COLIFAST®

USER MANUAL FIELD KIT

Water and Food Safety

RAPID | AUTOMATED | AT-LINE

Contents

1 Technology

Scope and Application

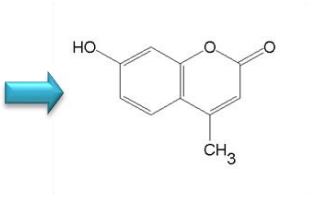
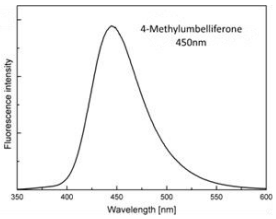
The Colifast Micro Detector (CMD) from Colifast AS is used together with bacterial growth media for rapid test for the detection of total coliforms, fecal coliforms and *E. coli* in water. It is based on the patented technology of Colifast AS. The system can detect down to 1 cfu (colony forming unit) per sample volume.

Method Principle

The Colifast technology consists of a fluorimeter and a bacterial growth medium. The method is based on an enzymatic reaction. The medium contains the substrate 4-methylumbellieryl (MU)-β-D-galactoside, and this substrate is hydrolysed by the enzyme β-D-galactosidase that is present in coliforms. The fluorescing product 4-methylumbelliferone (MU) is produced as a result of the hydrolysis reaction (see figure below). The media also contains inhibitors to inhibit growth of non-coliforms.

The results are measured with the fluorimeter. An increase in the number of coliforms means an increase in amount of β-D-galactosidase (enzyme). This brings an increase in the production of MU (the fluorescing product) which results in a higher fluorescence signal on the CMD.

Incubation temperatures selects for fecal coliforms (42 °C to 44.5 °C) and coliforms (35 °C to 39 °C). *E. coli* is detected by incubating the sample at 37 °C with different growth media.

COLIFORM BACTERIA	COLIFAST REAGENT(S)	REACTION	DETECTION
			
Coliform bacteria with faecal origin	**MU substrate** —*Enzymes*→ **substrate + MU**		
Coliform bacteria with specific enzymes TC/FC: β-D-galactosidase *E. coli*: β-glucuronidase	Nutrients, inducers, inhibitors, non fluorescent reagent (substrate that reacts together with specific bacterial enzyme)	Bacteria enzyme hydrolyze the substrate, the product is fluorescent (4-methylumbelliferone)	Fluorescence = presence of bacteria in the sample Excitation = 365 nm Fluorescence = 430 nm

2 Calibration

Colifast calibrates the instrument. The instrument should also be re-calibrated if the Blank and Cal values are found to be inaccurate. The accuracy can be controlled by reading the Blank soulution (3ml with 3 drops NaOH). If the blank reading differ with more than 10 RFU from 0, the unit should be re-calibrated. Background fluorescence can be altered by temperature so pre-heating of calibration solutions to 44 °C is recommended.

1. **Press ON/OFF on the Colifast Micro Detector. Press STD VAL and check that the value is 1.0 (calibration set). If not, use up and down arrows to set value. When set, press ESC or ENT to accept the value and return to the home screen.**

1. **Remove caps from Cal48 and Blank vial and transfer 3 mL to separate clean cuvettes. Use two plastic pipettes (with 3 mL mark, one for each vial). Cap vials.** ***These two 3 mL plastic pipettes should only be used for calibration. Avoid contamination.***

2. **Add 3 drops (100 µL) of 0.5 M NaOH solution to each cuvette from the dropper bottle.** ***Cap bottle after use / avoid contamination.***

3. **Cap cuvettes and turn 5-10 times to mix the content.**

4. **Open the black cover, insert the cuvette with Blank solution, close cover, and press CAL. Follow instructions on screen. Press ENT twice to read blank. Check standard value as 48.0. If not, use up and down arrows to set 48. Insert the Cal48 cuvette, close cover, and press ENT. Calibration is completed. Check unit set to RFU and use the arrows for any change and to accept the calibration (Cal Done). Press ENT to return to home screen.**

3 Methods

1.1 **Rapid Screening Test** - medium to high bacterial levels (> 500 cfu/100 mL)

Applications

- Screening of raw water sources
- Contamination tracking

Early preparations

The incubator should be pre-warmed (turned on) for about 60 minutes before start. The unit uses max. 3 A in combination with the 12 V car converter.

