Testing Waste Water for Fecal Coliforms and/or *E.coli* using Colilert® and Colilert®-18 & Quanti-Tray®

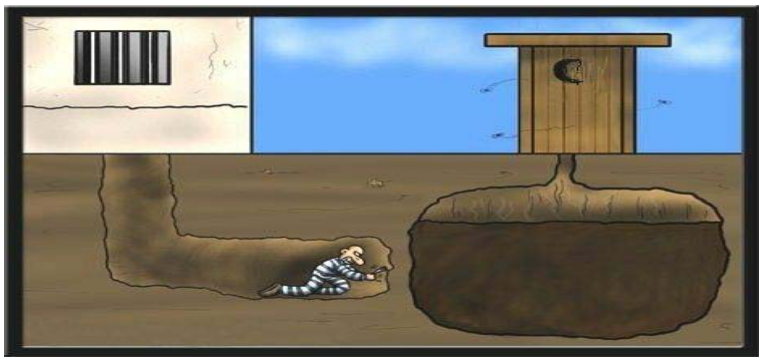
Gil Dichter

World Wide Technical Support Manager, Water

www.idexx.com/water

IDEXX

FOR ALL OF YOU WHO ARE HAVING A BAD DAY...



IDEXX

OBJECTIVES

- Define total coliforms, fecal and E.coli
- Colilert & Colilert-18 for WW
  - Theory
  - How to test
  - Reading results
  - Understanding MPN theory and relationship to MF
  - QC/QA
- Q&A

Bacteria Hierarchy

- Family
- Genus (type of bacteria)
- Species

- Enterobacteriaceae
- Escherichia, Klebsiella, Citrobacter
- E.coli, E. hermanii,
- K. pneumoniae,
- C. freundii

Coliform Genera

- Escherichia- human and animal feces
- Enterobacter- environment, feces
- Klebsiella- environment, feces
- Citrobacter- environment
- Serratia- environment

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Thermotolerant Coliforms [aka Fecal Coliforms]

- It is a subset of total coliforms
- Defined as coliform bacteria that can grow at 44.5°C
- Consists of the following coliforms:
  - *E. coli*
  - *K. pnuemoniae*
  - *Enterobacter spp.*

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Escherichia coli

- Named after the German Scientist who found this bacteria- Dr. Escherich
- A genus of Gram negative bacteria of the family Enterobacteriaceae
- A type of thermotolerant coliform bacteria commonly found in the intestines of warm blooded animals including humans
- Does not occur naturally in soil and vegetation
- May occur in soil and water as a result of fecal contamination

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E. coli (non-pathogenic)

- **Major inhabitant of gastrointestinal tract in warm-blooded animals, birds and humans**
- **Shed in feces at high levels**
- **Thus, indicator of true fecal contamination**
- **Most common *E. coli* detected in water**

