AMT RAPID BAC CF

AMT-I-B-006 / AMT-I-B-006-M

Total coliform Presumptive Identification test for Fresh Water, Marine Water, Milk, Food, pharmaceutical, nonsterile products and Laboratory cultures.

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

AMT RAPID BAC CF is a modification of the original bile salt broth recommended by MacConkey and contains less inhibitory Brom cresol purple (BCP) as an indicator of lactose fermentation and Oxgall to inhibit gram-positive microorganisms.

The BCP Indicator at 10ppm along with the inverted Durham tube (AMT-I-B-006 Only) also helps visualize the turbidity reaction and gas production caused by the growth of Microorganisms. Lactose fermenting microorganisms will grow very well causing the media to turn yellow. Non-lactose fermenting microorganisms will grow but not produce acid or gas. Positive growth of microorganisms is indicated by a turbid yellow color. Gas trapped in the Durham tube indicates CO_2 production.

SAMPLE COLLECTION AND HANDLING

For milk or food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location. For water samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location. For pharmaceutical samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.

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.
- 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 blue color. If no color change, discard the ampoule and repeat previous steps.
- Incubate the ampoule at 37 °C for 1 hour.
- Gently tap the tube to make sure that there is no air bubble in the inverted Durham tube (AMT-I-B-006 only).
- Continue incubating at 37 °C. Check hourly for a yellow color and gas production. When the yellow color develops, record the time in hours from start of the test to the appearance of the yellow color and check the chart below for coliform levels. Gas will also be produced (AMT-I-B-006 Only).
- 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.

Approximate bacteria population:

Elapsed Time (hrs)	Approximate bacteria population (CFU/mL)
2	$> 10^7$
8	$10^5 – 10^6$
16	$10^3 – 10^4$
24	10 – 100
>24 (Negative)	< 10

PURE CULTURE IDENTIFICATION

For proper results observe aseptic techniques throughout all procedures.
Colonies, from a primary isolation plate, suspected of being coliform, can be emulsified in 25 mL of sterile Butterfield's solution or other purified sterile dilution water. The suspected colonies are emulsified in purified sterile water at an equivalent of a 0.5 McFarland turbidity standard or higher. The sample is then placed in the ampoule as outlined above and incubated at 37 °C in an aerobic atmosphere. Samples with a heavy inoculum of coliforms should produce a reaction in 24 hours or less.

RESULTS

Coliform bacteria will produce acid and gas in the media. A positive reaction is indicated by a yellow color (acid production) and a gas bubble in the inverted Durham tube (AMT-I-B-006 only). Yellow color & no air bubble indicates coliform positive / acid production positive / gas production negative; Yellow color & an air bubble in the inverted Durham tube indicates coliform positive / acid production positive / gas production positive. A blue color with no air bubble indicates negative presence of coliform.

LIMITATIONS OF THE PROCEDURE

AMT RAPID BAC CF is used for cultivating gram-negative, lactose-fermenting bacteria and as a presumptive test for coliform bacteria. It has been used to analyze food, milk, and water samples for coliforms. In addition, this medium has also been used in the rapid detection of shiga-toxin

producing *E. coli* in fecal samples. MacConkey Broth is recommended in the USP as a test medium for *E. coli* in the microbiological examination of nonsterile products.

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

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 and air bubble formation of all inoculated ampoules, and record the time of the appearance of yellow color and air bubble up to 30 hours post inoculation.

QUALITY CONTROL ORGANISMS

Enterococcus faecalis ATCC 29212
Markedly inhibited, no acid production, no gas production
Escherichia coli ATCC 25922
Good growth, acid production, gas production

WARNING AND PRECAUTIONS

- For in vitro Diagnostic Use.
- For Laboratory and field use by trained professionals.
- The AMT RAPID BAC CF test is a glass ampoule with a sharp tip when activated. **USE EXTREME 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.

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