1. **Add 10 mL water sample directly to a prefilled vial (up to 4th marker-line on vial) alternatively by using a syringe. The syringe can be re-used after wash (aspirate and dispense clean water or water from new sample source three times).**

2. **Cap the vial, place it in the incubator and start the timer (check temperature: 44 ^{o}C).**

3. **After 15, 45 and 75 minutes* (or only 15 and 45 minutes for rough estimate): Fetch the vial from the incubator. Remove the cap and transfer 3 mL sub-sample to a new and clean cuvette using a plastic pipette (with 3 mL mark). Cap the vial and place it back into the incubator. The plastic pipette can be reused for all sub-samples from this vial. Avoid contamination.**
   **5 sub-sample readings (15, 45, 75, 105 and 135 minutes) gives increased control of linearity.*

4. **Add 3 drops (100 µL) of 0.5 M NaOH solution to the sub-sample from the dropper bottle. *The bottle contains approximately 100 sub-samples. Cap bottle after use / avoid contamination.***

5. **Cap cuvette and turn it 5-10 times to mix the content.**

6. **Press <u>ON/OFF</u> on the Colifast Micro Detector. Open black cover, insert cuvette, close cover and press <u>READ</u>. Write down fluorescence value (MU).**

Calculate slope when all 3 or 5 sub-samples are read: Linear increase in fluorescence signal per hour.
3 readings: Slope = 75 min reading –15 min reading
5 readings: Slope = (135 min reading – 15 min reading) / 2
Correlate calculated slope to guideline table and find corresponding bacterial level.

Example: 0.150 (15 min), 20.25 (135 min): 20.25 – 0.150 = 20.10 20.10 / 2 = 10.05 10.05 correlate to 1000 to 5000 fecal coliforms per 100 ml.

Note: Negative numbers may occur (linked to calibration). This is not a problem: calculate the increase in fluorescence per hour. If the calculated slope is a negative value, the water sample contains no coliforms.

Example: -7.500 (15 min), 1.550 (135 min): 1.550 – -7.500 = 9.050 9.050 / 2 = 4.525 4.525 correlate to 500 to1000 thermotolerant coliforms per 100 ml.

Discharge cuvettes and vials after use. For disinfection of content use Ethanol tissues.

Prefilled vials, calibration solutions and NaOH solutions should be stored at room temperature, in the dark. The reagents survive storage at higher temperatures (max. 40 °C). Shelf life is 6 months.

Do not use contaminated reagents (look for visible growth).

1.2 **Presence Absence Test** - low bacterial levels

Applications

- Clean/treated water
- Presence / Absence of coliforms

If water contains chlorine: Add 5 drops Na-thiosulfate solution (from dropper bottle) pr 100 mL sample.

1. **Add 10 mL water sample directly to prefilled vial (up to 4th marker-line on vial), alternatively by using a sterile or clean syringe.**

2. **Cap the filled cuvettes, place them in the incubator and start the timer. Check temperature: 37 °C for total coliforms and E. coli and 44 °C for fecal coliforms (See 1.4.1). *Note: The incubator should be pre-warmed (turned on) for ca. 60 minutes before start. The unit uses max. 3A in combination with the 12V car converter.***

3. **Read the sample after 8 hours (early warning) and 11 hours. See *section 1.1 Rapid screening test* for reading procedure. Fluorescence values equal to or over 200 mean that there are viable coliforms in the sample (1 or more per sample volume).**

1.3 **Most Probable Number test (MPN)** - low bacterial levels

MPN is a statistical number of coliforms per 100 ml.

