MICROPLATE MUD/SF

INTENDED USE

Microplate MUD/SF allows the practical identification and enumeration of intestinal enterococci in water, according to NF EN ISO 7899-1. 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 enterococci, the microtiter technique with MUD has been recognized as the most specific, precise, and rapid of all previously used methods. This technique represents an important evolution as regards to existing technologies for enterococci, which among the indicator microorganisms of fecal contamination, present a particular interest in water quality controls.

HISTORY

Among the numerous methods used for the isolation and enumeration of Group D streptococci, GTC media, originally developed by Donnelly & Hartman in 1978 was very selective, but did not allow the separation of fecal streptococci from non-fecal streptococci. The use of fluorogenic substrates has attracted numerous studies for the identification of microorganisms via simple and rapid procedures. In 1982, Littel & Hartman selected, from amongst numerous fluorogenic substrates, the one that allowed the characterization and differentiation of fecal streptococci from other streptococci. They demonstrated that 4-methylumbelliferyl-β-D-glucoside (MUD) was hydrolyzed by nearly all fecal streptococci with the exception of Streptococcus mitis. 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. This method can therefore be considered as a reliable means to detect enterococci in water.

PRINCIPLES

- Each microtiter plate contains 96 wells (12 rows of 8 wells).
- The substrate demonstrating the bacterial enzymatic activity in question is MUD (4-methylumbelliferyl-β-D-glucoside). This component is incorporated in a culture media specifically designed for the detection of enterococci. 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. Enterococci eventually present in the sample inoculum, hydrolyze the MUD into 4-methylumbelliferone and glucose. 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 enterococci in the analyzed sample.
- The media composition, with its high level of peptone and galactose, allows excellent recuperation.
- Polysorbate, monopotassium phosphate and sodium hydrogenocarbonate increase the performance of the medium.
- Nalidixic acid blocks replication of DNA in bacteria sensitive to it and thallium acetate inhibits nearly all other contaminating microflora.
- Incubation at 44°C was studied in order to inhibit the growth of the majority of contaminating microorganisms.

**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 oxidents. 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.
- Proceed with an initial 1:2 dilution by transferring 18 mL of the sample in a tube containing 18 mL of diluent: reconstituted synthetic sea salt (without bromophenol blue) BR003, or ready-to-use Synthetic sea salt (with bromophenol blue indicator).
  - For fresh water, bathing water and other surface water (salinity less than 30 g/Kg), use tubes containing 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 diluent BR003, BM088 or BM115. 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:

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Number of dilutions</th>
<th>Number of wells per dilution</th>
<th>Detection range for microorganisms present (per 100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bathing / swimming water</td>
<td>2</td>
<td>64 wells at 1:2 32 wells at 1:20</td>
<td>1.5 x 10^1 to 3.5 x 10^4</td>
</tr>
<tr>
<td>Standing fresh water</td>
<td>4</td>
<td>24 wells at 1:2 24 wells at 1:20 24 wells at 1:200 24 wells at 1:2000</td>
<td>4.0 x 10^1 to 3.2 x 10^6</td>
</tr>
<tr>
<td>Effluent and post-treatment waters</td>
<td>6</td>
<td>16 wells at 1:2 up to 16 wells at 1:200000</td>
<td>6.0 x 10^1 to 6.7 x 10^8</td>
</tr>
</tbody>
</table>
**SAMPLE INOCULATION**

- Transfer the initial dilution into an appropriate sterile container.
- By using a multi-channel pipette (8 sterile tips), inoculate 200 μL 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.

**INCUBATION**

Incubate the microtiter plates at (44°C ± 0.5)°C for at least 36 hours, not exceeding a maximum of 72 hours.

**READING**

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 (BT003) were developed for a visual reading and manual counting.

**RESULTS**

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.