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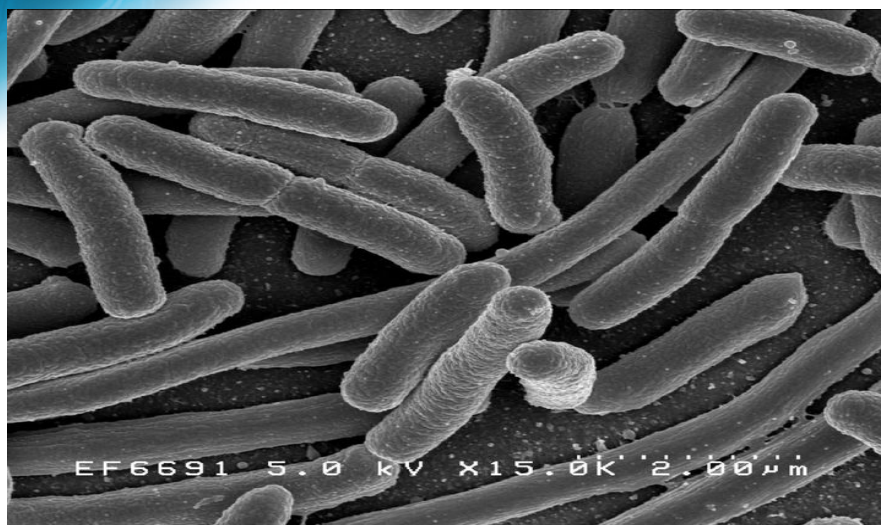
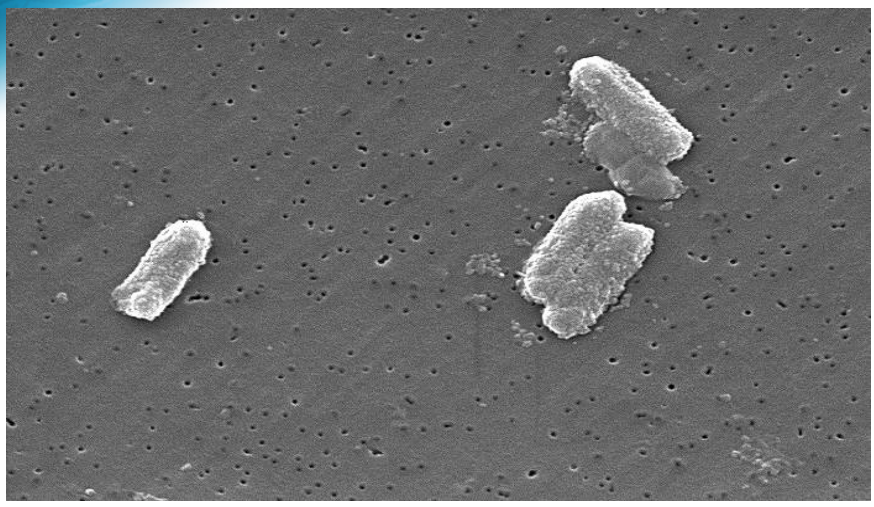
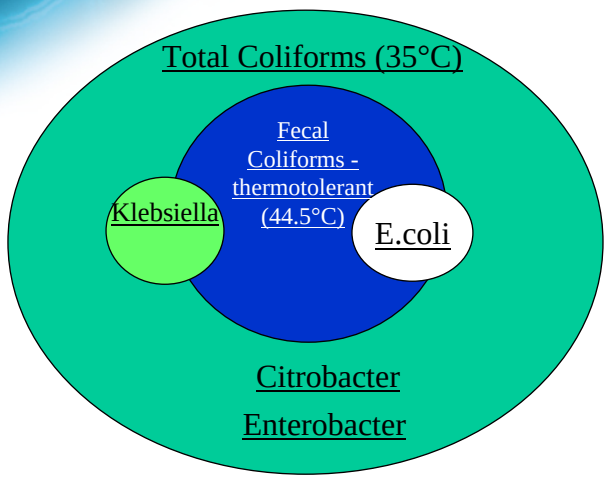
E. coli



Citrobacter freundii



Coliform Bacteria Group



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Definitions of Coliforms & E.coli

- Standard Methods for the Examination of Water and Wastewater, 21st Edition
  - **9223 Enzymatic:** The total coliform group is defined as all bacteria possessing the enzyme β-D galactosidase cleaving the chromogenic substrate resulting in the release of the chromogen.
  - *E.Coli* is defined as giving a total coliform response and possessing the enzyme β-D glucuronidase, cleaving the fluorogenic substrate resulting in the release of the fluorogen.

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Why Test for *E.coli*

Animal	# Tested	*E.coli*	Klebsiella spp	Enterobacter/ Citrobacter
Human	26	96.8%	1.5%	1.7%
Cow	15	99.9	-	0.1
Horse	3	100	-	-
Sheep	20	97	-	3
Pig	15	83.5	6.8	9.7
Average		94.5%		

Source:*E.coli*:Fecal Coliform A.P. Dufour. Special Technical Publication 65, ASRM. Pp48-58, 1977

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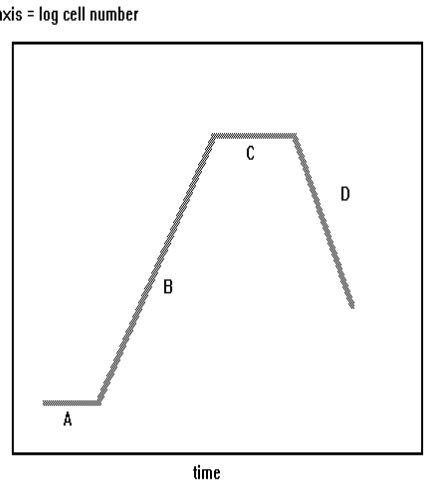
Standard Bacterial Growth Curve

- (A) Lag Phase
- (B) Log or Exponential Phase
- (C) Stationary Phase
- (D) Decline (Death) Phase



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Commonly Used Indicator Bacteria for Water Testing

