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colitag™
The First Definitive Water Test System

How Colitag works:

Colitag is a dehydrated test culture medium

- Colitag works through the presence of two indicators, ONPG and MUG.
- If coliform bacteria are present in the water sample, the ONPG will be hydrolyzed, producing a yellow-colored positive test.
- If *E. coli* are present, the MUG component will be hydrolyzed, causing the sample to emit a bright blue fluorescence when visualized in the dark under a long-wave (366 nm) UV light.

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Colitag Development Summary

Colitag was specifically engineered to:

- Enhance the current state-of-the-art in detecting minute quantities of chlorine-injured coliforms and *E.coli*
- Promote vigorous growth of target organisms producing a more robust positive result for *E. coli*

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Colitag is:

- A one-step ready to use medium.
- EPA-approved for TCR (Total Coliform Rule) Compliance Monitoring
- EPA approved for both P/A and MPN (enumerative) formats.
- Detects 1 CFU of total coliform or *E. coli* bacteria per 100 ml.
- Detects microbes in 24 (+/- 2) hours.
- No confirmation steps required.

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How Colitag works:

The procedure to test with Colitag is as follows:

1. Add powdered media packet to a 100 ml water sample.
2. Shake to dissolve and incubate at 35 degrees C for 22-26 hours.
3. Observe for yellow color and fluorescence under UV light:
   -Yellow color means the sample is positive for total coliforms
   -Fluorescence means the sample is positive for *E. coli*.

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Colitag is as easy as:

1. Snap

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Colitag is as easy as:

2. Apply

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Colitag is as easy as:

3.Incubate

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Colitag is as easy as:

4. Compare

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Colitag is as easy as:

5. UV Lamp

(room light)

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Colitag is as easy as:

5. UV Lamp

(ultraviolet light)

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Colitag has a patented system of “acid resuscitation”.

- Targeted pH works together with a special nutrient profile to resuscitate weak and injured microbes.
- Slightly acidic pH at the onset of the incubation improves recovery of E. coli and other coliforms which are sub lethally damaged by chlorine.
- This system allows for vigorous bacterial growth of *E. coli* injured by chlorination.

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Colitag also has built in flexibility and can be used as a:

1) Total Coliform/*E. coli*
2) Fecal Coliform/*E. coli* test.

-In the fecal coliform application (44.5 degrees C), Colitag can provide a 24-hour fecal coliform and *E. coli* determination.

-Detects both MUG-positive and MUG-negative *E. coli* in one test.
-Includes the built-in ability to detect *E. coli* using the reliable indole test.

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Fecal coliform and *E. coli* procedure:

1. Incubate water sample with medium at 35°C for 4 hours followed by a 20-hour incubation at 44.5°C (±0.2°C).

2. Read yellow color as *fecal* coliform positive.

3.a. Read fluorescence as *E. coli* positive

and / or

3.b. Perform indole test – red color indicates that sample is positive for *E. coli.*

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USEPA APPROVAL OF COLITAG

USEPA mandates that new methods for drinking water compliance monitoring be tested with a microbiological Alternative Test Procedure (the ATP) protocol.

The results from ATP comparability study are verified through extensive statistical analyses to validate that new methods can effectively recover chlorine-injured *E. coli* and other coliforms.

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Overview of ATP Comparability Study protocol:

1. Collect spike (sewage effluent).
2. Determine density of target organisms in spike using membrane filtration method (mENDO for coliforms, mFC for fecal coliforms).
3. Spike a drinking water sample to 10^5 target organisms per 100 ml.
4. Chlorinate spiked sample to stress target organisms: (1-4 log reduction in target microbes).
5. Dechlorinate sample.
6. Run 20 presence / absence replicates in parallel comparing the test method to an EPA approved reference method.
7. Repeat with different spikes and water samples from geographically dispersed areas.

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Agency approved reference methods that were used for comparison:

(LTB-BGLB) for coliforms

(LTB-EC-MUG) for *E. coli*

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Comparability Study Results Summary:

	Total Coliforms		*E. coli*	
	Positives	Negatives	Positives	Negatives
Colitag	115	85	126	125
Reference method	115	85	109	128

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USEPA findings and actions:

- Colitag method compared favorably to the approved reference methods. Federal Register, March 2002, p. 10538, Vol. 67 No. 45.