If water contains chlorine: Add 5 drops Na-thiosulfate solution (from dropper bottle) pr 100 mL sample.

1. **Add 10 mL water sample directly to prefilled vial (up to 4th marker-line on vial), alternatively by using a sterile or clean syringe.**

2. **Cap vial and turn 5-10 times to mix content. Remove cap and use a clean plastic pipette to distribute 3 mL to 6 separate cuvettes (total 18 mL).**
3. **Cap the filled cuvettes, place them in the incubator and start the timer. Check temperature: 37 °C for total coliforms and E. coli and 44 °C for fecal coliforms (See 1.4.1). *Note: The incubator should be pre-warmed (turned on) for ca. 60 minutes before start. The unit uses max. 3A in combination with the 12V car converter.***

4. **After 11 hours: Take out the 6 cuvettes from the incubator. Remove caps and add 3 drops (100 µl) of 0.5 M NaOH solution to each cuvette with the dropper bottle. Cap cuvettes and mix content.**

5. **Read the 6 cuvettes on the Colifast Micro Detector. See *section 1.1 Rapid screening test* for reading procedure. Fluorescence values equal to or over 200 means that the cuvette is positive. Values under 200 means that the cuvette is negative. Use the table under to find the number of coliform per 100 ml.**

Number of positive cuvettes (of 6)	Coliforms per 100 ml
0	**0 - 11**
1	**12**
2	**27**
3	**47**
4	**77**
5	**136**
6	**over 136**

The bacterial number is based on statistical calculation.

1.4 Test for all coliform bacteria

1.4.1 Adjust the temperature of the incubator to 37 °C. Turn the incubator on and turn the adjustment screw ¼ turn counter-clockwise (with flat screwdriver). Wait for about an hour and check temperature. Tweak

the adjustment screw more if necessary. Wait for 20 minutes after any smaller adjustment to ensure that the temperature is stable. The temperature should lie between 36 °C and 38 °C, or 43 - 45 °C for thermotolerant(fecal) coliforms.

1.4.2 Test for all coliform bacteria using test 2 (Presence-Absence Test) or test 3 (Most Probable Number Test) as described above. Increase incubation time to 12 hours. Results are calculated in the same way but referred to as number of total coliforms per 100 ml sample.

1.5 Test for *E. coli* bacteria

1.5.1 Adjust the temperature of the incubator to 37 °C as described above.

1.5.2 Test for *E. coli* bacteria using test tubes and calibration solutions marked with *E. coli.* For the analysis use test 2 – Presence-Absence test or 3 – Most Probable Number test as described above. Increase the incubation time to 12 hours. Results are calculated in the same way but referred to as number of *E. coli* bacteria per 100 mL sample.

4 Choice of method

Water source / tracing the most suitable source

A water source should contain the smallest amount of coliform bacteria as possible. A large amount of bacteria increase the risk of insufficient treatment and contaminated tap water/drinking water. Find the most suitable source if there are several options available. The amount of bacteria can vary from zero to millions per 100 mL water sample. Adjust treatment according to the quality of the raw water.
Use **test 1 – Rapid screening test** for comparison of bacterial content.
Use **test 3 – Most Probable Number test** when the amount of bacteria present is lower than 200 coliform bacteria per 100 mL sample.
Do several test rounds and if possible periodically over time to map temporal variations in bacterial content.

Water source / testing of raw water

Variations and incidents should be recorded in order to ensure optimal treatment of raw water. Incidents of high levels of coliform bacteria increase the risk of contaminated tap water/drinking water.
Use **test 1 – Rapid screening test** for comparison of bacterial content.
Use **test 3 – Most Probable Number test** when the amount of bacteria present is lower than 200 coliform bacteria per 100 mL sample.
Do several test rounds and if possible periodically over time to map variations in bacterial content.

Tap water / drinking water control

Drinking water should not contain coliform bacteria.
Use **test 2 – Presence-Absence test (**optionally with the Colifast 100 mL flask**)** or **test 3 MPN test**. Do the testing periodically. Avoid using the water if tests detect coliform bacteria.