- Most commonly used:
  - Total coliform
  - Fecal coliform
  - *E. coli*
  - *Enterococci*
- Total coliform used for >100 years
- Fecal coliform used for >80 years
- *E. coli* >20 years

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Indicator vs Specific Pathogen Testing

- In a perfect world we would test for all the pathogens in real time
- Technology not available
- Too expensive
- Too time consuming to safeguard against unknown or emerging pathogens

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Requirements for an Indicator Organism

- Present when pathogens are present
- Absent in uncontaminated waters
- Present in higher numbers than pathogens in contaminated water
- Better survival in water than pathogens
- Easy and Safe to analyze
- Rapid results
- Inexpensive
- Accurate

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Guidelines for Analysis of Pollutants; Analytical Methods for Biological Waste Water

- Federal Register Notice- August 16th, 2005
  - Comments on proposal ended on October 17th, 2005
  - Promulgated -March 26th,2007
  - Effective Date-April 25th, 2007
- Testing for E.coli and Enterococci added
  - Approved methods include Colilert, Colilert-18 and Enterolert and several MF methods
  - E.coli for fresh surface waters and enterococci for marine waters

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Defined Substrate Technology- Colilert & Colilert-18

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Product Approvals/Acceptances Worldwide*

ORGANIZATIONS

- American Society for Testing and Materials
- Association of Official Analytical Chemists International
- Food and Drug Administration
- *Standard Methods for the Examination of Water and Wastewater* (APHA-AWWA-WEF)
- United States Military
- The United States Pharmacopeia
- World Health Organization
- European Bottled Watercooler Association
- International Bottle Water Association

COUNTRIES

- Argentina
- Australia
- Belgium
- Brazil
- Canada
- Chile
- China
- Columbia
- Costa Rica
- Czech Republic
- Denmark
- El Salvador
- Germany
- Greenland (Denmark)
- Hungary
- Iceland
- Ireland
- Italy
- Japan
- Mexico
- New Zealand
- Norway
- Panama
- Philippines
- Poland
- Slovakia
- South Africa
- South Korea
- Spain
- Switzerland
- Taiwan
- UK
- Ukraine
- USA
- Zimbabwe

www.idexx.com/water

Easy. Rapid. Accurate.

IDEXX

ISO 9001:2000 CERTIFIED

*Approvals/Acceptances vary per region-effective as of May 2009. Contact IDEXX for references or updated information.

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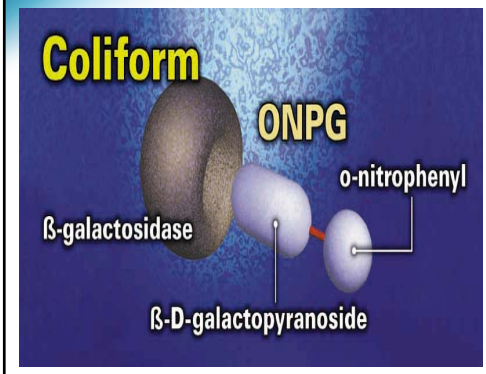
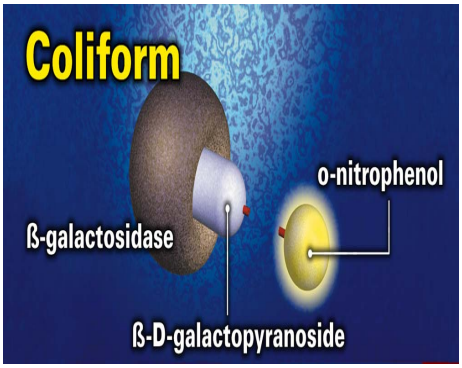
ONPG Positive Reaction

Colilert & Colilert-18





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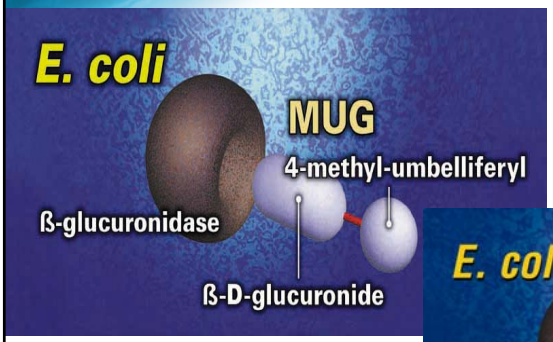
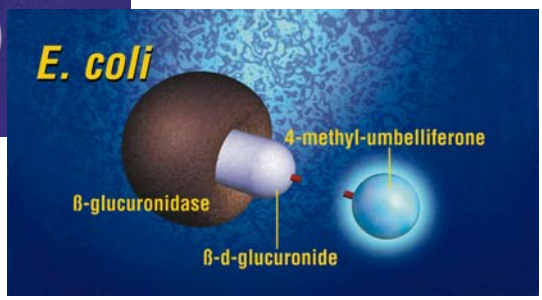
MUG Positive Reaction

