



Colitag™ Presence/Absence (P/A) Water Test Kit Fecal Coliform and *E. coli* Application

Product Overview Colitag™ is a selective and differential medium capable of simultaneously detecting Fecal Coliforms and *E. coli* in drinking water, surface, and source water samples. It is a ready-to-use medium to be combined with a water sample. Colitag provides results in 24 ± 2 hours and does not require further confirmation or verification steps. Colitag™ detects 1 colony-forming unit (CFU) of *E. coli* and Fecal Coliform bacteria in 100mL of water.

Fecal Coliform and *E. coli* Method Summary

Colitag™ has the capability to detect **Fecal Coliforms** simultaneously with *E. coli*. In this application, the medium is added to a 100-mL water sample, followed by pre-incubation at $35.0\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ for 4 hours. This initial period allows injured cells to recover. The sample is then incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ for 20 hours. This shift in incubation temperature can be accomplished by transferring the sample to a second pre-set incubator or electronic shift in temperature of the same incubator. At 44.5°C , only **fecal** coliforms grow and hydrolyze ONPG producing a yellow color. If any MUG-positive *E. coli* are present in the sample, a bright blue fluorescence is emitted when the sample is subjected to a long wavelength (366 nanometer) ultraviolet light placed 3-4 centimeters away. Nonfluorescing samples may be checked for MUG-negative *E. coli* by adding approximately 5-7 drops of Kovac's reagent to a 10-mL aliquot. A reddish-purple color forming on the surface of the solution indicates the presence of *E. coli*.

The Colitag™ fecal coliform and *E. coli* method is based on the detection of two enzymes, β -glucuronidase, characteristic of *E. coli*, and β -galactosidase, characteristic of the fecal coliform group at 44.5 degrees Celsius. Colitag™ detects fecal coliforms using the chromogenic substrate ortho-nitrophenyl- β -D-galactopyranoside (ONPG). Upon hydrolysis by β -galactosidase at 44.5°C , ONPG produces a distinct yellow color, confirming the presence of fecal coliforms in the sample. For detection of *E. coli*, Colitag™ utilizes the fluorogenic enzyme substrate 4-methylumbelliferyl- β -D-glucuronide (MUG). Upon hydrolysis by β -glucuronidase, MUG releases 4-methylumbelliferone. This reaction by-product fluoresces when exposed to UV light. The β -glucuronidase enzyme is specific to *E. coli* and observation of fluorescence differentiates this organism from other members of the coliform group.

Only the Colitag method offers a patented new technology called “acid resuscitation” for increased test sensitivity. In this cutting edge technology, a low pH works together with specialized nutrients to resuscitate weak and injured *E. coli* cells. After the bacteria are rejuvenated, Colitag utilizes another patented technology to self-adjust the pH to a neutral level. This rise in pH aids the growth of *E. coli* cells to levels readily detectable and allows the color and fluorescence to be optimally visualized.

Test Procedure for Fecal Coliforms and *E. coli* Bacteria

1. Aseptically add Colitag™ to a 100-mL test water sample. Agitate to begin dissolution. **Note: the solution can be straw-like in color. This is due to the rich nutrients in the medium. Dramatic color change to vibrant yellow indicates a positive sample.*
2. Pre-incubate the sample and medium mixture at 35.0° C ± 0.5° C for 4 hours.
3. Increase the incubation temperature to 44.5° C ± 0.2° C for 20 hours.

Sample Interpretation

1. Visually check each sample for yellow color. If the sample is yellow, **fecal** coliform bacteria are present. If no yellow color is observed, the sample should be recorded as negative for fecal coliforms.
2. Examine the sample for fluorescence using a long wavelength (366 nm) UV lamp. If bright blue fluorescence is observed, *E. coli* bacteria are present. If no fluorescence is observed, the sample should be recorded as negative for *E. coli*.
3. Examine the sample for MUG-negative *E. coli* by adding approximately 5-7 drops of Kovac's reagent to 10-mL aliquots of nonfluorescing samples showing either growth (visual turbidity) or yellow color. A reddish-purple color forming on the surface of the solution indicates the presence of MUG-negative *E. coli*. The sample should be recorded as positive for *E. coli*, regardless of whether fluorescence is observed. (**Note:** This test can be performed on a smaller aliquot of sample if desired. Simply add enough Kovac's to form a visible ring of reagent on the surface of the sample. Alternatively, approximately 5-7 mL of reagent may be added directly into the original reaction/collection vessel.)

Product Storage Store at 4°C to 30°C, away from light.

The Material Safety Data Sheet for Colitag™ is available upon request or by visiting us on-line at:

www.cpiinternational.com or,
www.colitag.com

Product shelf life is 22 months from the manufacture date. The product expiration dates are printed on the box and individual blisters.

The world version includes an inhibitor for *Aeromonas*, not yet approved for US drinking water compliance monitoring.

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