-Tray® two types
  - Quanti-Tray® /2000: 97 wells, counts 2419 per 100mL
    - 49 large and 48 small
  - Quanti-Tray® : 51 wells, counts 200 per 100mL
    - 51 wells total
- Reagent Colilert® or Colilert-18®
  - Yellow wells: Total coliform
  - Yellow and fluorescent wells: *E. coli*
- 24 or 18 hour incubation at 35.0 ± 0.5˚C

Media Quality Control

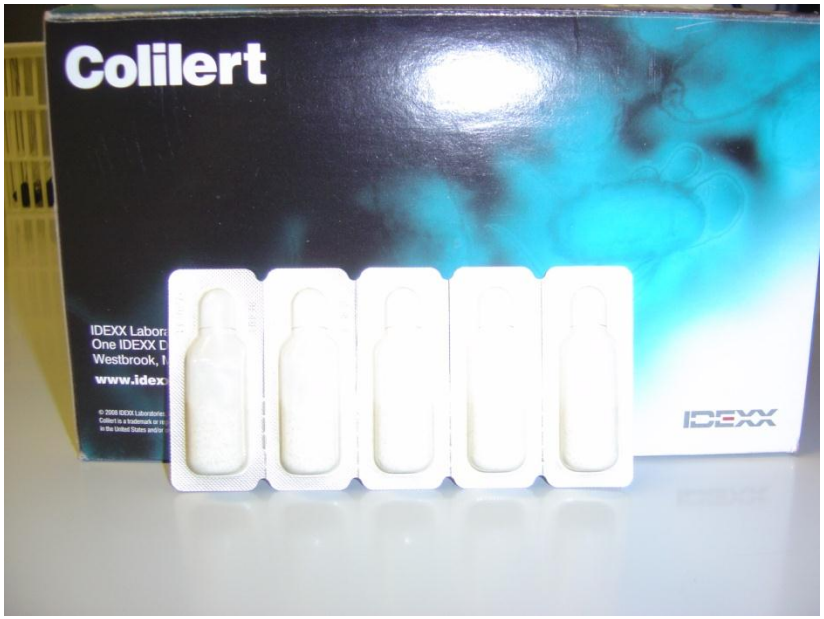
- Colilert® reagent must be checked prior to use
- Use known control bacteria to obtain the associated results
  - *Escherichia coli* - yellow (total coliform positive) and fluorescence (*E. coli* positive)
  - *Klebsiella pneumoniae* – no color, no fluorescence
  - *Pseudomonas aeruginosa* – yellow, no fluorescence

Media QC Procedure

- Aseptically fill three test vessels with 100mL sterile deionized or distilled water
- Inoculate each of the test vessels with one of the three control bacteria, see manufacture instructions
- Add a tube of the Colilert® reagent to each vessel and mix to dissolve
- Incubate at 35.0 ± 0.5˚C for 24 hrs

