# AMT RAPID BAC EC

## AMT-I-B-003 / AMT-I-B-003-M

*E. coli* Presumptive Identification Test for natural waters, drinking waters, process waters, and wastewater.

### BRINGING THE LABORATORY TO THE FIELD

AMT brings the testing laboratory where it is needed most—the field. Our tests are easy to use, fast and reliable, have an extended shelf life and present a unique, cost-effective way to perform microbiological analysis in the field or the laboratory.

Our tests allow you to identify microorganisms that traditionally could only be cultured or identified using expensive equipment in a laboratory setting for a fraction of what a laboratory charges. When using AMT tests, you can make the decisions in the field, where they count.

**Read all directions entirely before running this test.**

### SUMMARY AND EXPLANATION

Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination. Feng and Hartman2 developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG) at a final concentration of 100 µg/mL into Lauryl Tryptose Broth. AMT RAPID BAC EC is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the Presence / Absence or Multi Tube Method procedure by inoculating AMT RAPID BAC EC Ampoules with a sample suspected of containing *E. coli*.

AMT RAPID BAC EC is prepared according to the formula specified by the U.S. Environmental Protection Agency and published in Standard Methods for the Examination of Water and Wastewater.

AMT RAPID BAC EC contains a MUG Indicator to visualize the blue fluorescence caused by the growth of *E. coli* in the sample. Positive growth of *E. coli* is indicated by a fluorescent blue color when viewed under fluorescent light (366nm). When using the Multiple Tube Test; tubes that produce fluorescent blue are considered positive. When using presence absence method a positive result is indicated by a blue fluorescent color at the end of the test when viewed under fluorescent light (366nm).

When used to identify laboratory cultures, an inoculum is made with the suspected culture in purified sterile water and placed in the ampoule, incubated at 37°C and checked for fluorescent blue color at 366nm.

### PRESENCE / ABSENCE METHOD

If using the supplied sample cup, triple rinse the cup with the water sample prior to use.

- Gather the sample in the supplied sample cup filling to the 25 mL line or preferably in a single use sterile sample container.
- (Optional) if sample contains chlorine, add 5 drops of the De-chlorination solution for every 25 mL of sample. Swirl to mix and let sit 2 minutes.
- Remove ampoule from box and carefully remove and save the provided safety cap. Inspect the ampoule tip for breakage. If broken discard properly and get a new ampoule.
- With an unbroken ampoule, place the tip (without safety cap) in the sample container with the tip against the sample container wall holding the ampoule at a 45° angle. Gently push the tip against the sample container wall with a slight twisting motion. The ampoule tip will break and the sample will automatically be drawn into the ampoule. Make sure to keep the ampoule tip in the sample until it has finished filling.
- Remove the ampoule from the sample. Water sample in the ampoule should become pale yellow.
- Incubate for 24-48 hours at 37°C. At the end of incubation, check for the blue fluorescence viewed under long-wave (approximately 366 nm) UV light.
- Sterilize sample container with 10% bleach before next test. For example, to a 20 mL bacterial sample, add 2 mL or ~40 drops bleach solution. Mix and wait for 10 minutes. Rinse 3 times with sterile milli-Q water to remove residual bleach.

### TIME-BASED METHOD

If using the supplied sample cup, triple rinse the cup with the water sample prior to use.