Colilert & Colilert-18





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Quanti-Tray Demonstration

Add Colilert to sample and shake to dissolve

Pour mixture into a Quanti-Tray

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Quanti-Tray Demonstration

Seal and then incubate at 35°C for 24 hours

Count positive wells and refer to MPN table

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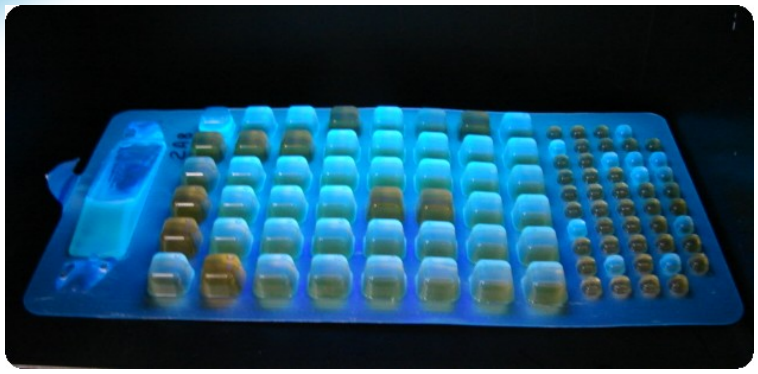
E.coli- Blue Fluorescence- Quanti-Tray under a 365nm UV Light



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Estimation of Bacterial Densities by MPN

- Dates back to 1915 when the concept was introduced by M.H. McCrady (J of Infectious Disease Vol 17, 1915*)
- Prior to this novel concept; no means of direct counting
  - Only had presence-absence of fermentation tubes
- The method is a means for estimating without any direct count, the density of organisms in a liquid.
- Multiple samples of the liquid are taken and incubated in suitable media
  - Record presence or absence of growth in each sample tube
  - Ingenious application of probability theory
  - Estimate the number of organisms from the number of negative tubes

*The Numerical Interpretation of Fermentation Tube Results

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MPN Theory – a short math lesson

Basic assumptions:

- The organisms are distributed randomly throughout
- the liquid
  - Sample is well shaken (important and often neglected)
- A sample will exhibit growth (in the culture media) whenever one or more of the target organisms is present
  - Media should be selective and sensitive
- Requires that at least 1 tube shows no growth (sterile)

If *n* samples, each of volume *v* mL are taken from a liquid, and *s* of these are sterile, then an estimate of the organism density *d* per mL in the original sample is:

$$d = -2.303/v \log(s/n)$$ **[Poisson distribution]**

d can be shown statistically to have the highest probability of estimating the actual organism density – the "**Most Probable Number" - MPN**

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Single dilution – Maximum counting range

Number of Tubes	Maximum MPN/100mL	Number of Tubes	Maximum MPN/100mL
5	8	35	125
10	23	40	148
15	40	45	171
20	60	50	196
25	80	75	324
30	102	100	461

n = 50, v = 2 mL, s = 1

$d = -2.303/v \log(s/n)$

d = -2.303/2 log (1/50)
= 1.956 MPN/mL
= 195.6 MPN/100 mL

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Uncertainty of MPN

- Skewed distribution & Uncertainty of Results
  - For microbiological tests, because of the complications of the skewed distribution, the estimate of uncertainty from a well-designed precision experiment will be regarded as a reasonable estimate of the uncertainty of results
- When MPN tables are used to obtain results, the 95% CL in the table will be regarded as a reasonable estimate of the uncertainty of the results
  - Labs are encourage to identify and question unusual combinations of positive tubes in the tables.
    - 15 tube MPN combination of + tubes
      - »1-2-3
      - »3-5-4

