TECHNICAL DATA SHEET

MICROPLATE MUG/EC

ENUMERATION OF *ESCHERICHIA COLI* IN WATER

1 INTENDED USE

Microplate MUG/EC allows the practical identification and enumeration of *Escherichia coli* in water, according to ISO standard NF EN ISO 9308-3.

The use of microtiter plates, with wells containing a specifically developed medium, was conceived for use in the analysis of several types of water, notably swimming and beach water in salt and fresh water, surface (still) water and post-treatment water. The method is applicable to all water samples, including those rich in suspended matter. For the enumeration of *Escherichia coli*, the microtiter technique with MUG has been recognized as a more specific, precise, and rapid compared to previously used method. By virtue of its role as an indicator microorganism of fecal contamination, this bacteria is of special interest due to its exclusively intestinal origin, which confers on it a more particular status as a priority bioindicator in water quality controls.

2 HISTORY

Buehler et al, in 1949, were the first to reveal the presence of a β-D-glucuronidase activity in *Escherichia coli*. In 1976, Kilian and Bülow demonstrated this enzymatic activity characterized only the genera *Escherichia*, *Shigella* and *Salmonella*. From these observations, Feng and Hartman, in 1982, developed procedures for the specific detection of *Escherichia coli* in water and food products. Following this, Trepeta and Edberg incorporated MUG in a culture medium in order to detect the presence of β-glucuronidase. The majority of studies have shown that from 94 to 97 % of *Escherichia coli* of human or environmental origin possess this activity. Recent data also shows that this enzyme can be detected in certain species of *Citrobacter*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella* and *Yersinia*, but its presence only concerns a limited number of strains in each of the aforementioned genera. The microtiter technique, as well as the statistical method of interpreting the results, was used and validated by Hernandez in 1988, with the aid of 5 partner laboratories for a comparative study between currently used methods and the new miniaturized fluorogenic method, on sea water off the French coasts. The level of recovery was found to be equal or superior to other methods in tubes or by membrane filtration. In particular, the miniaturized method has shown a higher specificity than membrane filtration. β-D-glucuronidase can therefore be considered as a reliable indicator for the detection of *Escherichia coli* in water.

3 PRINCIPLES

Each microplate contains 96 wells (12 rows of 8 wells).

The substrate demonstrating the bacterial enzymatic activity in question is MUG (4-methylumbelliferyl-β-D-glucuronide). This component is incorporated in a culture medium derived from A1 Medium cited by the American Public Health Association for the determination of the presence of fecal coliforms in water. The culture medium is dehydrated and fixed to the bottom of the microtiter wells. Actual rehydration of the medium is achieved when the water sample itself is introduced into the wells. *Escherichia coli*, eventually present in the sample inoculum hydrolyses the MUG into 4-methylumbelliferone and its glucuronide constituent. The production of 4-methylumbelliferone, indicated by a blue fluorescence, can be observed with the aid of a UV lamp at 366 nm. Once reading of the wells has been performed, the number of fluorescent wells is counted for each dilution. From an obtained characteristic number, a statistical analysis, based on Poisson's law allows the calculation of *Escherichia coli* in the analyzed sample. It is important to note that certain strains of *Escherichia coli* do not possess β-D-glucuronidase, in particular the enterohemorrhagic strain O157:H7.

The media composition, with its high level of peptone and salicin, allows excellent recuperation.

Triton X favors microorganism and fluorogen dispersion in the wells of the microtiter plate.

Incubation at 44°C was studied in order to inhibit the growth of the majority of contaminating microorganisms.

4 TYPICAL COMPOSITION

Each microtiter plate well is filled with 100 µL of medium, of which the formula can be adjusted to obtain optimal performances.

For 1 liter of media:

- Tryptone ..... 40,0 g
- Salicin ..... 1,0 g
- Triton X 100 ..... 1,0 g
- MUG ..... 0,1 g

pH of the ready-to-use media at 25 °C: 6,9 ± 0,2.

5 INSTRUCTIONS FOR USE

In order to proceed with the analysis of samples from chlorinated, brominated or ozonized waters, it is necessary to add, by sterile methods, excess sodium thiosulfate in the collecting container to neutralize the oxidants. In this way, total recuperation of the microorganisms to be detected can be obtained.

