The ATP Process for USEPA Approval of Colilert-18 for Fecal Coliform Testing in Wastewater and Review of Calculations

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IDEXX Laboratories
www.idexx.com/water

Flying Experience

Kulula Air comments from Flight Attendants

- As you exit the plane, make sure to gather all of your belongings. Anything left behind will be distributed evenly among the flight attendants. Please do not leave children or spouses..“
- Another flight attendant's comment on a less than perfect landing: "We ask you to please remain seated as Captain Kangaroo bounces us to the terminal."

Objectives

- ATP process for USEPA approval of Colilert-18 for fecal coliforms
- What is a number- MPN and MF & 95% confidence limits
- Review of calculations for microbiology tests

Colilert-18 Fecal Coliform ATP Study

- The study was based on USEPA ATP protocol (40CFR136)
- Colilert-18/Quanti-Tray compared against m-FC to determine fecals in waste water
- Based on the ATP Study, the EPA determined that Colilert-18 is a suitable method for the detection of fecal coliforms in wastewater.

Protocol Summary

- Primary wastewater effluent samples from 10 different locations within the New England area were approved for use as sources of fecal coliform bacteria for this study.
- Effluent samples were collected, processed and tested at a certified laboratory within six hours.

Colilert-18 -Fecal Coliform ATP Study

- Effluent samples were diluted to approximately 20-50 organisms per 100 mL of sample.
- Each sample was analyzed by Colilert-18 and SM9222D in replicates of 20 after incubation at 44.5 ±0.2°C.
- Sensitivity of fecal coliform detection was determined by comparing the number of presumptive positive responses for the Colilert-18 against the number of presumptive positive responses from m-FC
- Specificity was determined by confirming the identity of at least 200 presumptive positive and 200 presumptive negative determinations from each test method.

Colilert-18 Fecal Coliform ATP Study

- Mean values for the 10 sites

	M-FC
51.4/100mL	37.8/100mL

- Test of variance equality for each method across the 10 sites based on Bartlett's test & Levene's test p-values

Method	p-value (Bartletts's Test) $p > 0.05$	p-value (Leven's test) $p > 0.05$
Colilert-18	0.308	0.619
SM9222D	0.006	0.042

- False Positive and False Negative Rates

Method	Colilert-18	SM9222D
False Positive Rate	0%	0.5%
False Negative Rate	7.0%	21.5%

Colilert-18 Fecal Coliform ATP Study

- **Comments on study findings:**
  - The EPA determined that Colilert-18 is a suitable method for the detection of fecal coliforms in wastewater when incubated at 44.5°C.
  - The high false negative rate for SM9222D is hypothesized to be a result from SM9222D detecting fecal coliform bacteria via active lactose fermentation, which was found to be absent in 16.5% of the strains encountered in this study. These strains of anaerogenic fecal coliforms primarily belonged to the Escherichia and Klebsiella genus, and represent true fecal coliform bacterial and should have been detected.
  - Read subjectivity: Analysts performing the study noted that m-FC can be highly subjective due to the varying morphological characteristics of the colonies. Other fecal coliform colonies on m-FC plates may lack the blue color, due to non-target organisms competing for nutrients and interfere with the growth of fecal coliforms.

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Colilert®-18 Fecal Coliform Protocol Addendum
Quanti-Tray® Enumeration Procedure

1. Add contents of one snap pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray or Quanti-Tray/2000 and seal in an IDEXX Quanti-Tray Sealer.
4. Place the sealed tray in a 44.5˚C ±0.2˚C incubator for 18 hours (prewarming to 35˚C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the Quanti-Tray to obtain a Most Probable Number.

Result Interpretation

Less yellow than the comparator when incubated at 44.5°C ±0.2°C. Negative for fecal coliforms

Yellow equal to or greater than the comparator when incubated at 44.5°C ±0.2°C. Positive for fecal coliforms

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Separation of Snap Packs

Quanti-Tray Demonstration

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Add Colilert/Colilert-18 to sample and shake to dissolve

Pour mixture into a Quanti-Tray

Quanti-Tray Demonstration

cont.

