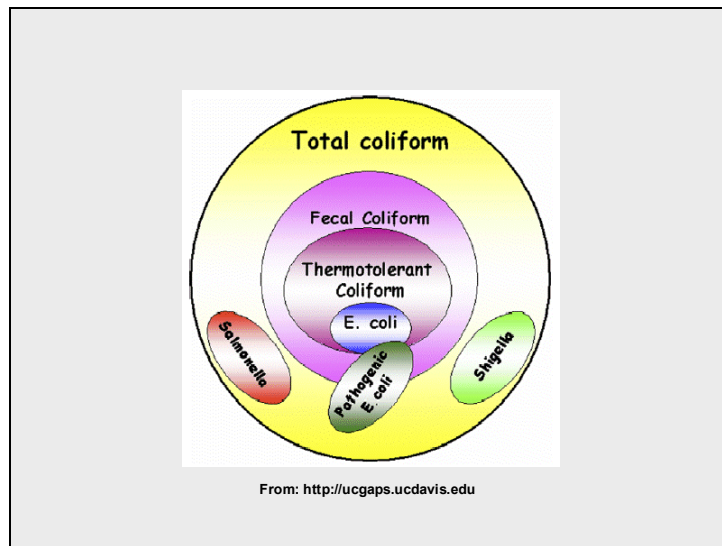


Slide 1

**Citizen Water Quality Monitoring:
Bacterial testing
using
defined substrates**

David W. Buckalew
Dept. of Natural Sciences
Kathleen M. Register
Clean Virginia Waterways
Longwood University

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Slide 3

A connection between water and public health...



Street map of area surrounding Broad Street pump
London, 1843

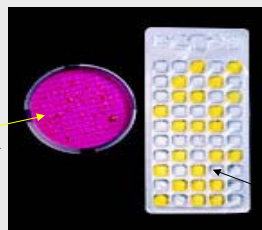
- 1854- **John Snow** mapped and correlated incidence of cholera with proximity to public water pumps
- 1880's- **Robert Koch** reported microbial disease agents
- 1880's- **Theodore Escherich** suggested "*Bacillus coli*" as an indicator of fecal contamination

Slide 4

A brief history of bacterial assays



Phenol red lactose broths



Membrane filter using m-endo broth

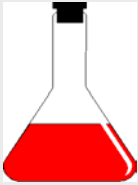
- **1904** – assays for *E. coli* using glucose broths (Eijkman)
- **1920's** – multiple tube fermentation with lactose broths (Leiter)
- **1950's**- membrane filtration (Goetz and Tsuneishi)
- **1988** – defined substrates developed (Edberg et al.)

Quant-i-tray using Colilert® medium

Slide 5

How do bacterial tests work?

They all utilize growth media with specific ingredients:

<p><u>Selective ingredients:</u></p> <ul style="list-style-type: none">•bile salts•selective dyes		<p><u>Differential ingredients:</u></p> <ul style="list-style-type: none">•lactose sugar•other sugar analog
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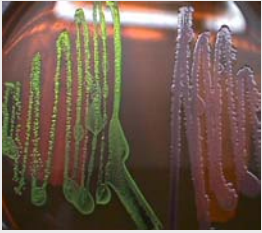
Plus...pH indicator (phenol red, combination of dyes)

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How are results read?

After a specified incubation temp (44.5° C) and time (24 hrs.), the following reaction is examined:

IF sugar(s) is fermented → CO₂↑ and a variety of acids are released into the medium

	
<p>CO₂ and acid production in broths</p>	<p>Acid production as evidenced by ppt. of dyes on agar media</p>

Slide 7

Then the number of bacteria
are quantified...(counted)

A) The number of “+” broth tubes for a sample → Broth tests
are compared to a Most-Probable Number table...

Or

B) The number of individual colonies are counted
on/in an agar plate and multiplied by the amount of → Plate tests
dilution....

Is this the end of the test? Usually not...

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To be sure of results, each assay
requires *Confirmation*...

**Isolated colonies are tested on media which
confirms their identity as E. coli**

Identity is confirmed via:

- a. testing on a variety of diagnostic agars or broths
(e.g. EMB, MAC, BGB, etc)
and/or
- b. performing a multiple test sequence (IMViC, etc)

Thus adding additional time and expense!!

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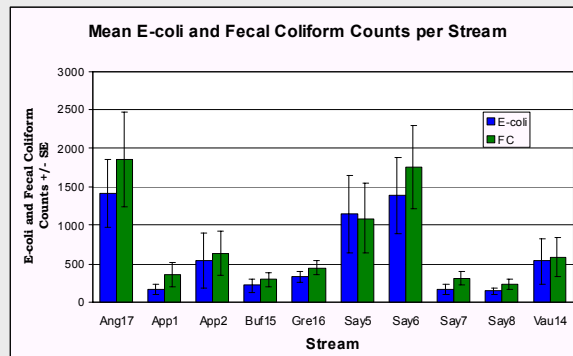
Colilert® provides a rapid, reliable test for coliforms & *E. coli*

- ONPG (sugar analog) → metabolized by coliform bacteria
- MUG → tests for presence of enzyme found ONLY* in *E. coli*
- Results are easily read:
 - Yellow indicates Coliform bacteria
 - Fluorescent Blue/green indicates *E. coli*