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IDEXX

Standard Methods MPN Table 15 Tube

TABLE 9221:IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)*

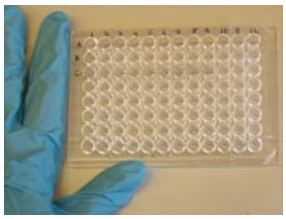
Combination of Positives	MPN Index/ 100 mL	Confidence Limits Low	Confidence Limits High	Combination of Positives	MPN Index/ 100 mL	Confidence Limits Low	Confidence Limits High
0-0-0	<1.8	—	6.8	4-0-3	25	9.8	70
0-0-1	1.8	0.090	6.8	4-1-0	17	6.0	40
0-1-0	1.8	0.090	6.9	4-1-1	21	6.8	42
0-1-1	3.6	0.70	10	4-1-2	26	9.8	70
0-2-0	3.7	0.70	10	4-1-3	31	10	70
0-2-1	5.5	1.8	15	4-2-0	22	6.8	50
0-3-0	5.6	1.8	15	4-2-1	26	9.8	70
1-0-0	2.0	0.10	10	4-2-2	32	10	70
1-0-1	4.0	0.70	10	4-2-3	38	14	100
1-0-2	6.0	1.8	15	4-3-0	27	9.9	70
1-1-0	4.0	0.71	12	4-3-1	33	10	70
1-1-1	6.1	1.8	15	4-3-2	39	14	100
1-1-2	8.1	3.4	22	4-4-0	34	14	100
1-2-0	6.1	1.8	15	4-4-1	40	14	100
1-2-1	8.2	3.4	22	4-4-2	47	15	120
1-3-0	8.3	3.4	22	4-5-0	41	14	100
1-3-1	10	3.5	22	4-5-1	48	15	120
1-4-0	10	3.5	22	5-0-0	23	6.8	70
2-0-0	4.5	0.79	15	5-0-1	31	10	70
2-0-1	6.8	1.8	15	5-0-2	43	14	100
2-0-2	9.1	3.4	22	5-0-3	58	22	150
2-1-0	6.8	1.8	17	5-1-0	33	10	100
2-1-1	9.2	3.4	22	5-1-1	46	14	120
2-1-2	12	4.1	26	5-1-2	63	22	150
2-2-0	9.3	3.4	22	5-1-3	84	34	220
2-2-1	12	4.1	26	5-2-0	49	15	150
2-2-2	14	5.9	36	5-2-1	70	22	170
2-3-0	12	4.1	26	5-2-2	94	34	230
2-3-1	14	5.9	36	5-2-3	120	36	250
2-4-0	15	5.9	36	5-2-4	150	58	400
3-0-0	7.8	2.1	22	5-3-0	79	22	220
3-0-1	11	3.5	23	5-3-1	110	34	250
3-0-2	13	5.6	35	5-3-2	140	52	400
3-1-0	11	3.5	26	5-3-3	170	70	400
3-1-1	14	5.6	36	5-3-4	210	70	400
3-1-2	17	6.0	36	5-4-0	130	36	400
3-2-0	14	5.7	36	5-4-1	170	58	400
3-2-1	17	6.8	40	5-4-2	220	70	440
3-2-2	20	6.8	40	5-4-3	280	100	710
3-3-0	17	6.8	40	5-4-4	350	100	710
3-3-1	21	6.8	40	5-4-5	430	150	1100
3-3-2	24	9.8	70	5-5-0	240	70	710
3-4-0	21	6.8	40	5-5-1	350	100	1100
3-4-1	24	9.8	70	5-5-2	540	150	1700
3-5-0	25	9.8	70	5-5-3	920	220	2600
4-0-0	13	4.1	35	5-5-4	1600	400	4600
4-0-1	17	5.9	36	5-5-5	>1600	700	—
4-0-2	21	6.8	40				

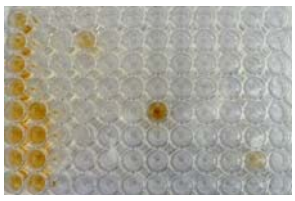
* Results to two significant figures.