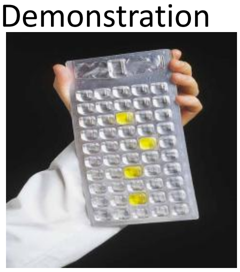
Seal and incubate at 44.5°C for fecal coliforms for 18 hours

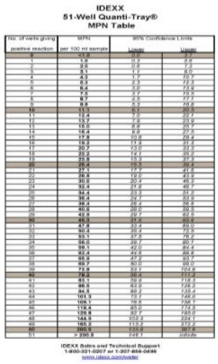
Incubate Samples at 44.5 ± 0.2ºC forColilert-18 for Fecal coliforms

Or A Water Bath for Fecal Coliforms at 44.5±0.2ºC

Quanti-Tray Demonstration

Count positive wells and refer to MPN table





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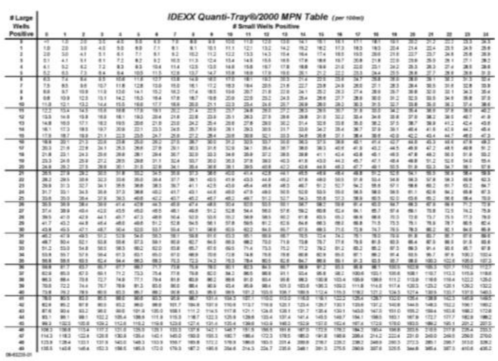
51-Well Quanti-Tray®
MPN Table

No. of wells giving positive reaction	MPN per 100 ml sample	95% Confidence Limits Lower	Upper
0	<1.0	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
12	13.7	7.9	23.9
13	15.0	8.8	25.7
14	16.4	9.8	27.5
15	17.8	10.8	29.4
16	19.2	11.9	31.3
17	20.7	13.0	33.3
18	22.2	14.1	35.2
19	23.8	15.3	37.3
20	25.4	16.5	39.4
21	27.1	17.7	41.6
22	28.8	19.0	43.9
23	30.6	20.4	46.3
24	32.4	21.8	48.7
25	34.4	23.3	51.2
26	36.4	24.7	53.9
27	38.4	26.4	56.6
28	40.6	28.0	59.5
29	42.9	29.7	62.5
30	45.3	31.5	65.6
31	47.8	33.4	69.0
32	50.4	35.4	72.5
33	53.1	37.5	76.2
34	56.0	39.7	80.1
35	59.1	42.0	84.4
36	62.4	44.6	88.8
37	65.9	47.2	93.7
38	69.7	50.0	99.0
39	73.8	53.1	104.8
40	78.2	56.4	111.2
41	83.1	59.9	118.3
42	88.5	63.9	126.2
43	94.5	68.2	135.4
44	101.3	73.1	146.0
45	109.1	78.6	158.7
46	118.4	85.0	174.5
47	129.8	92.7	195.0
48	144.5	102.3	224.1
49	165.2	115.2	272.2
50	200.5	135.8	387.6
51	> 200.5	146.1	infinite

IDEXX Sales and Technical Support
1-800-321-0207 or 1-207-856-0496
www.idexx.com/water



QA/QC

- Colilert-18 read results at 18 hours and up to 22 hours
- Be consistent with time to read results and reading of yellow wells
- Use Comparator as indicated as indicated in the package insert
- Proficiency Testing

Quality Control for Colilert-18

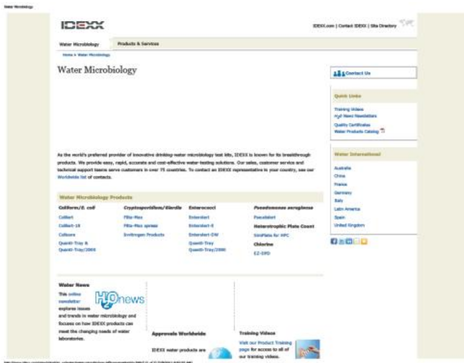
- Use of positive and negative controls
  - For Colilert-18, test upon receipt of each lot of product
- Quanti-Cult
  - 3 sets of a positive Coliform
  - 3 sets of positive coliform/E.coli
  - 3 sets of a negative control
- ATCC Strains

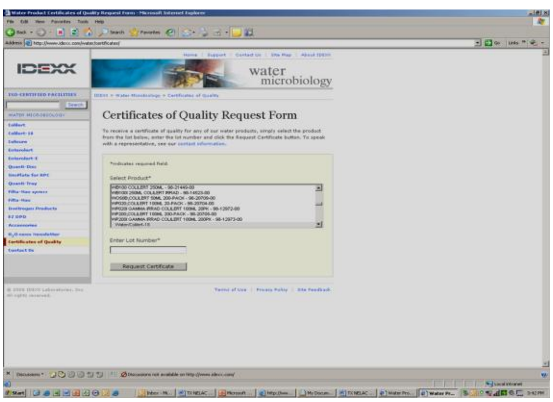
Internal Requirements for Products

- IDEXX is an ISO 9001-2009 certified facility
- IDEXX manufactures under 6 Sigma
  - = or< 3.4 defects/million opportunities
  - Goal is to improve processes to that level of quality or better
- All manufacturing follows approved manufacturing documentation
- All products must meet strict QC requirements
- QA responsible to review all batch records prior to releasing the product for sale.