Procedure

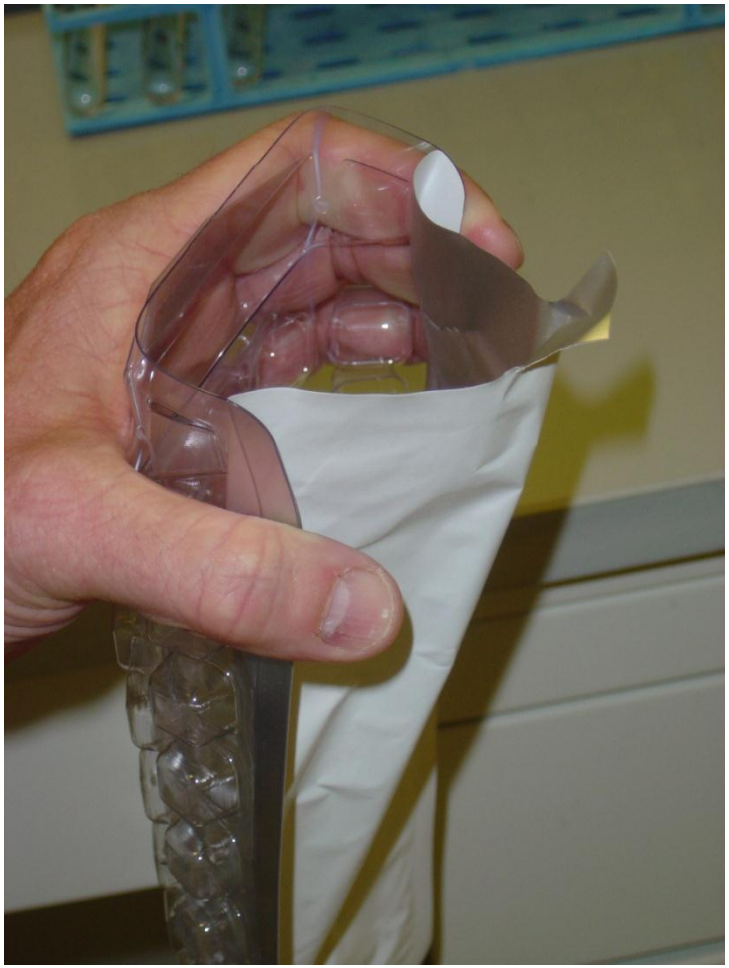
Sample Preparation



- Aseptically adjust the sample volume down to 100mL
- Add one packet of Idexx Colilert® reagent to each sample bottle
- Set up and analyze duplicate

