TECHNICAL DATA SHEET

MICROPLATE MUD/SF

DETECTION AND ENUMERATION OF ENTEROCOCCI IN WATER

1 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.

2 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.

3 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.

4 TYPICAL COMPOSITION

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

For 1 liter of media:

- 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 of the ready-to-use media at 25 °C: 7,5 ± 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 tubes containing 18 mL of the special microplate diluent (Synthetic Sea Salt - BR003 or BM088) with or without bromocresol blue and make a first 1:10 dilution.
- For sea water with a salinity greater than 30g/Kg (measured with the aide of a refractometer), use sterile distilled water (BM115) as a diluent.
- Perform successive dilution with synthetic sea salt. The number of dilutions to inoculate is a function of the nature and the contamination level of the water.
- Les dilutions and 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	Range of measures (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 water	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

Sample 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.

✓ **Repartition :**
200 µL per well

✓ **Incubation:**
36 to 72 h at 44 °C

- 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 44,0 ± 1,0 °C for 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 (BT003) were developed for a visual reading and manual counting manuel.

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 the standard NF EN ISO 7899-1.

NOTE:
An Excel file can be furnished on demand to determine the MPN and confidence levels.

7 QUALITY CONTROL

Fluorescence after incubation for 48 hours at 44 °C:

Analysis		Result / Growth
Background: distribution sterile DSM		Absence of positive wells, Average background < 25 % of positive threshold
Average level of fluorescence with *Enterococcus faecalis*	WDCM 00176	Fluorescence greater than the double of the positive threshold (variation < 10 %)
Fertility, Microorganisms		**Growth / Recovery level**
Enterococcus faecalis	WDCM 00176	*66 to 150 % of the target level*
Enterococcus faecium	WDCM 00178	*66 to 150 % of the target level*
Enterococcus hirae	WDCM 00089	*66 to 150 % of the target level*
Aerococcus viridans	WDCM 00061	Fluorescence < 25 % of the positive threshold
Lactococcus lactis	WDCM 00016	Fluorescence < 25 % of the positive threshold
Staphylococcus epidermidis	WDCM 00132	Fluorescence < 25 % of the positive threshold

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 BT00308

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.

Document code : MICROPLATES MUD SF_Env8.
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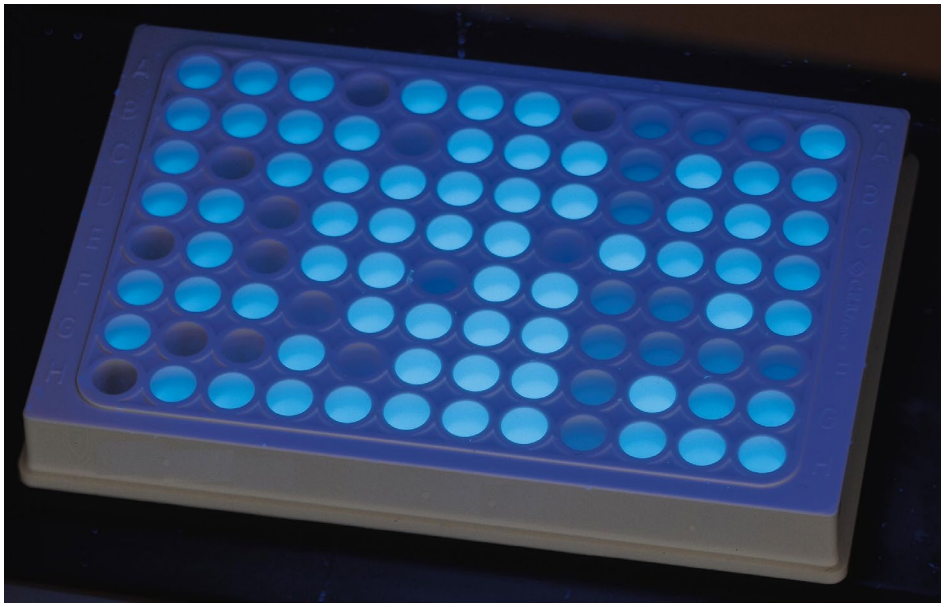
ANNEX 1: PHOTO SUPPORT

Microplate MUD/SF

Detection and enumeration of enterococci in water, following the method NF EN ISO 7899-1.

Results:

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



Characteristics: The wells presenting a blue fluorescence under UV light at 366 nm are considered positive.