Certificates of Quality

- **Obtain a C of Q for all our products from our website**
  - **http://www.idexx.com/water/certificates/**
  - Indicates all the testing performed for the specific product and lot
  - Signed by QC for completion date of testing along with QA approval
    - All products are under quarantined until the batch records are reviewed and approved by QA
    - Once approved by QA, the specific product and lot is released for distribution





IDEXX Laboratories, Inc.
Quality Control Certificate
Colilert®

Product and Company Contact Information

Part Number ☒ 98-12973-00 ☐ 98-12972-00
Product Catalog No. WP200I 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^{-6} 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

IDEXX Laboratories, Inc.
Quality Control Certificate
120ml Sterile Vessels Certificate w/Sodium Thiosulfate

Product and Company Contact Information

Part Number	☒ 98-09221-00	☐ 98-09220-00	☐ 98-09159-00	☐ 98-09203-00
Product Catalog No.	WV120SBST-200	WV120SBST-20	WV120ST-200	WV120ST-20

Lot Number: MF009

Expiration Date: 24 January 2014

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 — Lot has been tested using the gravimetric method. The 100 ml fill line is accurate to within ± 2.0%
2. Sterility — 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^{-3}. 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 — Lot is able to neutralize a 100 mL sample with up to 15 mg/L chlorine per *Standard Methods for the Examination of Water and Wastewater* section 9060A.2
5. Fluorescence Test — Result: Negative

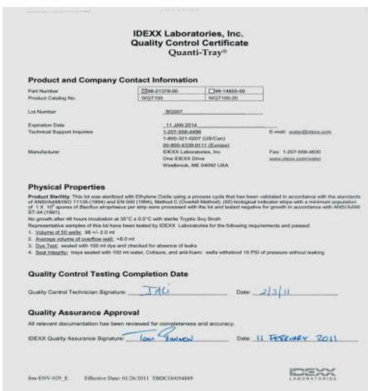
Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bannen Date: 07 FEBRUARY 2011

frm-ENV-022_H Effective Date: 01/26/2011 CO# 054889

IDEXX LABORATORIES



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

Product and Company Contact Information

Part Number	☒ 98-21378-00	☐ 98-14800-00
Product Catalog No.	WQT100	WQT100-20

Lot Number: BG007

Expiration Date: 11 JAN 2014

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/AAMI ST-34 (1991).

No growth after 48 hours incubation at 35°C ± 0.5°C with sterile Tryptic Soy Broth

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: TAC Date: 2/3/11

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bannen Date: 11 FEBRUARY 2011

frm-ENV-029_E Effective Date: 01/26/2011 TBDCO#054889

IDEXX LABORATORIES

IDEXX Laboratories, Inc.
Quality Control Certificate
Quanti-Tray®/2000

Product and Company Contact Information

Part Number	☒ 98-21675-00	☐ 98-14854-00
Product Catalog No.	WQT-2K	WQT2K-20

Lot Number: AS012

Expiration Date: 03JAN2014

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/AAMI ST-34 (1991).

No growth after 48 hours incubation at 35°C ± 0.5°C with sterile Tryptic Soy Broth

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

1. Volume of 96 wells: 98 +/- 2.0 ml
2. Average volume of overflow well: >10.0 ml
3. Volume ratio large wells to small wells: between 11:1 and 13:1
4. Dye Test: sealed with 100 ml dye and checked for absence of leaks
5. 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: Tamara Steel Testing Date: 2/7/11

Quality Assurance Approval

All relevant documentation has been reviewed for completeness and accuracy.

IDEXX Quality Assurance Signature: Tom Bannen Date: 09 FEBRUARY 2011

frm-ENV-030_E Effective Date: 01/26/2011 CO# 054889

IDEXX LABORATORIES

Requirements to use Colilert-18 & Quanti-Tray from USEPA region 4 and FL

- Region 4 Science and Division approves the use of IDEXX Colilert-18 for the detection and enumeration of fecal coliforms in wastewater samples as an Alternate Test Procedure (ATP) until it has been formally promulgated nationally as part of 40 CFR Part 136 under the Clean Water Act.
- Labs are to send their requests to use Colilert-18 for fecal coliform detection to Region 4 (next slides)
- FL is aware of this approval by Region 4; Contact accreditation body to confirm what is required such as DOC.
- **Private labs will need to list all facilities for which they will use this method**

Requirements to use Colilert-18 & Quanti-Tray from USEPA Region 4 and FL.