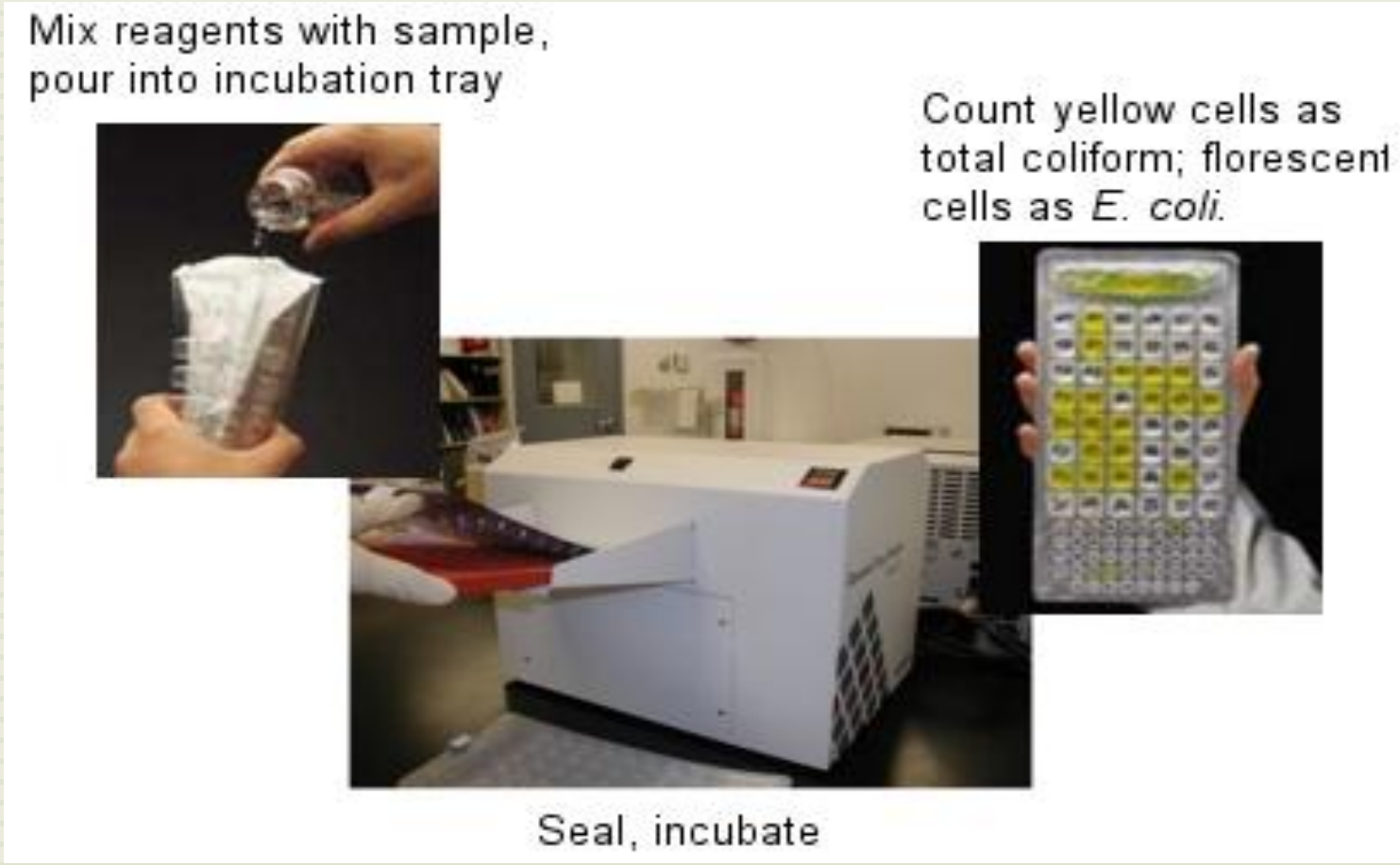
Q-Tray Preparation

- Turn on Quanti-Tray® sealer, allow to heat up
- Holding the Quanti-Tray® upright, well side to palm, squeeze upper part till the tray bends
- Gently pull foil tab to separate the foil from the tray
  - Avoid touching the inside
- Pour prepped sample into tray and tap out bubbles
  - A top layer of foam may remain



Processing Q-Tray

- Join tray and rubber holder together and place well side down on Quanti-Tray® sealer
- Spread sample through out Quanti-Tray® by running your hand over the foil before sealing
- Run tray trough sealer and remove sealed tray from opposite side
- Incubate trays well side down at 35.0 ± 0.5˚C for 24 hours