- USEPA approved Colitag for drinking water compliance monitoring. Federal Register, Feb 13, 2004, Vol. 69, No. 30

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Colitag / Colilert Comparative Study

Conducted for:
GAEPD Drinking Water Program

Conducted by:
Cartersville Water Department
Drinking Water Laboratory

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Methodology

Sample types:

- Distribution: Bartow County and Cartersville City
- Wells: Bartow and surrounding counties
- Pre-chlorination wastewater effluent
- Post-chlorination wastewater effluent
- Raw water: Lake Allatoona
- Industrial

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Methodology

Collection and Storage:

- All samples collected in pre-sterilized 100 ml sample bottles
- Each lot of bottles checked for sterility and volume
- Each lot of media was tested with positive and negative controls:
- *E. coli*, *Pseudomonas aureus*, and *Klebsiella*
- Samples transported to laboratory on ice

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Methodology

Sample Prep:

- Counter top cleaned with Sporocidin and samples placed on the sanitized counter to come to room temperature
- Pre and post blank bottles were labeled
- Colitag and Colilert media were counted and separated
- Hands were sanitized with Alcoscrub and new gloves were donned

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The First Definitive Water Test System

Methodology

Test Procedure:

1. Add Colitag or Colilert to 100 ml sample container
2. Shake a minimum of 25 times to mix thoroughly
3. Place in 35±0.5°C Incubator for 24±4 hours
4. Yellow color development was recorded as a Total Coliform positive
5. Fluorescence under a 6 watt, 365 nm UV light within 5 inches of the sample was recorded as *E. coli* positive.

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Results:

	ONPG +	ONPG -	MUG +	MUG -
Colitag	42	107	38	111
Colilert	43	108	37	112

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International Evaluations

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Evaluation of Coliform Rapid Test Methods by the Japan Water Works Association

Findings of the Microbiology Subcommittee of the Expert Committee Investigating Water Quality Testing Methods

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In 1992, the Japanese government revised both tap water quality standards and testing methods. As part of the changes, new regulations were implemented to allow for the use of rapid techniques to evaluate water quality.

In 2001, a special subcommittee of the JWWA carried out an evaluation study to compare other currently available methods with the two previously officially certified methods, the MMO-MUG method (Colilert) and the Lactose Broth / BGLB method.

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In this study, five media were compared:

LB-BGLB
MMO-MUG (Colilert, IDEXX)
X-gal / pyruvate medium (Nissui)
Readycult (Merck)
Colitag (CPI International)

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The study was divided into 3 portions:

- "Basic" research
- "Practical" research
- Chlorine injury comparative study

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Findings of the Basic Research:

- The committee found that the effects of co-existing bacteria in the same reaction vessel did not significantly affect the test results.

- Colitag produced positive results in an equal or shorter timeframe than the two previously certified methods (LB-BGLB, MMO-MUG/Colilert)

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Practical Research:

- Each method was compared with the two officially certified methods using control strains and both untreated environmental water and natural source water samples treated with 0.5 mg/L of chlorine for 20-1800 seconds. Recoveries were reported in the most probable number format, (MPN/100 ml).

- The data distributions were confirmed to be normal, and a correlation coefficient was found between each method and the two officially certified methods.

- The ratio between the results provided by each method was found, and a statistical *t* test was performed to ascertain whether the ratio between them was statistically significant.