IDEXX

Overcoming the tube numbers problem

- To increase counting range need more tubes
- For 100mL sample could use 50 tubes x 2mL
- Would give counting range of ~200MPN/100mL
  - Big headache for lab – lots of tubes – lots of pipettes -lots of washing! Not really practical.
  - Microtitre plate (96 wells) – MPN range 438/100mL





IDEXX

Quanti-Tray®

- Use multiple well concept but automate the sealing

51 well Quanti-Tray
Total sample volume 100mL

$d = -2.303/v \log(s/n)$

$n = 51$, $v = 1.96$ mL, $s = 1$

$d = -2.303/1.96 \log (1/51)$
$= 2.006$ MPN/mL
$= 200.6$ MPN/100 mL

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IDEXX

Multiple Dilutions

- Not practical to extend the counting range by increasing the number of wells beyond a certain point.
- Sample serially diluted and each dilution is inoculated into a similar number of tubes. Commonly used configurations:
  - 3 tubes x 3 decimal dilutions – food industry
  - 5 tubes x 3 decimal dilutions – food, water wastewater (Max MPN = 1600/100mL)
- With multiple dilutions the statistical calculation of the MPN value becomes more complex. **Thomas** provided an **approximation** that could be used for any combination of tubes

$$\text{MPN/100mL} = \frac{\text{\# of positive tubes} \times 100}{\sqrt{(\text{\# of mL in negative tubes}) \times (\text{\# of mL in all tubes})}}$$

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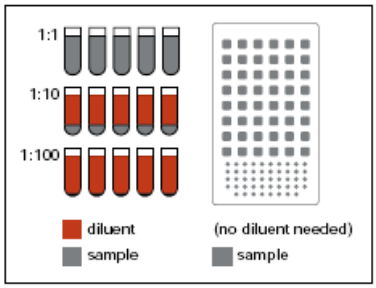
IDEXX

Quanti-Tray 2000®

- Innovative approach to provide a multiple dilution system without adding dilution water
- Small wells are 1/10th the volume of the large wells
  - Effectively a 49 x 48 tube serial dilution



Using the Thomas formula:
(MPN is an approximate, deviates from the MPN table and is not significant)
Number of +ve tubes = 96
Volume of –ve tubes = 0.186mL
Volume in all tubes = 100mL

$$MPN_{max} = \frac{96 \times 100}{\sqrt{(0.186) \times (100)}}$$

= 2232/100mL vs 2419

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IDEXX

Confidence Limits

- Distribution of MPN estimates is approximately log normal (distribution).
- Confidence limits can be calculated (95% limits = +/- 1.96σ)
- Precision is low when the number of tubes is small.
  - For 10 x 10mL tubes with 9 positive: MPN = 28/100mL
  - the 95% confidence limits are: 11.5 – 68.1 MPN/100mL
- Confidence limits for 51 well Quanti-tray
  - When increased to 51 x 1.96mL with 22 positive: MPN = 28.8/100mL
  - The 95% confidence limits are: 19.0 – 44.3 MPN/100mL
  - Interesting fact: If had 100 x 1mL with 25 positive: MPN = 29/100mL
  - The 95% confidence limits are: 19.6 – 43.0 MPN/100mL
  - Not much improvement – 50 tubes is about optimum

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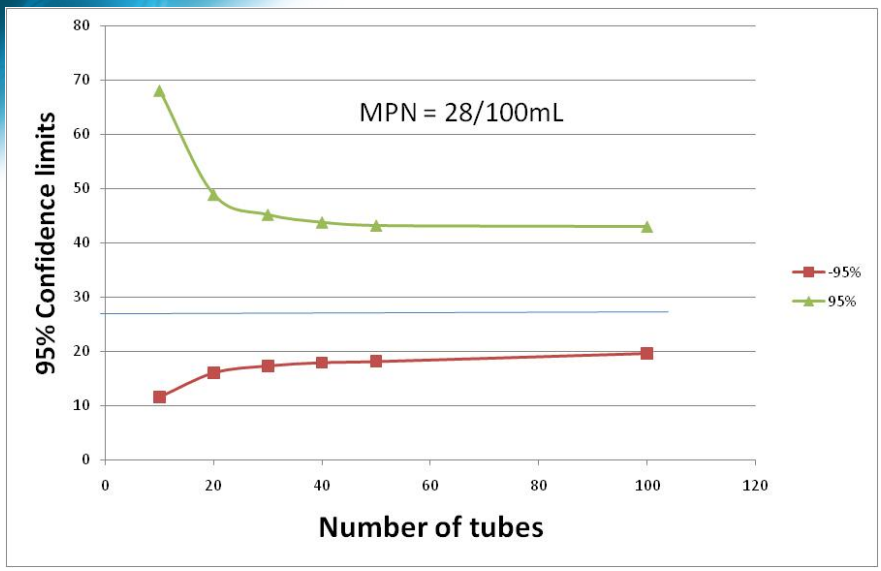
IDEXX



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95% Confidence Limit Comparison