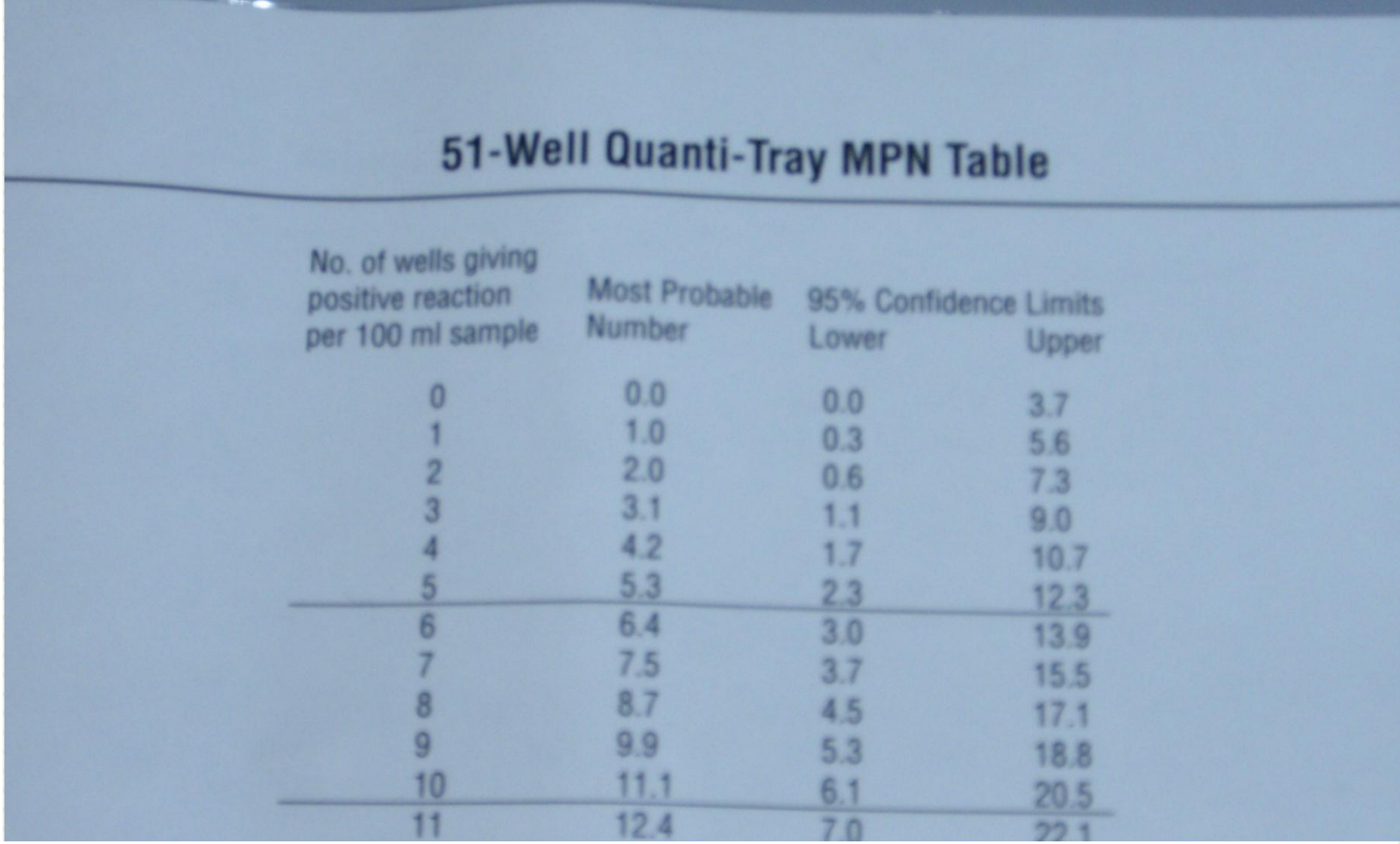


Quanti-Tray sample tray and sealer

Pictures courtesy of Idexx Corp. , Westbrook, Maine; Colilert Quanti-Tray/2000®

Post Incubation

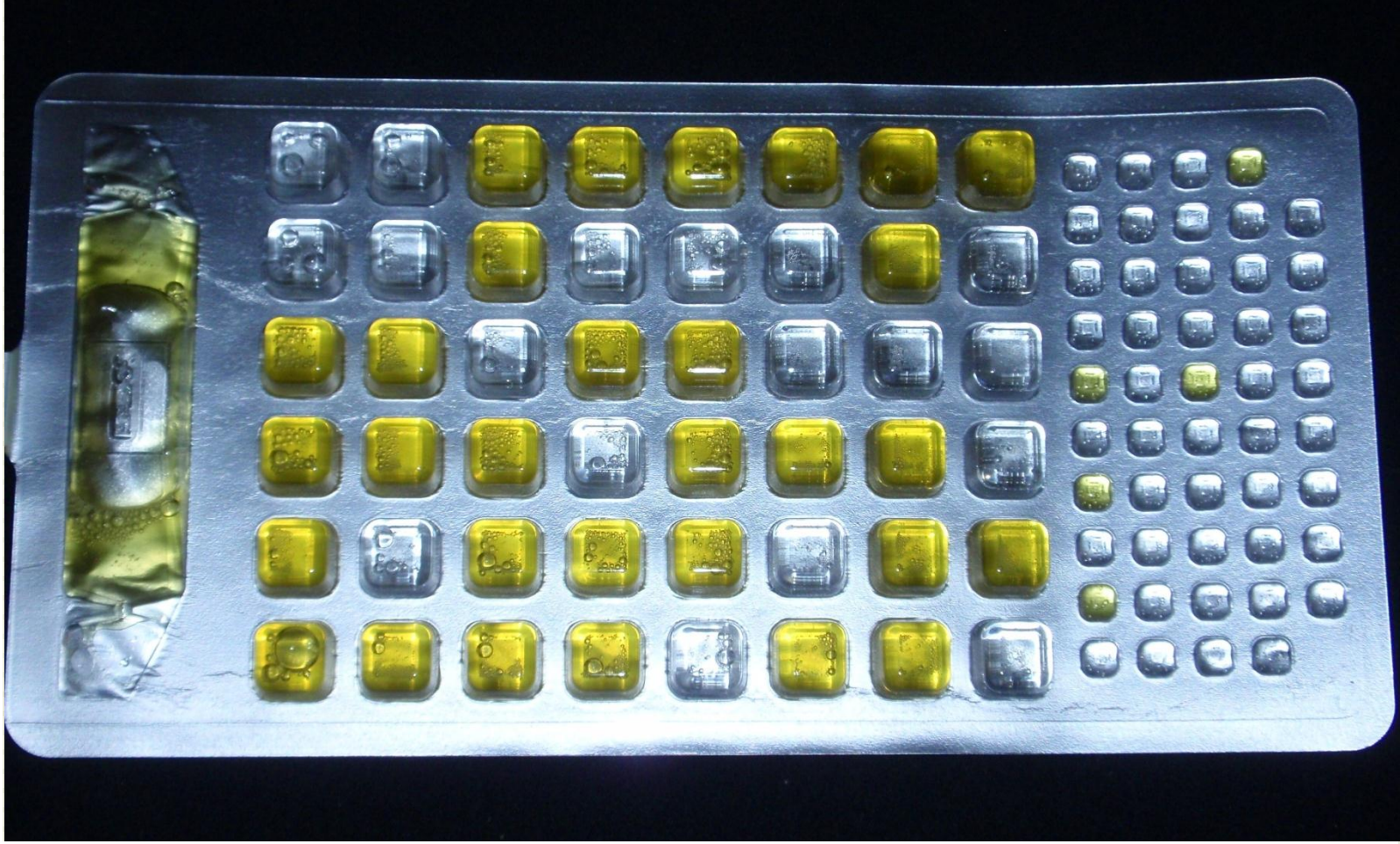
- Remove trays from incubator and using a UV light count the number of florescent wells
  - Yellow wells show the presence of total coliform
  - Only yellow wells that fluoresce have *E. coli*
  - Intensity of color and fluorescence will vary
- Once counted refer to the Quanti-Tray® MPN table
- Dispose of media in accordance with good laboratory practices



