ALLIANCE BIO EXPERTISE

Reference: BCWR3016 **A.B.E. - Technical Data Sheet**

Product: **VRBL + TLHTh Agar**

Specification

Selective and diferential medium with neutralisers, for the detection and enumeration of coliforms.

Presentation

	Packaging Details	Shelf Life	Storage
30 Contact Plates Contact Plates - Double Wrapping with: 15 ± 2 ml	1 box with 5 blisters (PET laminated and PPBO bag) with 6 contact plates/blister.	7 months	2-25 ºC

Composition

Composition (g/l):

Yeast extract	3.000
Peptone from gelatin	7.000
Bile salts mixture	1.500
Lactose	10.00
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Lecithine	0.700
Polysorbate 80	5.000
Histidin	1.000
Sodium Thiosulfate $5H_2O$	0.500
Agar	13.000

Description /Technique

Description

The Violet Red Bile Agar corresponds to the classic formulation of standardized media for the screening of coliforms in milk and other dairy products. This medium has been adopted for the enumeration of coliforms as well as for differentiating between lactose-fermenting and non-lactose fermenting organisms, due to its contents of crystal violet and bile salts, whose inhibiting or selective properties have been widely confirmed.

The addition of he neutralizing agents TLHTh (Tween 80 - Lecithin - Histidine - Sodium Thiosulphate) may inactivate a variety of disinfectants.

* The combination of lecithin, polysorbate 80 and histidine neutralizes aldehydes and phenolic compounds.
* The combination of lecithin and polysorbate 80 neutralizes the quaternary ammonium compounds.
* The polysorbate 80 neutralizes hexachlorophene and mercurial derivates.
* Sodium thiosulphate neutralizers halogen compounds.
* Lecithin neutralizes clorhexidine.
* Histidine neutralizes formaldehyde.

Technique

Contact plates are used in the microbiological control of disinfection and cleaning of surfaces. It acts simultaneously as a sampler and incubation culture medium without the need for any other intermediate steps.

The plates come in a form appropriate for this function and can be used with different culture media depending on the type of microbe that needs to be controlled. On average the plates provide a contact surface of approximately 25 cm2.

To use, remove the cover and gently press the culture medium on the surface to be controlled, ensuring contact between the two surfaces. The Contact plate is removed and covered with the lid to prevent air contamination. It is advisable that the lid is secured with adhesive tape and the bottom labelled with the sampling data (place, date and time).

If the sample surfaces are rough, the contact plates will not make good contact, even when the pressure is increased. In these cases it is advisable to delineate an sample surface area of 25 cm squared and rub this area vigorously with a wet sterile swab and then rub the swab over the Contact plate.

If verifying the effectiveness of a cleaning or disinfection process, contact plates should be used within two hours after the end of the process, ensuring that the sample surface is dry. It is advisable to always include positive controls, sampling the area before disinfection or dirty areas beside the disinfected area.

The technician will determine the frequency of sampling and disinfection according to performance criteria. Apply the agar directly onto surface to be monitored ensuring that the pressure is distributed over the whole plate for 10 seconds. Clean the surface where the sample was collected in order to remove any traces of agar.

The inoculated plates are incubated at 30±1º C for 18-24 h.

Note: Contact plates are used for monitoring the microbiological contamination of surface and air inside cleanrooms, isolators, RABS, food industries and hospitals. The double/triple irradiated wrapping ensures that the package itself doesn't contaminate the environment as the first wrapper is removed just before entering the clean area.

The plates must be kept in their original packaging (blisters) to guarantee their stability at the end of their expiration date.

Quality control

Physical/Chemical control

Color : Red - Brownish pH: 7.4 ± 0.2 at 25ºC

Microbiological control

Inoculate: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)/ 10^4-10^6 (selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 30 ± 1 ºC during 25 ± 1 h.

Microorganism	Growth
Enterococcus faecalis ATCC® 19433, WDCM 00009	Inhibited
Ps. aeruginosa ATCC® 9027, WDCM 00026	Colourless to beige colonies
Salmonella typhimurium ATCC® 14028, WDCM 00031	Colourless to beige colonies
Escherichia coli ATCC® 8739, WDCM 00012	Good (≥50%)- Red purple colonies
Escherichia coli ATCC® 25922, WDCM 00013	Good (≥50%)- Red purple colonies

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA, Washington. DC.
· FIL-IDF. (1998) Standard 73B. Enumeration of coliform bacteria. ICMSF (1978). Microorganisms in Food, University of Toronto Press.
· ISO (1986) Standard 5541-1 Milk and Milk Products. enumeration of coliforms. Colony-count technique at 30ºC.
· ISO (2006) Standard 4832: 2006 (E) - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliformes - Colony-count technique.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA, Washington. DC.
· PASCUAL ANDERSON, Mª R. (1992) Microbiología Alimentaria. Díaz de Santos, S.A., Madrid.