- This letter should be submitted in writing via fax, e-mail, or standard mail to RayTerhune, the Regional DMR-QA Coordinator, for Regional approval of this ATP process.
- EPA Region 4
- Science and Ecosystem Support Division
- Quality Assurance Section
- 980 College Station Road
- Athens, Georgia 30605-2720
- *Telephone:(706) 355-8557*
- *Email address: Terhune.Ray@epa.gov*
- The facility should keep a copy of their request and approval letter on file for State Inspection purposes.

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

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**

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

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

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

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

Uncertainty of MPN

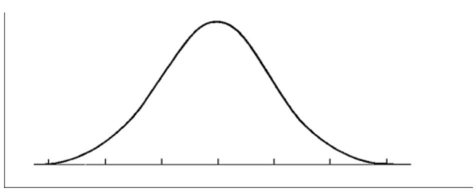
- Skewed distribution & Uncertainty of Result

  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

Frequency curve (positively skewed distribution)

Normal Distribution (Bell Shape)



Standard Methods MPN Table 15 Tube

9-54 MICROBIOLOGICAL EXAMINATION (9000)

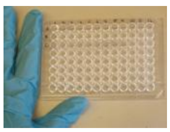
TABLE 9221:IV. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN FIVE TUBES ARE USED PER DILUTION (10 mL, 1.0 mL, 0.1 mL)*

Combination of Positives	MPN Index/ 100 mL	Confidence Limits Low	High	Combination of Positives	MPN Index/ 100 mL	Confidence Limits Low	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.

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



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

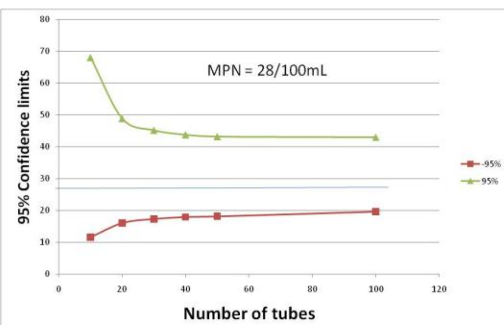
Multiple Dilutions

- 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

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

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



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	

MPN vs. CFU

- Is there a difference different between the two?
  - A simple answer is that the use of either term is based on the method the lab uses for the detection of bacteria
  - Additional information

IDEXX

Distribution of Bacterial Populations

- For chemical analysis the distribution of results usually follows a bell shape curve-symmetrical distribution about the mean
- Microbial distribution are usually not symmetrical and have a skewed distribution . This can be a result of many low values and some high values
  - This can lead to the arithmetic mean to be much larger than the median
- Statistical techniques requires the assumption of symmetrical distributions such as a normal curve and the skewed data is converted to log values resemble a normal distribution.

IDEXX

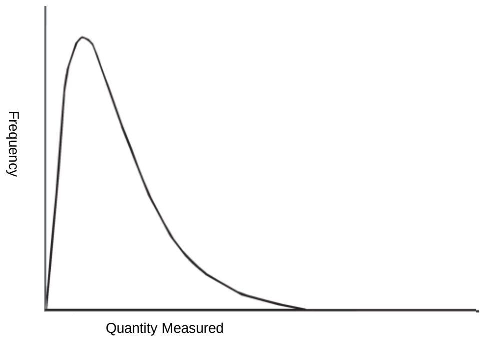
Calculate the coliform density of all the samples and convert to Log_{10} to obtain a geometric mean. Average the 7 samples and take the anti-log to obtain the final value for the 7 samples.

Sample No.	Coliform Density	Log_{10}
1	6.0×10^5	5.78
2	4.2×10^6	6.63
3	1.6×10^6	6.22
4	1.4×10^5	6.14
5	4.0×10^5	5.60
6	1.0×10^6	6.02
7	5.1×10^5	5.71

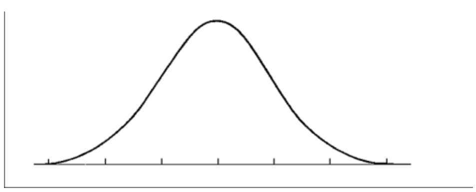
The average value Log_{10} = 6.01 and the antilog = 1.03×10^6

IDEXX

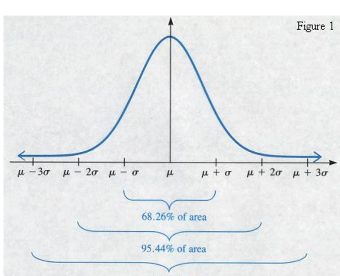
Frequency curve (positively skewed distribution)



Normal Distribution (Bell Shape)



•The graph of the distribution is a bell-shaped curve (a.k.a. a normal curve).