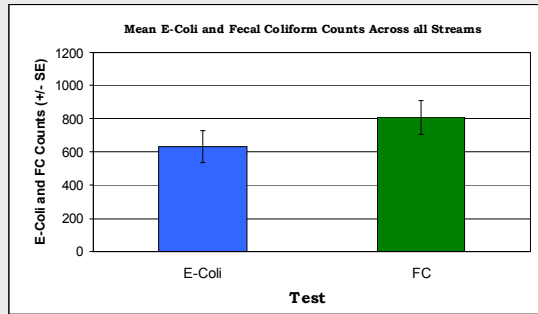
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Test comparisons: Colilert® vs. membrane filtration



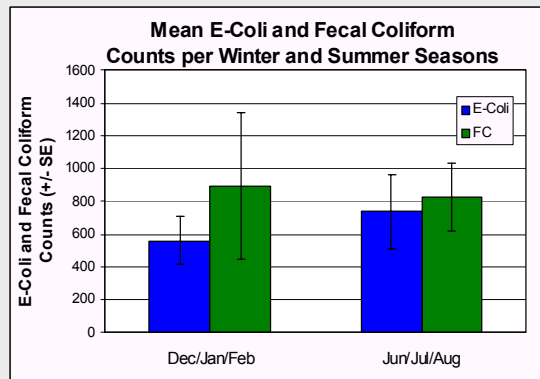
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Test comparisons:
Colilert® vs. membrane filtration



Slide 12

Test comparisons:
Colilert® vs. membrane filtration



Slide 13

Test comparisons:
Colilert® vs. membrane filtration

	Independent Samples Test									
	Levene's Test for Equality of Variances		t-test for Equality of Means							
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		
								Lower	Upper	
ANK0	Equal variances assumed	1.435	.236	-.615	62	.541	-396.6594	645.21527	-1086.38	893.1576
	Equal variances not assumed			-.615	56.662	.541	-396.6594	645.21527	-1088.80	895.5837
APP1	Equal variances assumed	1.646	.204	-1.118	62	.268	-169.8125	151.92710	-473.510	133.8854
	Equal variances not assumed			-1.118	45.592	.270	-169.8125	151.92710	-475.700	136.0748
APP2	Equal variances assumed	.019	.889	-.360	66	.720	-132.3824	367.46614	-866.052	601.2875
	Equal variances not assumed			-.360	63.795	.720	-132.3824	367.46614	-866.526	601.7610
BLF15	Equal variances assumed	.100	.753	-.683	64	.497	-72.2727	105.74677	-283.526	138.9807
	Equal variances not assumed			-.683	63.902	.497	-72.2727	105.74677	-283.532	138.9869
GRE16	Equal variances assumed	.025	.876	-.307	64	.760	-125.7576	409.59898	-944.025	692.5099
	Equal variances not assumed			-.307	63.989	.760	-125.7576	409.59898	-944.025	692.5100
SAY5	Equal variances assumed	.041	.840	-.248	64	.805	-151.7576	613.12643	-1376.62	1073.103
	Equal variances not assumed			-.248	63.761	.805	-151.7576	613.12643	-1376.71	1073.162
SAY6	Equal variances assumed	.288	.593	-.556	64	.580	-390.7576	702.36769	-1793.90	1012.383
	Equal variances not assumed			-.556	63.789	.580	-390.7576	702.36769	-1793.99	1012.473
SAY7	Equal variances assumed	.028	.873	-.278	62	.782	-118.9375	428.00274	-974.503	736.6278
	Equal variances not assumed			-.278	61.989	.782	-118.9375	428.00274	-974.503	736.6279
SAY8	Equal variances assumed	3.622	.062	-1.199	62	.235	-75.9375	63.31834	-202.509	59.62496
	Equal variances not assumed			-1.199	50.613	.236	-75.9375	63.31834	-203.078	51.20209
VAUF4	Equal variances assumed	.090	.765	-.274	64	.785	-88.2727	322.39632	-732.333	555.7890
	Equal variances not assumed			-.274	63.176	.785	-88.2727	322.39632	-732.495	555.9498

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Confirmatory* results

- For Colilert, all (100%) of MUG + wells contained culturable *E. coli*.
- For Membrane Filtration using m-FC broth, most of blue pigmented colonies tested as *E. coli*.

Other colonies tested as:

Enterobacter spp.

Citrobacter spp.

*Confirmatory tests included 1) indole test, and 2) reaction on MacConkey's agar. Pure culture isolates further identified using BDL Crystal multi-test system.

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Discussion:

Colilert® benefits

- Specificity
- Simultaneous enumeration of both Total Coliforms and *E. coli* (EC)
- Time savings
 - less time for Presumptive setup
 - Confirmatory test not required
- EC is a reliable indicator for Fecal Coliforms
- Reduced chance for accidental contamination
- Extended shelf life of medium
- Overall ease of interpretation

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Discussion:

Colilert® liabilities

- Cost comparison
 - \$5.60+/sample (Colilert) vs \$1.75+/sample (memb. flt.)
 - Possible false positives with turbid samples
- Reduced reliability for assays of brackish or saline waters
- Restricted enumeration of Fecal Coliforms

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Conclusions:

Versatility

Colilert® provides similar fecal coliform counts in freshwater samples as compared with membrane filtration methods over a range of environmental conditions including:

- quality of stream water
- variations in temperature
- variations in streamflow (not shown here)

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Questions?

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Westbrook, Maine 04092
1-800-321-0207