- Gather the sample in the supplied sample cup filling to the 25 mL line or preferably in a single use sterile sample container.
- (Optional) If samples contain chlorine, add 5 drops of the De-chlorination solution for every 25 mL of sample. Swirl to mix and let sit 2 minutes.
- Remove ampoule from box and carefully remove and save the provided safety cap. Inspect the ampoule tip for breakage. If broken discard properly and get a new ampoule.
- With an unbroken ampoule, place the tip (without safety cap) in the sample container with the tip against the sample container wall holding the ampoule at a 45° angle. Gently push the tip against the sample container wall with a slight twisting motion. The ampoule tip will break and the sample will automatically be drawn into the ampoule. Make sure to keep the ampoule tip in the sample until it has finished filling.
- Remove the ampoule from the sample.
- Incubate the ampoule at 37 °C. Check hourly for a blue fluorescence (viewed using long-wave UV light ~366 nm). When the blue fluorescence develops, record the time in hours from start of the test to the appearance of the light pink color and check the chart below for bacterial levels.
- Sterilize sample container with 10% bleach before next test. For example, to a 20 mL bacterial sample, add 2 mL or ~40 drops bleach solution. Rinse 3 times with sterile milli-Q water to remove residual bleach.

### Approximate bacteria population:

| Elapsed Time (hrs) | Approximate bacteria population (CFU/mL) |
|---|---|
| 2 | $10^8$ |
| 4 | $10^7$ |
| 6 | $10^6$ |
| 9 | $10^5$ |
| 13 | $10^4$ |
| 19 | $10^3$ |
| 25 | $10^2$ |
| 30 | 1 – 10 |
| 48 or more | < 1 |

## EXPECTED RESULTS

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light.
Typical strains of *E. coli* (red with a green metallic sheen on m Endo Agar LES) exhibit blue fluorescence. Non-*E. coli* coliforms do not fluoresce.

## LIMITATIONS OF THE PROCEDURE

Glucuronidase-negative strains of *E. coli* have been encountered.

Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.

Strains of *Salmonella* and *Shigella* species that produce glucuronidase may infrequently be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters; i.e., gas production, lactose fermentation or growth at 44.5°C.

To reduce false positives when testing marine waters the sample should first be diluted 1:10 using appropriate dilution water.

## STORAGE

Upon receipt, store tubes in the dark at 2 – 25°C. Avoid freezing and overheating. Ampoulated media stored as indicated may be inoculated up to the expiration date. Minimize exposure to light.

**Product Deterioration:** Do not use ampoules if they show evidence of microbial contamination, discoloration, or other signs of deterioration.

## EXPIRATION DATE

The product is stable if stored properly for 1 year from manufacture. The expiry date applies to media stored at or below 30°C out of direct sunlight.

## Laboratory Quality Control Procedure

Incubate the listed bacterial strains for about 16 hours, until reach 2.0 absorbance @600nm (~$10^9$ CFU/mL).
Perform CFU counting to determine the cell density of bacterial cultures.
Mix bacterial cultures at 50%/50% ratio.
Dilute the mixed bacterial culture with sterile phosphate buffered saline (PBS) to the final CFU/mL of $10^8$, $10^7$, $10^6$, $10^5$, $10^4$, $10^3$, $10^2$, 10, and 1. Use sterile PBS buffer as negative control.
Inoculate ampoules with these bacterial cultures and incubate in an aerobic environment at 37 °C. Perform biological triplicates for each cell density and negative control.
Examine the color development of all inoculated ampoules and record the time of the appearance of blue fluorescence up to 30 hours post inoculation.

## QUALITY CONTROL ORGANISMS

*Enterobacter aerogenes* ATCC 13408
Growth No Fluorescent Reaction.
*Escherichia coli* ATCC 25922
Growth Fluorescent Reaction.

## WARNING AND PRECAUTIONS

- For in vitro Diagnostic Use.
- For Laboratory and field use by trained professionals.
- The AMT RAPID BAC E. coli test is a glass ampoule with a sharp tip when activated. USE EXTREEM CAUTION when breaking the tip. Always carefully apply the provided safety cap.
- Dispose of broken unused ampoules in a broken glass receptacle.
- Dispose of used ampoules in an appropriate sharps container or sealed puncture resistant receptacle then offer for biohazard processing according to local, state and Federal regulations.
- Keep away from children
- Not for use as a diagnostic tool on humans or animals.
- Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures before, during and after use.
- Prepared ampoules, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

## Contact Us:

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