¼ Strength Ringers Solution Media & Tablets

Codes:

Media: KM100
Tablets: KM100T

Description

An osmotically controlled solution for the preparation of suspensions of food samples and for use as a diluent in dilution techniques for bacterial enumeration. The solution can also be used in the sampling of food production apparatus by the rinse and swab technique.

Typical formula	**g/ltr**
Sodium chloride	2.25
Potassium chloride	0.10
Calcium chloride	0.12
Sodium bicarbonate	0.05

Method in use

Media: Dissolve 2.5 grams of powder in 1 litre of deionised water, when completely dissolved dispense into containers as required and sterilise by autoclaving at 121ºC for 15 minutes.

Tablets: Dissolve 1 tablet in 500mls of deionised water.

ROSE BENGAL AGAR BASE

A basal medium for the enumeration of yeasts and moulds.

Code: KM1095

Typical formula	**(g/l)**
Mycological Peptone	5.00
Potassium Phosphate Bibasic	1.00
Magnesium Sulphate	0.50
Glucose	10.0
Rose Bengal	0.05
Agar	12.0

pH 7.2 +/- 0.2

Directions
Suspend 28.5g in 1000ml of cold distilled water and bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes and cool to approximately 50°C. Reconstitute one vial of Chloramphenicol Supplement with 3ml of acetone and add to the base medium. Final concentration 100 mg/l

Description
Rose Bengal Agar with the addition of chloramphenicol is recommended for the enumeration of yeasts and moulds in foods, when it is necessary to limit the diameter and height of the mould colonies, along with the production of aerial mycelia.

Method
Prepare suitable decimal dilutions of the samples. Add 1ml to empty petri dishes using two dishes for each dilution. Add to each dish approximately 15ml of melted medium cooled to 50°C. Mix gently, allowing the medium to gel then incubate at 22°C for 5 days. Count the colonies in the plates containing 50 -100 colonies.

Quality assurance (25°C-3 days)

Productivity control
C.albicans ATCC 10231: good growth
A.niger ATCC 16404: good growth
P.cyclopium ATCC 16025: good growth
S.cerevisiae ATCC 9763: good growth

Selectivity control
E.coli ATCC 25922: inhibited
B,subtilis ATCC 6633: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared flasks: 1 month at 2-8°C

References
Banks, J.C., Board, R.G., Carter, J. and A.D. Dodge (1935) - J. App. Bacteriol. 58, 391.
Jarvis, B. (1973) - J. App. Bacteriol. 36, 723.

R2A Medium

Used to determine the bacterial count in potable waters during treatment and distribution, and has been shown to give significantly higher counts than plate count agar (PCA) or similar high-nutrient media

Code: KM1090

Typical formula	**(g/l)**
Dipotassium hydrogen Phosphate	0.3
Magnesium sulphate	0.05
Sodium pyruvate	0.3
Yeast Extract	0.5
Meat Peptone	0.5
Acid Casein	0.5
Glucose	0.5
Starch	0.5
Agar	15.0

pH: 7.2 + 0.2

Directions
Weigh 18 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise at 121ºC for 15 minutes. (If required, bring to the boil to dissolve the agar, and pour into smaller volumes before sterilising). Cool to 44-46ºC for not more than 3 hours before use. Inoculation: Pour 15ml into a petri dish containing 1ml of sample, mix well and allow to set. Pour a further 10ml as an overlay and again allow to set. Alternatively it may be used as a spread plate, inoculating 0.1ml onto the plate and spreading over the entire surface of the medium. It can also be used with membrane filters if required. Incubation: When plates have set, incubate at 22ºC for 5-7 days or 30ûC for 3 days. Other incubation temperatures between 20ºC and 28ºC may be used.

Description
R2A medium was developed to determine the bacterial count in potable waters during treatment and distribution. The standard plate count (SPC) method using PCA provides an enumeration of bacteria, which grow best at, or near, body temperature and this estimation at best, may correlate to the coliforms present in the sample. However, there will be a population of heterotrophic bacteria, which cannot grow at all under the conditions of the SPC method or may grow so slowly that the colonies fail to reach a size detectable to the eye in the 48-hour incubation period. In order to enumerate this section of the bacterial population in water, a medium of low nutritional content (R2A) and extended incubation times are required. R2A medium is recommended in Report 71, Methods for the Examination of Waters and Associated Materials, and Standard Methods for the Enumeration of Water and Wastewater.

Interpretation: Count all colonies and report the number of bacteria in the original sample as the heterotrophic plate count

Quality assurance organisms: *Pseudomonas fluorescens, Aeromonas hydrophila*

References:
Reasoner D.J., Geldreich E.E. (1985) A New Medium for the Enumeration and Subculture of Bacteria from potable water. App & Env. Microbiol. Jan. 1985 p. 1-7.
American Public Health Association (1985) Standard Methods for the Enumeration of Water and Wastewater. 16th Edition. American Public Health Association Inc. Washington DC.
Report on Public Health and Medical Subjects No. 71. (1994) Methods for the Examination of Waters and Associated Materials. HMSO.

RAPPAPORT VASSILIADIS (Single Component) ENRICHMENT BROTH

Selective liquid medium for the enrichment of *Salmonella* spp.

Code: KM1092

Typical formula	**(g/l)**
Soy Peptone	4.50
Sodium Chloride	7.20
Monopotassium Phosphate	1.26
Dipotassiurn Phosphate	0.18
Magnesium Chloride	13.58
Malachite Green Oxalate	0.036

pH 5.2 +/- 0.2

Directions
Suspend 26.8g in 1000ml of cold distilled water. Heat to dissolve, then distribute 10ml into screw-cap bottles or tubes and sterilise by autoclaving at 115°C for 15 minutes.