Preparation and dilutions

- Mix the sample well in order to obtain a homogeneous repartition of the microorganisms.
- For fresh water, bathing water and other surface water (salinity less than 30 g/Kg), use 18 mL of the special microplate diluent (Synthetic Sea Salt - BR003 or BM088).
- For sea water with a salinity greater than 30g/Kg (measured with the aid of a refractometer), use sterile distilled water (BM115) as a diluent.
- Perform successive serial dilutions with the Synthetic Sea Salt. The number of dilutions to be inoculated is a function of the level of contamination in the tested sample.
- The detection ranges for the number of microorganisms present in each sample category are listed in the following table:

Nature of the sample	Number of dilutions	Number of wells / dilution	Detection range (microorganisms / 100 mL)
Bathing / swimming water	2	64 wells at 1:2 32 wells at 1:20	1,5 x 10^1 to 3,5 x 10^4
Other surface water	4	24 wells at 1:2 24 wells at 1:20 24 wells at 1:200 24 wells at 1:2000	4,0 x 10^1 to 3,2 x 10^6
Effluent and post-treatment waters	6	16 wells at 1:2 up to 16 wells at 1:200 000	6,0 x 10^1 to 6,7 x 10^8

Inoculation

- Transfer the initial dilution into an appropriate sterile container.
- By using a multi-channel pipette (8 sterile tips), inoculate 200µL into each microtiter plate well.
- In the same fashion, inoculate the subsequent dilutions (1:20, 1:200, 1:2000, etc.) by using a new recipient and new sterile pipette tips.
- Well inoculation should be performed with care in order to avoid cross-contamination.
- Cover each microtiter plate with a sterile adhesive cover furnished in the kit. This measure limits dehydration of the media in the wells and protects the plate from external contamination during the incubation period.

✓ **Répartition:**
200 µL par puits

✓ **Incubation:**
36 à 72 h à 44 °C

Incubation

Incubate the microplates at 44,0 ± 1,0 °C for at least 36 hours, not exceeding a maximum of 72 hours.

6 RESULTS

The wells showing a blue fluorescence under 366 nm UV light are considered positive. Reading may be performed after the minimal period of incubation as the fluorescence does not diminish over time.
The opaque microtiter plates (BT001) were developed for a visual reading and manual counting.

See ANNEX 1: PHOTO SUPPORT.

Determine the characteristic number from the number of positive wells for each of the chosen dilutions. In the event that more than 3 dilutions are inoculated, a characteristic number comprised of 3 numbers (if possible ending in 0) should be retained.
Refer to the annex of method NF EN ISO 9308-3.

NOTE:
An Excel file can be furnished on demand for the calculation of the MPN determination and confidence intervals.

7 QUALITY CONTROL

Fluorescence after incubation for 48 hours at 44 ±1 °C:

Analysis	Result / Growth
Background: distribution sterile DSM	Absence of positive wells. Background average < 25 % of positive threshold
Average level of fluorescence with *Escherichia coli* WDCM 00179	Fluorescence greater than the double of the positive threshold (variation < 10 %)
Fertility, Microorganisms	Growth / Level of recovery
Escherichia coli WDCM 00179	66 to 150 % of the target value

8 STORAGE / SHELF LIFE

Microplates: 2-8 °C.
The expiration date is indicated on the label.

9 PACKAGING

White, opaque Microplates :
25 microplates + 25 sterile, transparent adhesive covers BT00108

10 BIBLIOGRAPHY

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11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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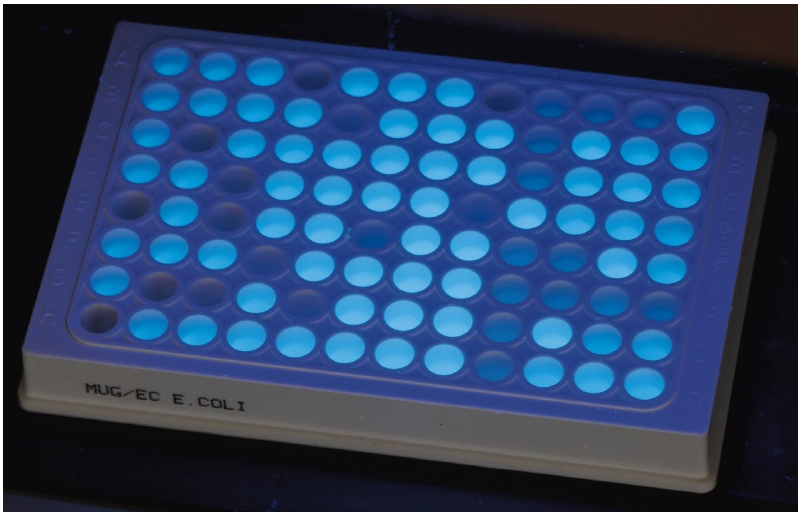
ANNEX 1 : PHOTO SUPPORT

Microplate MUG/EC

Detection and enumeration of *Escherichia coli* in water, following the method NF EN ISO 9308-3.

Results :

Growth obtained after 36 hours of incubation at 44 °C.



Characteristics : Wells presenting a blue fluorescence under UV light at 366 nm are considered positive (presence of *Escherichia coli* in water).