51-Well Quanti-Tray MPN Table

No. of wells giving positive reaction per 100 ml sample	Most Probable Number	95% Confidence Limits Lower	Upper
0	0.0	0.0	3.7
1	1.0	0.3	5.6
2	2.0	0.6	7.3
3	3.1	1.1	9.0
4	4.2	1.7	10.7
5	5.3	2.3	12.3
6	6.4	3.0	13.9
7	7.5	3.7	15.5
8	8.7	4.5	17.1
9	9.9	5.3	18.8
10	11.1	6.1	20.5
11	12.4	7.0	22.1

Quanti-Tray Most Probable Number Table



Sample

Sample

Florescence shows *E. coli*

Duplicate Recovery

Applicable to all microbiology methods

- Logarithmic calculation allows for greater variability between duplicates than the standard %RPD method of ± 20%
- Analyze a duplicate
  - 10% of sample run
  - Minimum of 1 per week, if <10 per week
- Excel file will note “OUT” in OK? column if duplicate exceeds the control limit
  - Identify and resolve the analytical problem before making further analyses.

Initial Demonstration

- Analyze duplicates for the first 15 positive samples
- Record as Dup Value 1 and Dup Value 2
- Input into Excel to calculate the log difference of each result
  - If either results is zero add 1 to both values before calculating
- Program will calculate the average of the log difference and then multiply it by 3.27 to determine the control limit to be used for the next set of 15 duplicates

Continued Duplication

- Update the active control limit
  - Based off of the previously used 15 sample duplicates
- Standard Methods 18th Edition, Section 9020 B, pg 9-10

Historical
Modified mTEC
Field Duplicate Correlation

Log Diff	
Average:	0.0644
Standard Dev:	0.0354
Control Limit:	0.2106

#	Date	Dup Value 1	Dup Value 2	Log Difference
1	9/28/2009	270	390	0.1597
2	9/28/2009	36000	39000	0.0348
3	9/30/2009	320	270	0.0738
4	10/1/2009	230	260	0.0532
5	10/7/2009	270	240	0.0512
6	10/7/2009	340	310	0.0401
7	10/20/2009	280	350	0.0969
8	10/26/2009	220	260	0.0726
9	10/29/2009	2400	2500	0.0177
10	3/9/2010	250	220	0.0555
11	3/9/2010	230	270	0.0696
12	5/18/2010	690	600	0.0607
13	5/20/2010	3700	2900	0.1058
14	6/1/2010	210	230	0.0395
15	6/8/2010	650	600	0.0348
16				
17				
18				
19				
20				