Quanti-Tray MPN/100mL		Quanti-Tray 2000 MPN/100mL		MTF MPN/100mL		MF CFU/100mL	
Value	CL	Value	CL	Value	CL	Value	CL
5.3	2.3-12.3	5.2	1.8-10.8	4.5	0.79-15	5	1.6-7.2
17.8	10.8-29.4	17.9	10.7-28.2	17	6.0-40	17	9.9-27.2
20.7	13-33.3	20.6	12.7-31.8	20	6.8-40	20	12.2-30.8
50.4	35.4-72.5	50.4	35.0-69.1	49	15-150		
78.2	56.4-111.2	78.0	55.6-103.8	79	22-220		

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QA/QC

- On line Quality Control Certificate for each product by lot number can be obtained from the web at **www.idexx.com/water**
- Read results at 24 hours. Window to 28 hours for Colilert For Colilert-18 it is 18 hours up to 22 hours
- Be consistent with time to read results and reading of Y/F wells
- Use Comparator as indicated as indicated in the package insert
- Proficiency Testing

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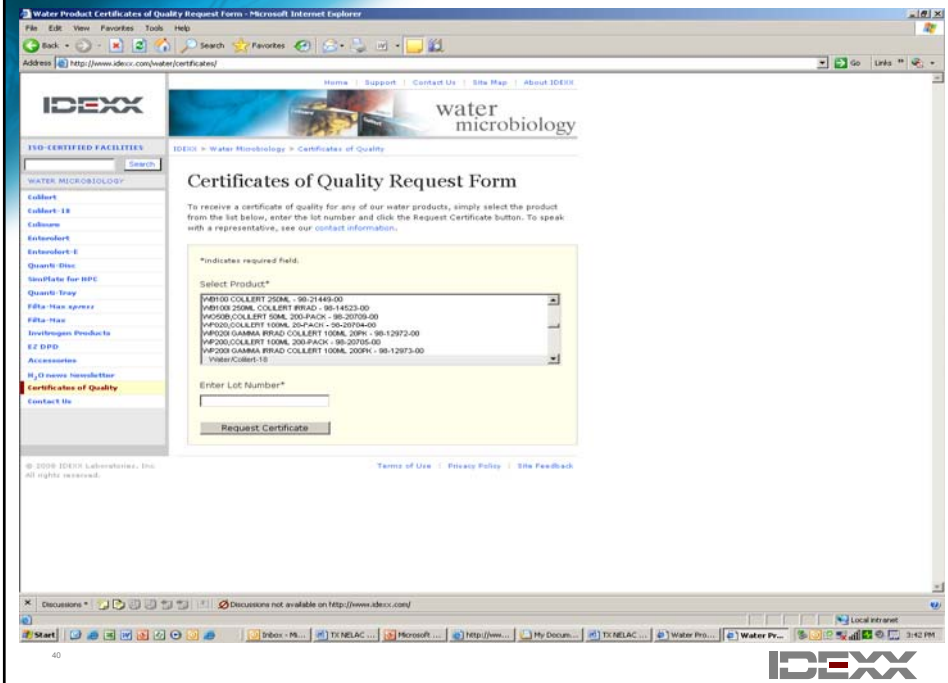

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IDEXX Laboratories, Inc.
Quality Control Certificate
Colilert®

Product and Company Contact Information

Part Number Product Catalog No.	☒ 98-12973-00 WP200I	☐ 98-12972-00 WP020I
Lot Number	FD408	
Expiration Date	25 Jun 2009	Store at 2-30 °C
Technical Support Inquiries	1-207-556-4496 1-800-321-0207 (US/Can) 00-800-4339-9111 (Europe)	E-mail: water@idexx.com
Manufacturer	IDEXX Laboratories, Inc One IDEXX Drive Westbrook, ME 04092 USA	Fax: 1-207-556-4630 www.idexx.com/water

Physical Properties

Powder Appearance: White to off-white, free flowing, granulated powder, free of foreign particles. Results: Passed
pH: Tested for pH range 7.0 – 7.6 Results: Passed

Product Performance

Representative samples of this lot have been tested with the organisms listed below. Ten samples per organism are incubated at 35 +/- 0.5 °C with reads at 24 hours for target organisms and 28 hours for non-target organisms. The inoculum level for target bacteria is approximately 20-50 cfu/100mL. Quantitative testing is performed on all target organisms using IDEXX Quanti-Tray MPN compared to spread plate counts using Tryptic Soy Agar with 5% Sheep's Blood.

