Tissue Culture Agar C

Code: GA1028

Tissue Culture Agar C, has been selected specifically for use as a gelling agent in plant tissue culture techniques. The product is selected first and foremost on gel strength, a parameter of particular importance for this application, and then tested to ensure it meets the parameters set by a major plant producer. The agar contains no nutrients for plant growth and is intended for use in traditional formulations such as: Murashige and Skoog, as well as customer's own formulations.

Typical analysis

Ash	2.30%
Acid Insoluble Ash	0.16%
Calcium	0.31%
Magnesium	0.12%
Iron	0.018%
Total Nitrogen	0.15%
Melting Point	88-91ºC
Setting Point	32-33ºC
Mesh	80
pH (1.5% at 20ºC)	6.8 ± 0.5

Gel Strength (1.5% W/V) >700g/cm2
Recommended Concentration 0.75 - 1.5%

Bacteriological Agar-Agar

Code: GA1006

Colour:	White/Cream
Odour:	Nil

Moisture	Max. 20%
pH	7.0 ± 0.5
Particle size	80 Mesh
Total ash	Max. 6%
Acid insoluble ash	. 0.5%
Foreign organic matters	<1.0%
Foreign insoluble matters	<1.0%
Solubility	Total when boiled
Foreign starch	Absent
Gelatin	Absent
Gel Strength	900 - 1100 g/cm^2
Transparency	less than 50 NTU (at 1.5%)
Viscosity	5 - 50 cps (Brookfield, 1.5%)
Melting point	90°C - 96°C (at 1.5%)
Setting point	Max. 36°C (at 1.5%)

Microbiology

Total plate count	<1000/g
Coliforms	< 3/g
Yeast/Moulds	< 100/g
E. coli	Nil

ALKALINE PEPTONE WATER

A transport and enrichment medium for *Vibrio* spp

Code: KM3321

Typical formula	**(g/l)**
Tryptone	10.0
Sodium Chloride	10.0

pH 8.5 +/- 0.2

Directions
Suspend 20 g in 1000 ml of cold distilled water, heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
Alkaline Peptone Water is recommended by OMS, APHA and FDA as a transport and enrichment medium for *Vibrio* spp. Generally the test sample is prepared by mixing 25 (or 50) g of sample with 225 (or 450) ml of Alkaline Peptone Water. The inoculated flasks are incubated at 37°C and plated on TCBS Agar after 6-8 hours and 16-24 hours of incubation. The final verification of characteristic colonies cultivated onto TCBS Agar may be performed according to the criteria given by the quoted literature. The differentiation between *V.cholerae*, *V.parahaemolyticus*, *V.vulnificus* and *V. alginolyticus* is obtained with the following test: Gram staining, motility, Oxidase test, OF test, Growth at 42°C, inhibition by O/129, reduction of nitrate to nitrite, arginine dehydrolase, lysine decarboxylase, ONPG, acid/gas from glucose, sucrose, cellobiose, halophytic characteristics. The so called 'cholera red reaction'' (red colouration due to nitrose - indole formation following to addition of a few drops of concentrated sulphuric acid) may be tested on Alkaline Peptone Water culture after incubation at 37°C for 24 hours. However, the test has a poor diagnostic value because a few non-pathogenic vibrios and, generally all the indole producing and nitrate reducing organisms also give a positive reaction. A microscopic examination in dark field (X400) that permits to observe the rapid and typical arrow mobility of vibrios is more useful for an early diagnosis. The appearance of the microscopic field was compared to that of a summer sky with shooting stars. The microscopic diagnosis can be confirmed by Benenson technique, based on the specific inhibition of Vibrio motility by O-group polyvalent antiserum.

User quality assurance (37°C-24hrs)

Productivity control
V.cholerae NCTC 11218: growth

Selectivity control
E.coli ATCC 25922: partially inhibited

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

References
APHA (1985) - Standard Methods for the Examination of Water and Wastewater, 16th ed.
Benenson, A.S., Islam, M.R. & Greenough, W.B. (1964Bull, WHO, 30, 827.
NMKL method n° 156, 2nd ed. 1997.

