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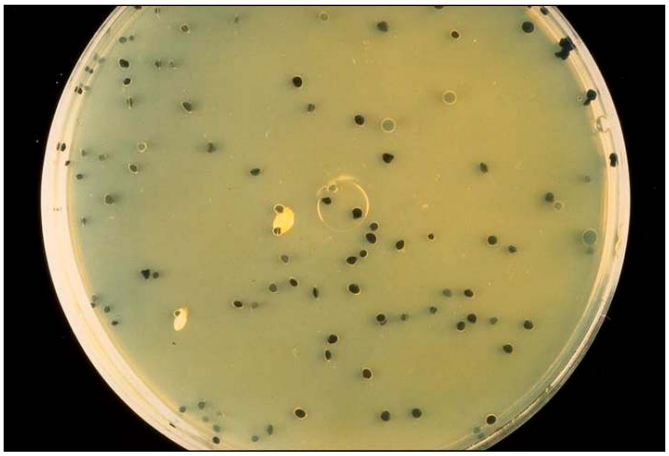
TSC AGAR (BASE) and TSC-MUP Agar
AGAR TRIPTONE-SULPHITE-CICLOSERINE

Detection and enumeration of *Clostridium perfringens* (UNE IN 13401:2000, UNE-IN 26461-2:1995, ISO/CD 6461-2:2002)

COMPOSITION

Triptose	15.00 g
Soya papainic peptone	5.0 g
Yeast extract	5.0 g
Ammonial ferric citrate	1.0 g
Sodium metabisulphite	1.0 g
Agar-agar	18.0 g

(Formula per liter)
Final pH : 7.6 ± 0.2

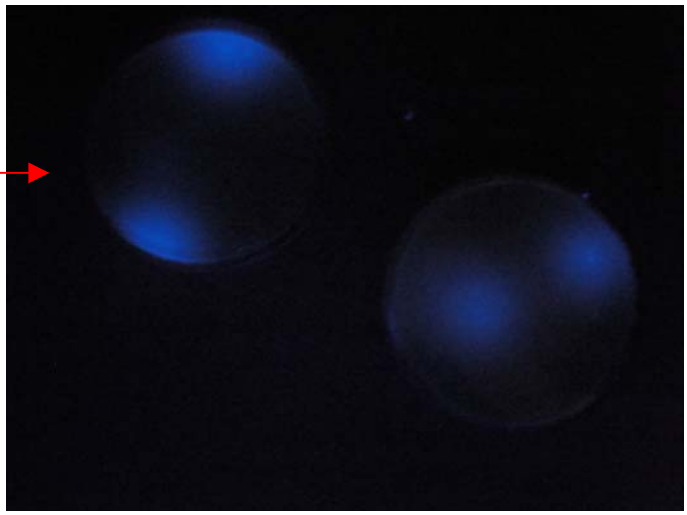


PREPARATION

Dissolve 45 g of medium in 1 l of bidestiled water. Heat, shaking, till boiling point, for its total homogenisation. Autoclave to 121 ºC during 15 minutes or better to 116 ºC, during 15 minutes. Final colour of medium is cream. Cool down to 50 ºC and add asepticaly 400 mg of D- Cicloserine Sterile (4 vials of 100 mg of SMS252). Pour immediately in plates and no overheat. Use immediately to its preparation to avoid its lethal oxigenation.

NOTE 1: To improve the growing of black colonies of Clostridios, add 1 vial of sterile supplement VMT136 to each 100-1000 ml of sterile media, boiled for desoxigenating itand cool down to 45-50 ºC.

NOTE 2: To follow next ISO,Normative and UE Directive add to each 500 ml of medium (cold at 50°C), 55-100 mg of MUP (Metil-Umbeliferil Phosfate suppl, Ref: MICROKIT SMT009); it reactions with *Cl.perfringens* colonies showing a fluorescence (yellow or blue) under 366 nm UVA light (MICROKIT lantern



VMT050).

FOR EXCLUSIVE USE IN LABORATORY. KEEP BOTTLE CLOSE IN A DRY, FRESH AND DARK PLACE. SHAKE BOTTLE BEFORE USE IT.
DEHYDRATED CODE: DMT175, ready to use plates with MUP: PPLM29

QUALITY CONTROL

Elaborated in our laboratory; it is prudent repeat in your laboratory always conditions change (more than three months without use it, after disinfect your laboratory, after keep it to higher temperature, when it acquires bizard aspect although expire date is correct,…)
DEHYDRATED: Thick powder , Yellow
PREPARED: Sterile, Beige
CUANTITATIVE GROWING CONTROL 24-48 h to **44**°C more or less, in anaerobiosis:
Clostridium perfringens MKTA 13124, Grey-black colonies. Regarding to standard* PCA, counting 75-252%, but in a more selective and differential way.
Bacillus subtillis MKTA 6633, Inhibited
Staphylococcus aureus MKTA 6538P, Inhibited.
E.coli MKTA 25922, Inhibited.
* The one which carry out with a recuperation higher than 92-125% according to cuantitative strains traceable to type strain. Uncertainties detected among all batches during a year (the most of uncertainties is due to strain and its inoculated companion strain, not to the media).

PRESENTATION: DEHYDRATED MEDIA (BASE) and SUPPLEMENTS. Also there are prepared tubes, ready to use Plates with MUP, hermetic small Plaquis and paraffined big bottles 200 ml for counting in 100 ml of sample). In liquid version, vials MF and puncturable vials.

INSTRUCTIONS AND INTERPRETATION OF RESULTS

Modification of TSN more selective and with less diffusion of darkness. Spread with Digralsky loop in surface 0'1 ml of sample and its decimal serial dilutions, adding a second layer of TSC. Or better spread in depth 1 ml even in tube or 100 ml in paraffined big bottle. Incubate 18-20 hours to 44-46 ºC aprox, in anaerobiosis and count as *Clostridium perfringens* all black colonie. White or grey colonies are suspected if anaerobiosis is not correct. In medium with MUP, count fluorescent colonies. For counting in water, according UNE-EN 26461-2, heat the sample 15 minutes to 70-80 ºC. Filter 100 ml of water across 0,22 µm (VAC022). Add in Petri plates and pour 18 ml of medium cool down to 50 ºC (and if you wish, prepared to double concentration), without form bubbles. To spread directly without filtration, of chlorined water, add 0.6 g of Sodium tiosulphate to inactivate the chlorine. Incubate 16-24 h and 40-48 hours to 36-38 ºC aprox or, more selectively, to 43-45°C aprox (it is quickler than SPS), in anaerobiosis if membrane is not in depth.

Final user is the only responsible of elimination of microrganism according current environmental legislation. Autoclave before throw it to the rubbish.

Last review in April-2013