Target Bacteria	P/A Result	Quantitative Result
Escherichia coli ATCC 25922	Yellow color change and fluorescence ≥ comparator	Confirmation plate counts within 95% confidence limits of Quanti-Tray MPN
Citrobacter freundii ATCC 8090	Yellow color change ≥ comparator, no fluorescence	Confirmation plate counts within 95% confidence limits of Quanti-Tray MPN
Klebsiella pneumoniae ATCC 31488		
Non-Target Bacteria	**P/A Result**	
Pseudomonas aeruginosa ATCC 10145	No color change, no fluorescence at inoculation level of approximately 20,000 cfu/mL	
Coliform-free	Lot has been subjected to gamma irradiation. In accordance with ISO 11137-02, post-irradiated product has a minimum sterility assurance level (SAL) of 10^{-2} for coliforms.	

Quality Control Testing Completion Date

Quality Control Technician Signature: Nicole Jewett Testing Date: 7/15/08

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bannen Date: 25 JULY 2008

fm-ENV-33_C CO#: 37970 Effective Date: 03/25/08

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IDEXX Laboratories, Inc.
Quality Control Certificate
Quanti-Tray®

Product and Company Contact Information

Part Number Product Catalog No.	98-21378-00 WQT100	
Lot Number	HD021	
Expiration Date	10 July 2011	
Technical Support Inquiries	1-207-556-4496 1-800-321-0207 (US/Can) 00-800-4339-9111 (Europe)	E-mail: water@idexx.com
Manufacturer	IDEXX Laboratories, Inc One IDEXX Drive Westbrook, ME 04092 USA	Fax: 1-207-556-4630 www.idexx.com/water

Physical Properties

Product Sterility: This lot was sterilized with Ethylene Oxide using a process cycle that has been validated in accordance with the standards of ANSI/AAMI/ISO 11135 (1994) and EN 550 (1994), Method C (Overkill Method). (50) biological indicator strips with a minimum population of 1 X 10^6 spores of *Bacillus atrophaeus* per strip were processed with the lot and tested negative for growth in accordance with ANSI/AAM ST-34 (1991).

Representative samples of this lot have been tested by IDEXX Laboratories for the following requirements and passed:

1. Volume of 50 wells: 98 +/- 2.0 ml
2. Average volume of overflow well: >8.0 ml
3. Dye Test: sealed with 100 ml dye and checked for absence of leaks
4. Seal Integrity: trays sealed with 100 ml water, Colisure, and anti-foam: wells withstood 18 PSI of pressure without leaking

Quality Control Testing Completion Date

Quality Control Technician Signature: [signature] Date: 8-6-08

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bannen Date: 06 AUGUST 2008

fm-ENV-29_C Effective Date: 10/29/07 CO# 36055

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IDEXX Laboratories, Inc.
Quality Control Certificate
Sterile Vessels with Sodium Thiosulfate

Product and Company Contact Information

Part Number / Product Catalog No.	☒ 98-09221-00 WV120SBST-200	☐ 98-09220-00 WV120SBST-20	☐ 98-06159-00 WV120ST-200	☐ 98-09203-00 WV120ST-20

Lot Number: GD029
Expiration Date: 24 Jun 2011
Technical Support Inquiries: 1-207-556-4496, 1-800-321-0207 (US/Can), 00-800-4339-9111 (Europe) — E-mail: water@idexx.com
Manufacturer: IDEXX Laboratories, Inc, One IDEXX Drive, Westbrook, ME 04092 USA — Fax: 1-207-556-4630, www.idexx.com/water

Physical Properties

1. Fill Line Accuracy	100 ml ± 2.0 ml
2. Sterility Testing	No growth after 48 hours incubation at 35°C ± 0.5°C with sterile Tryptic Soy Broth
3. Appearance	Absence of nicks, scratches, and cracks
4. Sodium Thiosulfate Content	Able to neutralize 100 ml of 10 ppm chlorine solution

Quality Control Testing Completion Date

Quality Control Technician Signature: Nicole Jewett — Testing Date: 7/16/08

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bawden — Date: 17 JULY 2008

frm-ENV-22_B Effective Date: 10/18/06 CO# 31748

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IDEXX Support

1-800-321-0207

- #1 Customer Support
- #2 Technical Service
- #3 Select extension
- www.idexx.com/water

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That's all folks!

Thank You

Questions