Recreational water / environmental monitoring

Water (lakes, ponds, rivers and watercourse) used by humans should not contain high levels of coliform bacteria. Avoid use/bathing if the level exceeds 1000 coliforms per 100 mL sample.
Use **test 1 – Rapid screening test** for comparison of bacterial content.
Use **test 3 – Most Probable Number test** when the amount of bacteria present is lower than 200 coliform bacteria per 100 ml sample.
Do several test rounds and if possible periodically over time to map temporal variations in bacterial content.

Tracing a source of contamination

Any source of contamination should be traced. Assess the level of coliforms in different locations connected to same lake or watercourse and trace the highest results to find source of contamination.
Use **test 1 – Rapid screening test**.
Do sufficient amounts of testing rounds to ensure secure detection of source(s).

Sewage control

High levels of coliform bacteria from sewage can contaminate the environment. Avoid use/bathing if the level exceeds 1000 coliform bacteria per 100 mL. Find bacterial level in discharges and affected areas and adjust treatment accordingly to minimize bacterial contamination of the environment.
Use test 1 – Rapid screening test.
Do sufficient amount of testing and record temporal variations.

5 Troubleshooting

1.6 General

1.6.1 Temperature Considerations

Fluorescence is temperature sensitive. As the temperature of the sample increases, the fluorescence decreases. This may lead to a slightly negative slope when there are no bacteria in sample.

1.6.2 Positioning Samples

For low concentration samples, cuvettes often will give slightly different, but not significant, measurements depending upon their orientation in the sample compartment. This is due to defects in the shape of the cuvette that are not visible to the human eye. Avoid touching the lower part of the cuvette (detection area - sensitive to dirt/fingerprints).

1.6.3 Data Quality

The Micro Detector is only as accurate as the standards that are used to calibrate it. This is why it is important to take care when working with standards, samples and blank.

1.7 Testing and results

1.7.1 Slope linearity

The readings during the test run should have an approximate linear increase in fluorescence (if there are bacteria in sample). Readings with values significantly below the previous reading could have one of the following explanations:

1. NaOH solution was not added to sub sample or the content in cuvette was not mixed before reading.
2. The volume of sub sample in cuvette is not correct.
3. The cuvette is dirty.
4. The instrument reading cell is dirty.
5. The incubator temperature is not correct or it was not pre-warmed before start.
6. The time between adding NaOH and reading results exceeded 3 minutes.

1.7.2 Negative readings

The chemical content in the water sample may be different from the pre-added water in the calibration solutions. This may lead to negative values for sample readings but should not alter the slope result. Calculate the increase in fluorescence. Negative slope value: See 1.6.1 Temperature Considerations. Storage time and storage temperature could alter the background fluorescence in the Colifast media. Remember to calibrate from time to time. It is recommended to pre-warm blank and Cal48 to 44 °C before calibration (to avoid negative

readings). See 1.6.1 Temperature considerations. Samples with no coliforms normally gives negative slope values.

1.7.3 Growth test gives high fluorescence value, but below 200

There is enzymatic activity in the sample but no growth after incubation for 9 or 11 hours. Set sample back in incubator and do a new reading after a total of 12 hours. If still not positive (200 or over) there is no cultivable bacteria in the sample. The recorded enzyme activity may originate from dead or injured bacteria. High concentrations of some type of algae are also known to produce fluorescence. Remember to add Na-thiosulfate to ensure bacterial growth in samples containing chlorine. Also remember to control temperature in incubator.

1.7.4 Inconsistent results

The values read by the instrument are based on the values from the calibration. The calibration solutions or the selected calibration value might be wrong. Check and set correct value and recalibrate if necessary. Check 1 - 6 in 1.7.1 Slope linearity. Check reagents for contamination. NaOH solutions, calibration solutions and unused pre-filled test vials should not be used if they contain visible growth/turbidity. This could lead to error in calibration or false test results. Note: When stored cold the reagents could develop some turbidity due to precipitation. This turbidity should disappear when the temperature rises.