Description
Rappaport Vassiliadis (RV) Broth is used as a selective enrichment medium for the isolation of *Salmonella* from food, water and environmental specimens. RV Broth is based on the revised formula described by van Schothorst et al. Rappaport Vassiliadis Broth is recommended by ISO/DIS 6579:2000 the horizontal method for detection *Salmonella* spp. (including *S.typhi*) in foodstuffs, together with Mulller Kauffman Tetrathionate Broth.

Method
1. The procedure recommended by ISO/DIS 6579:2000, is as follows:
2. Add 25g sample portion to 225ml of Buffered Peptone Water. If the required test portion is other than 25g, use a suitable quantity of Buffered Peptone Water to yield approximately 1/10 dilution (m/v).
3. Incubate the initial suspension at 37°C for a minimum of 16 hours and not more than 20 hours.
4. Transfer 0.1ml of the pre-enriched culture to a tube containing 10ml of Rappaport Vassiliadis Broth and 1ml to a flask containing 10ml of Mueller Kauffmann Tet. Broth (MKTB).
5. Incubate the inoculated RV Broth at 41.5°C +/- 1°C for 24hrs +/- 3hrs.
6. Incubate the inoculated MKTB at 37°C +/- 1 for 24hrs +/- 3.
7. Using a culture obtained from the RV Broth inoculate by means of a 3mm loop; a large-size petri dish or two 90mm petri dishes containing XLD Medium, proceed in the same way from the enrichment tube by inoculating a second plating medium (e.g. Chromogenic Salmonella Agar, or another suitable selective *Salmonella* plating-out medium chosen by the laboratory).
8. Using the cultures obtained in MKTB after 24 hours of incubation, repeat the procedure with the same two selective plating-out media.
9. Invert the dishes and incubate at 37°C for 24hrs. +/- 3 hrs.
10. Examine for the presence of typical colonies. For confirmation take from each dish of each selective medium at least one typical or suspected colony and a further 4 colonies if the first is negative. Streak the selected colonies onto the surface of Nutrient Agar and incubate at

37°C for 24hrs. Use pure cultures for biochemical and serological confirmation. Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of β-galactosidase, VP reaction, indole detection. Serological confirmation includes the detection of the presence of *Salmonella* O-, Vi- and H antigens by slide agglutination test.

Quality assurance (42°C-24hrs, subculture on TSA)

Productivity Control
S.typhimurium ATCC 14028: growth

Selectivity control
E.coli ATCC 25922: partially or completely inhibited

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 6 months at 2-8°C

References
ISO/DIS 6579 Microbiology of food and animal foodstuff - Horizontal method for the detection of Salmonella spp. 2000.
Van Schothorsts, M., Renaud, A., van Beek, C. (1987) Food Microbiology 4, 11-18

Reinforced Clostridial Medium (R.C.M.)

A semi-solid medium for the propagation of anaerobes in food and veterinary specimens.

Code: KM1094

Typical formula	**(g/l)**
Yeast Extract	3.0
Beef Extract	10.0
Peptone	10.0
Soluble Starch	1.0
Glucose	5.0
L-Cysteine hydrochloride	0.5
Sodium chloride	5.0
Sodium acetate	3.0
Agar	0.75

pH: 6.8 ± 0.2

Directions
Weigh 38 grams of powder, disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix then bring to the boil to dissolve. Distribute 25ml into 1oz Universal containers. Sterilise for 15 minutes at 121ºC.

Description
This medium was formulated by Hirsch and Grinstead to recover small numbers of Clostridium spp. from a variety of sources. Various workers have reported its efficiency with many products and specimens, R.C.M. is rich, non-selective and uses cysteine hydrochloride and glucose as reducing agents. The small amount of agar reduces diffusion of oxygen through the fluid.

Quality assurance organisms: *C. perfringens*

Storage: Capped containers up to 3 months at 15-20ºC in the dark.

Inoculation: Homogenised food sample to a ratio of 1:10 with R.C.M.

Incubation: 30ºC for up to 72 hours.

Growth Indicators
Turbidity, colonies in medium, gas production.

References
Hirsch, A. and Grinstead, E. 1954. Methods for the growth and enumeration of anaerobic spore-formers from cheese. J. Dairy Res. 21: 101-110.

REINFORED CLOSTRIDIUM AGAR

For the cultivation of Clostridia and other anaerobic microorgansims.

Code: KM1093

Typical formula	**(g/l)**
Yeast Extract	3.0
Beef Extract	10.0
Tryptone	10.0
D- Glucose	5.0
Soluble Starch	1.0
Sodium Chloride	5.0
Sodium Acetate	3.0
L- Cystine HCl	0.5
Agar	12.0
Phenol Red	0.025

pH: 6.8 ± 0.2

Directions
Suspend 49.5 g of Clostridium Agar in 1000 ml of cold distilled water. Allow to stand for 10 minutes. Swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and distribute into sterile dishes or tubes containing decimal dilutions of the sample under test. This medium should be used the day it is prepared.

Quality assurance organisms: *C. perfringens*

Storage: Capped container up to 3 months at 15-20°C in the dark.

Inoculation: Pour plate technique or tube culture.

Incubation: 30°C for up to 72 hours. Anaerobic conditions for pour plate. Count as early as possible as prolonged incubation may result in the medium being disrupted due to gas production.

Interpretation: Count all colonies as presumptive clostridia.

References:
Miller, N. J., Garrett, O. W. and Prickett, P. S. 1939. Anaerobic technique Ð a modified deep agar shake. Food Res. 4: 447-451. Ingram, M. and Barnes, E. M. 1956. A simple modification of the deep shake tube for counting anaerobic bacteria. Lab. Pract. 5: 145.