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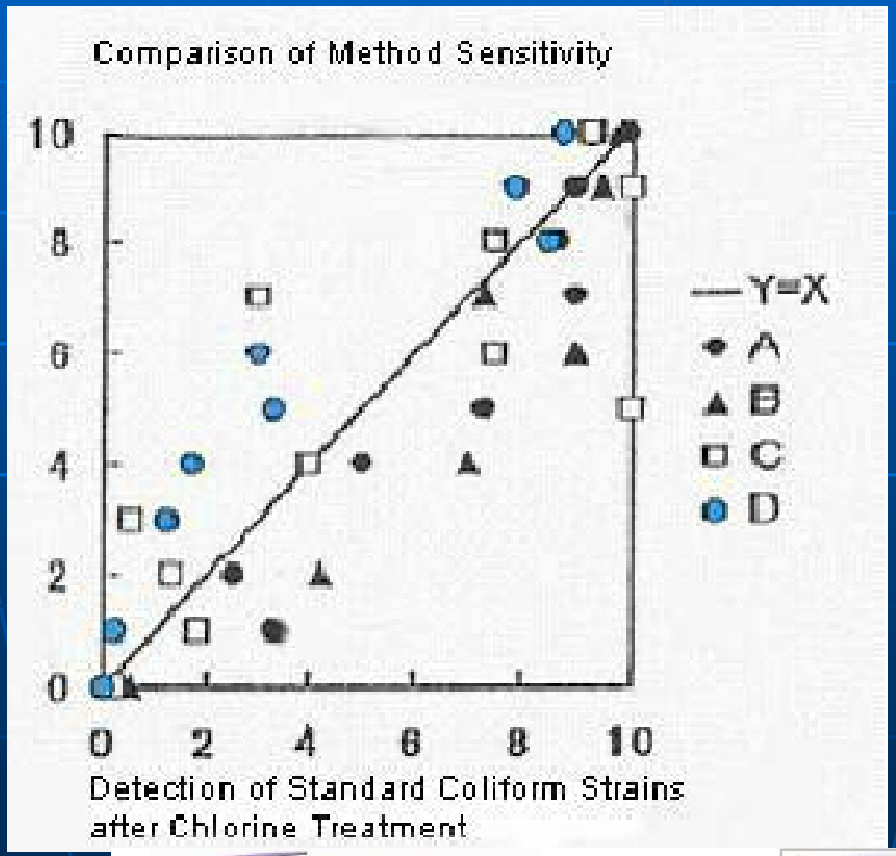
Sensitivity of Colitag using chlorine-injured coliform Control strains :

X =Y Colilert
A = Ready Cult (Merck)
B = LB-BGLB
C = X-gal-pyruvate (Nissui)
D = Colitag



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Findings of the Practical Research:

Culture medium (n= no. of samples)	24 hour	28 hour	48 hour	72 hour
Readycult	63**	88	128*	170**
LB-BGLB	61**	76*	103	118
X-gal-pyruvate	137*	165**	220**	237**
Colitag	127**	161**	245**	258**

Comparison of MPN (coliforms) of the natural source water
**Significance level 0.01, *Significance level 0.05

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Summary of the Environmental Water Study:

When coliform analyses were performed with each culture medium using environmental water to which no chlorine had been added, MPN values ranked in the following order of recovery efficiencies:

X-gal + pyruvate (Nissui) ≥ Colitag ≥ Readycult with 48 hour incubation ≥ Colilert ≥
Readycult with 24 hour incubation ≥ LB-BGLB method.

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Findings of the Chlorine Injury Study with natural source waters:

Culture medium (n= no. of samples)	24 hour	28 hour	48 hour	72 hour
Readycult	31**	44**	78	83
LB-BGLB	48**	68*	95	105
X-gal-pyruvate	91	103	126	142*
Colitag	260**	317**	466**	566**

X=Y Colilert

Comparison of MPN (coliforms) of the natural source water (after chlorine treatment)

**Significance level 0.01, *Significance level 0.05

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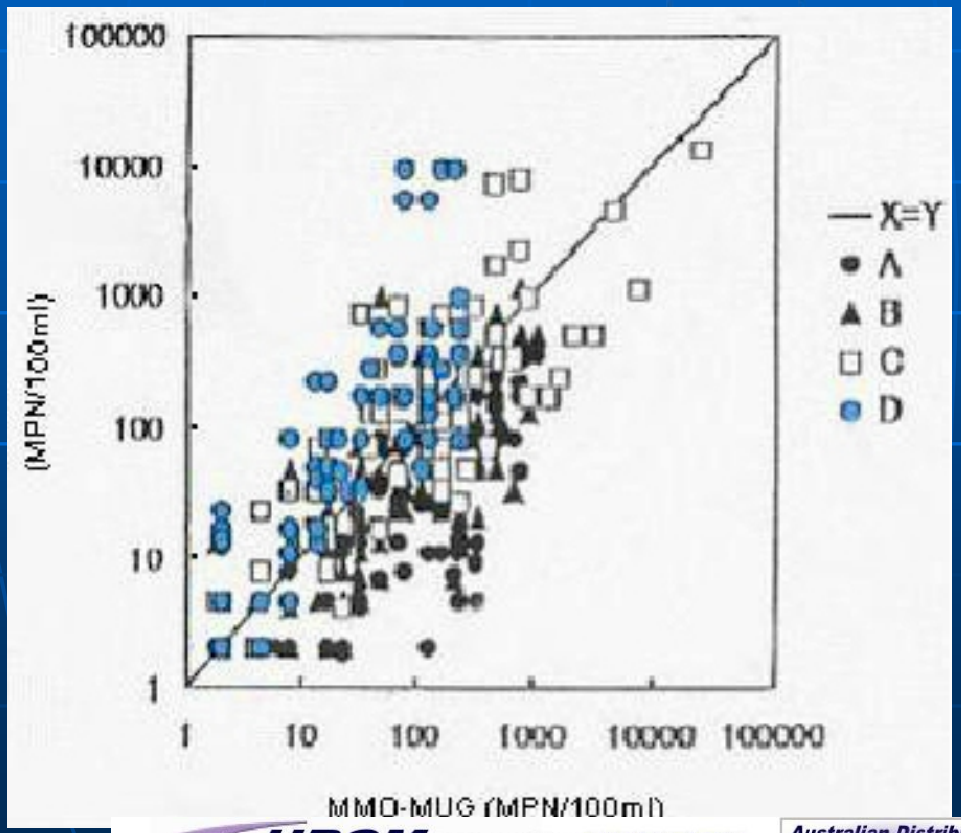
Findings of the Chlorine Injury Sensitivity Study:



X =Y Colilert
A = Ready Cult (Merck)
B = LB-BGLB
C = X-gal-pyruvate (Nissui)
D = Colitag

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Summary of the Chlorine Injury Study:

When media were compared using chlorine treated environmental water, MPN values ranked in the following order of recovery efficiencies:

Colitag ≥ Colilert ≥ X-gal with pyruvate medium ≥ Readycult with 48-hour incubation ≥ LB-BGLB ≥ Readycult with 24 hour incubation.

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Committee Conclusions:

The low detection rate obtained with the LB-BGLB method was due in part to the fact that the method fails to detect non-gas producing strains.

The committee recommended that both Colitag and X-gal with pyruvate (Nissui) medium may be used to measure coliforms in tap water.

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Final Result:

Colitag is now formally approved as an accepted method for tap water compliance monitoring in Japan, as published in the Drinking Water Testing Methods, 2001 by the Japan Water Works Association, Tokyo, Japan.

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Primary French Water Utility Evaluation of Colitag

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Paris Distribution Water Sample Data

1) Distribution water spiked with *E. coli*

	Water 1					Water 2					Water 3				
	Colitag			Colilert	Negative Control	Colitag			Colilert	Negative Control	Colitag			Colilert	Negative Control
Spike 1 (10 cfu / 100 mL)	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-
Spike 2 (5 cfu / 100 mL)	+	-	-	-		+	+	-	+		-	+	+	+	
Spike 3 (1 cfu / 100 mL)	+	+	-	-		+	-	-	-		+	+	-	+	

2) Distribution water spiked with contaminated water source

	Water 1		Water 2	
	Colitag	Colilert	Colitag	Colilert
Spike 1	+			
Spike 2	+			

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Spiked Paris Water Sample

		Colitag (CPI)					Colilert (IDEXX)				
Test number		1	2	3	4	5	1	2	3	4	5
cfu/100 ml		8	6	5	1	4	8	6	5	1	4
Sand-filtered Water	Coliforms	+	+	+	+	+	+	+	+	+	+
	E. coli (fluorescence at 365 nm)	+	+	+	+	+	+	+	+	+	+
Coliform spiked water sample	Coliforms	+	+	+	+	+	+	+	+	+	+
	E. coli (fluorescence at 365 nm)	-	-	-	-	-	-	-	-	-	-
Water spiked with *Enterococcus faecalis*	Coliforms	-	-	-	-	-	-	-	-	-	-
	E. coli (fluorescence at 365 nm)	-	-	-	-	-	-	-	-	-	-
Water spiked with *Klebsiella planticola*	Coliforms	+	+	+	+	+	+	+	+	+	+
	E. coli (fluorescence at 365 nm)	-	-	-	-	-	-	-	-	-	-
Water spiked with *Enterobacter clocae*	Coliforms	+	+	+	+	+	+	+	+	+	+
	E. coli (fluorescence at 365 nm)	-	-	-	-	-	-	-	-	-	-

Sand-filtered water : origin : C… …erational Center (COBE)

Coliform spiked water sample : … …gitol

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In summary:

Colitag is a simple to use, effective medium for the detection of E. coli and other coliforms.

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colitag™

The First Definitive Water Test System

Thanks for your interest!

CPI International resources:

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