1.7.5 Water samples and correlation

Sunlight and high temperatures might kill or damage bacteria. Water samples should be collected from water in motion and preferably below the surface. The holding time and storage conditions of the water sample can have a significant impact on the number of bacteria in the sample. Samples should be stored cold (water source temperature) and dark and the holding time should not exceed 8 hours. The guideline for slope value related to bacterial number is based on data from river water. Water from other sources may not correlate to this guideline.

1.8 Instrument and equipment

1.8.1 CMD Value <OVER>

The Colifast Micro Detector can read up to approximately 800 ppbMU. When the bacterial levels in the sample is very high (over 300 000 per 100 mL), the fluorescence can increase to over 800 during the 2 hour test run. Calculate the slope value based on the results collected before the <OVER> reading. Remember that slope equals increase in fluorescence per hour. When performing the bacterial growth test with prolonged incubation, the <OVER> value can be considered as positive.

1.8.2 CMD Message: <Recalibrate>

The calibration was not accepted by the instrument. Try again with new cuvettes containing Blank and Cal48. Remember NaOH solution and mixing. Check 1 - 6 in 1.7.1 Slope linearity.

1.8.3 CMD low battery level or no power

1. On the back of the instrument, loosen the screw and remove the battery panel.
2. Install 4 AAA batteries into the appropriate spaces.
3. Replace the battery panel and tighten the screw. The panel has an o-ring, which creates a watertight seal. The battery panel may be difficult to install if there is no lubrication on the o-ring. Use a silicon based grease to lightly lubricate the o-ring if necessary.

1.8.4 Incubator temperature is not 44 °C

The incubator needs to be on for approximately 60 minutes to reach 44 °C. Check that the unit has power (light in on/off switch). Check that the door is properly closed. If the temperature is still incorrect after 90 minutes, adjust the temperature. Use a screwdriver or coin to rotate the yellow button (1/4 turn = ca 5 °C). Follow the arrows to adjust the temperature up (clockwise) or down (counter-clockwise). The surrounding temperature can have an impact on the incubator temperature. The incubator should not be used at temperatures below 10 °C and above 40 °C.

1.8.5 12-220 V converter error

Check converter power switch (and green led lamp) and 12 V car contact (battery power). The converter turns off (noise alarm) if the battery voltage becomes to low. Start the car or charge battery. It also turns off (noise alarm) if the load is too big. Remove other equipment connected to converter and turn it off for a couple of minutes. Turn it on and check status (green led). When it is back to normal operation, continue incubation.