Precision of Quantitative Methods

- Perform duplicate analyses on first 15 positive samples of each matrix type, with each set of duplicates analyzed by a single analyst. If there is more than one analyst, include all analysts regularly running the tests, with each analyst performing an approximately equal number of tests
- Calculate precision of replicate analyses for each different type of sample examined
- Record duplicate analyses as *D*1 and *D*2 & calculate the logarithm of each result.
- If either of a set of duplicate results is <1, add 1 to both values before calculating the log
- Calculate the range (*r*) for each pair of transformed duplicates as the overall mean (*R)* of these ranges. Transform the duplicates and calculate their range

Precision of Quantitative Methods

- If the range is greater than >3.27*R* , there is greater than 99% probability that the laboratory variability is excessive; in such a case, discard all analytical results since the last precision
- Identify and resolve the analytical problem before making further analyses.
- Example

Calculations

- Distribution of Bacterial Population-Geometric Mean
- Precision of Quantitative Methods
- Membrane Filtration
- MPN

Sample No.	Duplicate Analyses *D*1	*D*2	Logarithms of Counts *L*1	*L*2	Range of Logarithms (*r* log) (*L*1-*L*2)
1	89	71	1.9494	1.8513	0.0981
2	38	34	1.5798	1.5315	0.0483
3	58	67	1.7634	1.8261	0.0627
14	7	6	0.8451	0.7782	0.0669
15	110	121	2.0414	2.0828	0.0414

Precision of Quantitative Methods

- Calculations from the table to determine the precision

  $\sum$ of r_{log} = 0.0981+ 0/0483 + 0.0627 + ………+0.0669 +0.0414 = 0.7189

  R = $\sum r_{log}/n$ = 0.7189/15 = 0.479

  Precision Criterion = 3.27(R) = 3.27(0.0479) = 0.157

Precision on Duplicates

- Thereafter, analyze 10% of routine samples in duplicate or one per test run
- Calculations:

Analyses	Duplicates D1	D2	Log of D1	D2	Range of Log	Results
1	71	65	1.85	1.81	0.04	A
2	74	50	1.88	1.70	0.18	U

Precision Criterion = 3.27(R) = 0.157

A =Acceptable, U = Unacceptable

Membrane Filtration

- Standard methods for Water & Waste Water (20 Edition)
  - MF for total coliforms requires counts between 20-80(coliforms) and 20-60 (fecal coliforms)with no more than 200 bacteria on a plate
  - If the total number exceeds 200 (coliforms & non-coliforms), or the colonies are not distinct enough to be counted &/or confluent growth, report results as TNTC or confluent
  - ill usually be absent of coliforms or minimal number
  - Clean/good quality water- If no colonies are observed, report the results as <1/100mL; if results are <20, calculate the results based on the number of colonies on the plate
    - Total colonies/100mL = Number of colonies x100/mL sample filtered
    - Example 8 colonies, 100 mL filtered:

      Colonies/100mL = 8X100/100mL = 8/100mL

Membrane Filtration con't

- For duplicate samples; 2 X 50mL samples filtered and the 2 membranes had counts of 5 & 3 then calculate the colonies/100mL as:
  - total colonies/100mL = [(5+3 X100]/(50 +50) =

    8 colonies/100mL
- For waste, recreational or raw waters , 3 plates are usually necessary to achieve the 20-80 or 20-60 range to find the total colonies/100mL
  - Examples:

Number of Colonies on MF Plates; 3 dilutions/sample

	0.1 mL Filtration	1 mL Filtration	10 mL Filtration
Sample No.			
1	0	1	23
2	2	18	TNTC
3	0	8	65
4	0	5	58
5	0	1	17
6	0	1	39
7	0	1	20

Coliform density is calculated for totals from samples 1, 3, 4, 6 & 7 and for fecals 1,4,6,& 7
For sample 1: 1 plate is within the 20-80 & 20-60 colony range

$$\text{Colonies/100mL} = \frac{23 \times 100}{10\text{mL}} = 230$$

If the total number of colonies is less than or greater than 20-80 or 20-60, total the counts on all countable filters;
For sample 2:

$$\text{Colonies/100mL} = \frac{[(2+18) \times 100]}{(1\text{ ml} + 0.1\text{mL})} = 2727$$

IDEXX Support

1-800-321-0207

- #1 Customer Support
- #2 Technical Service
- #3 Select extension

-www.idexx.com/water

That's all folks!

Thank You

Questions