**MPN (Most Probable Number) and CONFIDENCE INTERVAL CALCULATION**

- The most probable number represents a statistical estimation of the concentration of microorganisms in a given sample. This estimation follows Poisson's Law. The upper and lower limits correspond to a 95% confidence level.
- In the case of bathing water where 2 successive dilutions at 1:2 and 1:20 are inoculated into 64 and 32 wells, respectively, the following example demonstrates the determination of the characteristic number, the MPN and its upper and lower limits.

\[
\begin{align*}
1 : 2 & \text{ dilution : 25 positive wells out of 64} \\
1 : 20 & \text{ dilution : 3 positive wells out of 32}
\end{align*}
\]

The characteristic number is **25/3**

In referring to the statistical table furnished in Annex 1, the example **25/3** indicates:

- MPN : 524 enterococci / 100 mL
- Lower limit : 360 enterococci / 100 mL
- Upper limit : 763 enterococci / 100 mL

For all other cases, refer to the annexes A and B of norm NF EN ISO 7899-1 : March 1999. For still water and effluent testing, MPN tables are supplied upon demand.
TYPICAL COMPOSITION

MUD/SF medium
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 medium:
- Tryptose .......................................................... 40.0 g
- Galactose .......................................................... 2.0 g
- Polysorbate (Tween 80) ..................................... 1.5 mL
- Monopotassium phosphate ............................. 10.0 g
- Sodium hydrogenocarbonate ........................ 4.0 g
- Nalidixic acid .................................................. 0.25 g
- Thallium acetate .............................................. 2.0 g
- 2,3,5 triphenyltetrazolium chloride (TTC) ........ 0.1 g
- MUD ............................................................. 0.15 g

pH : 7.5 ± 0.2

Synthetic Sea Salt
The diluent is composed of synthetic sea salt, presented in dehydrated form without bromophenol blue, BR003, or as ready-to-use 18 mL tubes with bromophenol blue, BM088.

The reconstitution of the dehydrated diluent is as follows:
- Dissolve 22.5 g of synthetic sea salt in 1 liter of distilled or demineralized water.
- Mix slowly until complete dissolution.
- Distribute into 20 x 200 mm tubes; 9 mL or 18 mL per tube, according to the specific need.
- Sterilize in an autoclave at 121°C for 15 minutes.

NOTE:
After preparation, the medium is clear and limpid. Occasionally after prolonged storage, a slight precipitate can be observed that has no incidence on the performance of the method.

QUALITY CONTROL (following NF EN ISO 7899-1: March 1999, annex E):
- Background less than 25% of the positive threshold.
- Fluorescence more than twice the positive threshold.
- Fertility: 0.66 to 1.5 times the target value.
- Interfering microorganisms: fluorescence less than 25% of the positive threshold; absence of turbidity or formazan precipitate.
 STORAGE / SHELF LIFE

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

Synthetic sea salt (without bromophenol blue), dehydrated: 2-30°C.
- The expiration date is indicated on the label.

Synthetic sea salt (without bromophenol blue), reconstituted (benchmark value*):
- Medium in vials or in tubes: 6 months at 2-25°C.

Diluent (Synthetic sea salt, with bromophenol blue), ready-to-use,
Sterile distilled water, ready to use:
- Store between 2-25°C, shielded from light.
- The expiration dates are indicated on the labels.

PRESENTATION

Microplates, solid white
- Kit containing 25 microtiter plates +
  25 sterile transparent adhesive covers

Synthetic Sea Salt (without bromophenol blue), dehydrated:
- 100 g bottle

Synthetic Sea Salt (with 0.004 g/L bromophenol blue), ready-to-use:
- 50 x 18 mL tubes

Sterile distilled water, ready-to-use:
- 50 x 18 mL tubes

BIBLIOGRAPHY


**SUPPORT PHOTO**

**Reference**: BT00308

**Intended use**: Identification and enumeration of intestinal enterococci in water, according to NF EN ISO 7899-1.

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**Enterococci in water**

**Microplate MUD/SF**

**Ref.**: BT00308

**Incubation**: 36 hours / 44°C

**Characteristics**: Wells demonstrating a blue fluorescence under UV light at 366nm are considered positive (presence of enterococci in the water sample).

*Benchmark value refers to the expected shelf life when prepared under standard laboratory conditions following manufacturer’s instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.*

The information provided on the package or instructions takes precedence over the formulations or instructions described in this document. The information and specifications contained in this technical data sheet date from 2009-02-17. They are susceptible to modification at any time, without warning.