6 Content

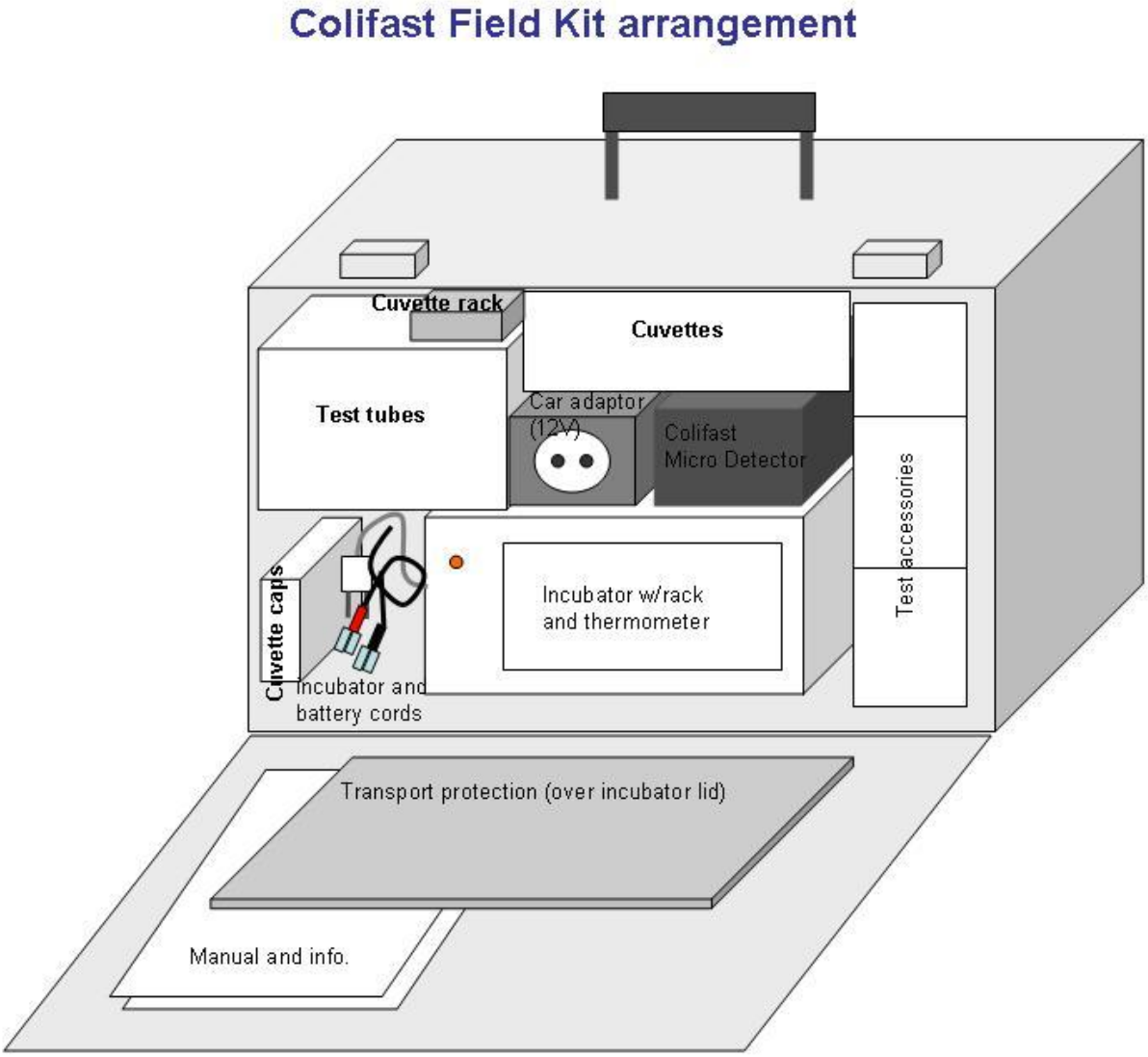
- **1 Colifast Micro Detector with storage pouch**
- **1 incubator with thermometer. 220 V, 44 ºC**
- **1 car converter 12 V – 220 V**
- **1 cable for battery poles**

- **18 prefilled vials with Colifast 6 medium**
- **2 dropper bottles with 0,5 M NaOH solution**
- **1 vial with ”blank” (calibration)**
- **1 vial with ”cal48” (calibration)**
- **1 dropper bottle with 10% Na-thiosulfate solution (chlorine neutralization)**

- **100 cuvettes (disposable)**
- **100 cuvette caps (disposable)**
- **1 cuvette rack**
- **20 large plastic pipettes for sub-sampling (3 mL, one for each test)**
- **3 sterile 10 mL syringes for addition of water sample (can be reused after wash)**
- **Ethanol tissues (disinfection)**
- **2 sterile filters (for control samples, fits on syringe)**

- **1 quick guide**
- **10 result sheets**
- **Manual**

7 Field Kit Arrangement



Test accessories

Pastettes			
NaOH dropper bottles		Syringes	
Virkon	Sterile filters	Na-thios. Dropper bottle	

8 Appendix

- Quick guide – Rapid Screening Test
- Quick guide – Most Probable Number test (MPN)
- Quick guide – Presence / Absence
- Quick guide – PA
- Safety Data Sheets