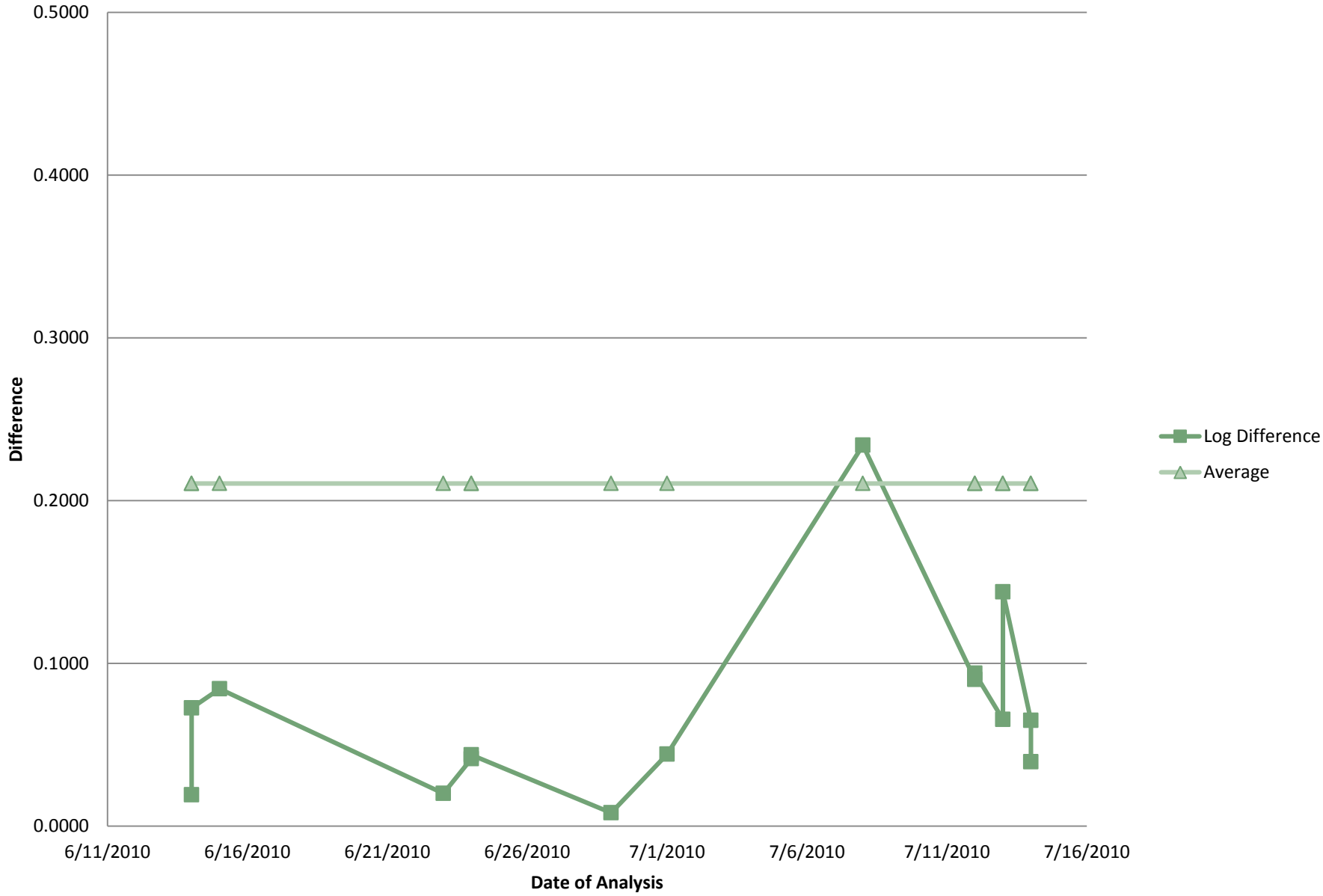
Active

Modified mTEC

Field Duplicate Correlation

	Average	Control Limit
	0.0711	0.2106
Previous 15 pts	0.0644	

#	Date	Dup Value 1	Dup Value 2	Difference	OK?	Log Difference	Control Limit
1	6/14/2010	220	230	10		0.0193	0.2106
2	6/14/2010	220	260	40		0.0726	0.2106
3	6/15/2010	2800	3400	600		0.0843	0.2106
4	6/23/2010	220	210	10		0.0202	0.2106
5	6/24/2010	200	220	20		0.0414	0.2106
6	6/24/2010	660	730	70		0.0438	0.2106
7	6/29/2010	530	520	10		0.0083	0.2106
8	7/1/2010	280	310	30		0.0442	0.2106
9	7/8/2010	210	360	150	OUT	0.2341	0.2106
10	7/12/2010	3200	2600	600		0.0902	0.2106
11	7/12/2010	290	360	70		0.0939	0.2106
12	7/13/2010	500	430	70		0.0655	0.2106
13	7/13/2010	2800	3900	1100		0.1439	0.2106
14	7/14/2010	310	360	50		0.0649	0.2106
15	7/14/2010	2100	2300	200		0.0395	0.2106


Field Duplicate Correlation Chart Modified mTEC
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
Difference
6/11/2010
6/16/2010
6/21/2010
6/26/2010
7/1/2010
7/6/2010
7/11/2010
7/16/2010
Date of Analysis
Log Difference
Average

Summation

- Two types of methods available
  - Membrane filtration (Modified mTEC and m-ColiBlue24®)
  - Most Probable Number (Quanti-Tray®)
- Modified mTEC single step process, similar to fecal analysis
- m-ColiBlue24® single step process and single incubation
- Quanti-Tray® pricey start up but saves time and colony questioning

Media/Method	Initial Start up Cost	Cost per Test
Modified mTEC (EPA 1603)	$660	$4.42
m-ColiBlue24® (Hach 10029)		$2.65
Colilert® (Idexx)	$3,600	$6.72

Crane, R., Jackson,C.,Sedlacek, R., and Walker, D. EPA Approves New Test Procedures for the Analysis of Microbiological Pollutants in Wastewater and Sludge. Hach Company, Loveland , CO. www.hach.com

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