M17 AGAR

For selective enumeration of *Streptococcus thermophilus* from yoghurt and for the improved growth of lactic streptococci and their bacteriophages

Typical formula (g/l)

Tryptic Digest of Casein	2.50
Peptone	2.50
Soy Peptone	5.00
Yeast Extract	2.50
Beef Extract	5.00
Sodium Glycerophosphate	19.00
Magnesium Sulphate	0.25
Ascorbic Acid	0.50
Lactose	5.00
Agar	13.00

DIRECTIONS

Suspend 55.2g in 1000ml of cold distilled water. Heat to boiling, distribute and sterilise by autoclaving at 121˚C for 15 minutes.
Final pH 7.0 ± 0.2

DESCRIPTION

M17 Agar is used for the isolation and the enumeration of the lactic streptococci from yoghurt, cheese, starter cultures and other dairy products. The medium is recommended by International Dairy Federation, for the selective isolation and enumeration of *S. thermophilus* in yoghurt.
The high concentration of sodium glycerophosphate inhibits the growth of *L.bulgaricus* while the high buffering power allows the cultivation of lactic streptococci. M17 Agar is prepared according to the formulation described by Terzaghi and Sandine and IDF. Terzaghi and Sandine recommend M17 Agar also for the demonstration of lactic bacteriophage activity. When this method is adopted (for details see the relevant reference: Appl. Microbiol 29,807, 1975), 100ml of medium is supplemented with 10ml $CaCl.6H_2O$ 1.0 M.

TECHNIQUE

The enumeration of *S. thermophilus* in yoghurt is carried out according to the following procedure:

1. Accurately mix the contents of the yoghurt pot by using a sterile spatula. In the case of fruit-yoghurt homogenise the contents of the pot for one minute.
2. Weigh 10g of product in an appropriate 200ml bottle and bring to 50g with an autoclaved peptone solution having the following composition:

Tryptone	0.5g
Peptone	0.5g
Distilled water	1000ml

3. Homogenise for 1 minute. Bring to 100g by using the same diluent a 1:10 dilution being this obtained.
4. Prepare a suitable series of decimal dilutions of the sample suspension in 9ml of 0.1% peptone solution.
5. From each tube, pipette 1ml of the appropriate dilution in a 90 or 100mm Petri dish in duplicate.
6. Add 14ml of Ml7 Agar, cooled to 43°C +/-1 to each dish. Mix and leave to solidify.
7. Incubate for two days at 35°C for 48 hours.
8. Examine the plates after 24 and 48 hours incubation. Under these conditions *S. thermophilus* grows with lenticulate appraisable colonies after 18-24 hours and reach 1-2mm diameter within 48 hours. *L. bulgaricus* may grow in the form of very small colonies.
9. Carry out the enumeration in each Petri dish that does not contain more than 400 colonies and express the result taking the dilution factor into account as the number of colony forming units per gram of specimen.
10. From each dish considered suitable for the expression of results, take up a number of colonies correspondent to the square root of the total number examined. Lay on a slide and perform a Gram stain to verify that these are Gram-positive cocci. Perform catalase test (negative).

For the assay of phage activity, consult the paper of Terzaghi and Sandine.

User quality assurance (37°C - 48hrs)
Productivity Control
S.thermophilus ATCC 14485: growth
Selectivity control
L.bulgaricus ATCC 11842: partially or completely inhibited

Storage
Dehydrated medium: 10-30°C

REFERENCES
• International Dairy Federation (1981) Joint IDF/1 SO/AOAC Group E 44
• Terzaghi, B.E., Sandine, W.E. (1975) App. Microbiol. **29**, 807

PACKAGING
4017192 M17 Agar 500 g (9.1 l)