

# COLITAG™

## TEST FOR TOTAL COLIFORMS AND *E.coli* IN P/A FORMAT

Colitag™ is a selective and differential medium for the determination of the presence or absence of total conformers and *E.coli* in drinking water, surface, and source water samples. It is a one-step, ready to use medium, to be combined with a water sample. This product is designed to test water samples for coliform and/or *E.coli* bacteria in  $24 \pm 2$  hours and does not require further confirmation or verification steps. Colitag™ detects 1 colony-forming unit (CFU) of *E.coli* and other coliform bacteria in 100 ml of water.

### Principle

The Colitag™ method is based on the detection of two enzymes,  $\beta$ -glucuronidase and R-galactosidase, which are characteristic of *E.coli* and the coliform groups, respectively. Colitag™ detects **Total Coliforms** using the chromogenic substrate ortho-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG). Upon hydrolysis by  $\beta$ -galactosidase, ONPG produces a distinct yellow colour, confirming the presence of coliforms in the sample. For detection of *E.coli*, Colitag™ utilizes the fluorogenic enzyme substrate 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG). Upon hydrolysis by  $\beta$ -glucuronidase, MUG releases 4-methylumbelliferone. This reaction by-product fluoresces when exposed to UV light. The  $\beta$ -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 colour and fluorescence to be optimally visualised.

### Format of P/A Test

A 100 ml water sample is mixed with Colitag™ followed by incubation at  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for  $24 \pm 2$  hours. If coliform bacteria are present, the medium changes colour from near colourless to a vibrant yellow. In addition, if any *E.coli* bacteria are present in the sample, a bright blue fluorescence is emitted when the sample is subjected to long wavelength (366 nm) ultraviolet (UV) light placed 3 - 4 cms away.

## **Reagents and Equipment**

Palintest Colitag 100 ml P/A Pack (CT 220)

Sterile Sample Container (CT 104/CT 105)

Longwave UV Lamp (CT 102)

Incubator at 35° - 37°C

## **Storage and Shelf-Life**

Store Palintest Colitag tubes at 4° - 30°C and away from light. Product cartons are marked with the product expiry date.

## **Sample Collection**

Samples for bacteriological examination must be aseptically collected using sterile sample containers. The sample collection procedures recommended are those as described in The Microbiology of Water 1994, Part 1 - Drinking Water (Reference 1).

## **General Procedures**

To ensure optimum results and safe use of this product the following general procedures should be observed :-

- 1 Avoid touching or otherwise contaminating the reagent, sample or inside of the reaction tubes prior to or after inoculation with the water sample.
- 2 Adhere to good laboratory practice throughout the test procedure and work in accordance with established codes of practice for bacteriological testing.
- 3 Do not pipette by mouth.
- 4 Thoroughly mix all samples immediately before inoculating.
- 5 Never autoclave the Colitag system prior to use. This process will destroy the reagent system.
- 6 Avoid prolonged exposure of the inoculated Colitag system to direct sunlight. The indicator compounds may be hydrolysed, creating a false-positive (particularly a yellow) result.
- 7 Do not dilute sample in buffered water for addition to the Colitag system. Colitag is already buffered and additional buffer compounds can adversely affect the growth of the target microbes and test performance.
- 8 Colitag is a primary water test. Colitag system performance characteristics do not apply to samples altered by any form of pre-treatment. Pre-treatment includes any method such as growth in lactose-based broth in which there is a non-specific growth enhancing step, or any pre-filtration method such as filtering the sample through a membrane filter then using the filter to inoculate in the Colitag system.
- 9 If additional confirmation is desired after incubating 24 to 48 hours and reading results, immediately transfer 0.1 ml with a pipette to the confirmation medium.
- 10 Dispose of used Colitag reagents and tubes only in the manner recommended.

## Test Instructions - P/A Tests

- 1 Aseptically add Colitag™ to a 100 ml test water sample in a sterile container.
- 2 A fine coating of powder may adhere to the inside of the tube after dispensing. This does not affect the performance of the test.
- 3 Cap the container tightly and mix vigorously to dissolve the reagent. Ignore any particles which remain undissolved. Mark the sample details on the container.
- 4 Incubate the container at  $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  for 24 hours. Incubation should commence within 30 minutes of inoculation. Incubation should not exceed 28 hours.
- 5 Observe the container at 24 hours. For interpretation of results see Test Results and Interpretation section.

## Test Results and Interpretation

- 1 Visually check each sample for yellow colour. If the test sample is yellow then coliform bacteria are present. If no yellow colour is observed, the sample should be recorded as negative for coliform bacteria.
- 2 Examine the solution 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*.

Some water samples may have an inherent colour due to the presence of humic or other organic materials. If the water sample has a background colour, compare the incubated Colitag sample to a controlled blank of the same water sample.

If an inoculated Colitag sample is inadvertently incubated for over 28 hours the following guidelines apply. No yellow colour is a valid negative test. A yellow colour, with or without fluorescence, after this incubation period should be confirmed by repeating the test.

## Disposal

After Colitag test results have been recorded, open each positive test tube or container and add liquid bleach. Cap tightly and mix. Allow to stand for 20 minutes, then flush away contents. Rinse out tubes or containers and caps, then dispose of in the dustbin.

## Quality Control Procedures

It is standard practice in bacteriological laboratories to carry out routine quality control procedures on incoming batches of the reagent or media. The following quality control procedure is recommended for Palintest Colitag packs.

- 1 Empty contents of a Colitag 100 ml P/A tube into a sterile vessel containing 100 ml of sterile distilled water. Mix to dissolve and then aseptically divide the solution evenly into three sterile glass tubes or equivalent.
- 2 Label the tubes '*Escherichia coli*', '*Klebsiella pneumoniae*' and '*Pseudomonas aeruginosa*' respectively.
- 3 Touch a sterile inoculating loop or needle to an 18-24 hour pure culture slant of one of the bacteria listed. Alternatively a commercially available disk of the respective organism may be used as the inoculum.
- 4 Transfer the inoculum to the appropriately labelled tube, then repeat the procedure for the two remaining control organisms.
- 5 Incubate the inoculated tubes at  $35^{\circ} \pm 0.5^{\circ}\text{C}$  for 24 hours. The following results should be observed :-

*E.coli* - yellow and fluorescent

*Klebsiella pneumoniae* - yellow

*Pseudomonas aeruginosa* - no colour, no fluorescence.

If the above results are not obtained, repeat the test on additional Colitag samples from the same batch. If correct results are still not obtained please contact Palintest Ltd.

## References

- 1 The Microbiology of Water 1994, Part 1 - Drinking Water. Reports on Public Health and Medical Subjects No 71, HMSO.

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