AMIES TRANSPORT MEDIUM

For the collection and transport of specimens

Code: KM1002

Typical formula	**(g/l)**
Charcoal	10.00
Sodium Chloride	3.00
Calcium Chloride	0.10
Potassium Chloride	0.20
Magnesium Chloride	0.10
Monopotassium Phosphate	0.20
Disodium Phosphate	1.15
Sodium Thioglycollate	1.00
Agar	4.00

pH 7.0 +/- 0.1

Directions
Suspend 19.75 g in 1000 ml of cold distilled water. Heat to boiling with frequent agitation, distribute into small screw-cap tubes or bottles and sterilise by autoclaving at 121° for 15 minutes. The charcoal must be properly suspended in the medium; invert the bottles or the tubes when they are cool but the agar still liquid.

Description
Amies Transport Medium is a semisolid medium used for the transport of clinical specimens to be subjected to bacteriological analysis. The charcoal present in the medium permits a high survival rate of the bacteria from faecal specimens and *Neisseria gonorrhoea*e, whilst the sodium thioglycollate lowers the redox potential of the medium, thereby allowing a better conservation of anaerobic bacteria. The glycerophosphate in Stuart's medium, used as a source of energy for the growth of coliforms and other Gram-negative bacteria, is substituted for in Amies Trasport Medium by a saline buffer solution. In this way, the overgrowth of common contaminants, especially in specimens for gonococcus research is avoided. Amies medium contains 3 g/l of sodium chloride to allow the optimal preservation of *N. gonorrhoea*. Amies Transport Medium permits conservation of haemolytic streptococci for more than 3 days, of *N. gonorrhoea, Bordetella pertussis,* and *Fusobacterium* for 48-72 hours. Amies Transport medium is available also in ready to use tubes.

Method
To transport specimens, insert a third of the swab with which the material has been collected into the centre of the medium, then cut the rod and screw the test-tube stopper down to clamp the swab.

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 6 month at 15-30°C.

Reference
Amies, C.R. (1967), Can. J. Pub. Hth.. 58, 296-300.

Anaerobe Identification Agar Base

A medium for testing the fermentation capabilities of non-sporing anaerobes.

Code: KM1566

Typical formula	**(g/l)**
Beef Extract	4.0
Peptone mixture	16.0
Sodium chloride	5.0
Agar	15.0

pH: 7.2 ± 0.2

Directions
Weigh 40 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 48ºC and aseptically add 50-70mls of sterile defibrinated horse blood. Mix well and pour. Before use, flood the surface with 1 ml of a sterile solution of the substrate under test.

Q.C. organisms:
B. *fragilis*

Storage: Plates up to 7 days at 2-8ºC in the dark. Capped Container up to 3 months at 15-20ºC in the dark.

Inoculation: Heavily inoculate a small area of the plate with a loopful of a fresh culture of the test organism. Up to 4 organisms per plate can be tested.

Incubation: 37ºC anaerobically for 24-48 hours.

Recognition of fermentation: Remove a small plug of agar from below the growth. Cover the plug with bromothymol blue indicator (0.04%). Colour changes due to production of acidity will develop in a few seconds and should be viewed against a white background. Comparison with controls is useful; a plug taken from an area well away from any growth can be used as a negative control.

References
Phillips K.D. 1976. A Simple and sensitive technique for determining the fermentation reactions of non-sporing anaerobes. J. Appl. Bact. 41: 325-328.

Anaerobe Isolation Agar

Designed to give optimum growth of demanding anaerobes.

Code: KM1000

Typical formula	**(g/l)**
Agar	15.0g
Peptone mixture	23.0g
Sodium chloride	5.0g
Sodium bicarbonate	0.5g
Dextrose	1.0g
Sodium pyruvate	0.5g
Amino acid factors	2.0g
Soluable pyrophosphate	0.13g
Vitamins and growth	11.00 mg

pH: 7.2 +/- 0.2

Directions:
Add 46 grams of dehydrated culture medium to 1 litre of distilled water. Allow to soak for 10 minutes, swirl to mix then sterilise by autoclaving at 121°C for 15 minutes. Cool to 48ºC then aseptically add 5-10% of sterile defibrinated horse blood, mix well and pour into petri dishes.

Description:
This medium has a high nutritive value due to its richness in peptones, extracts, haemin and amino acids. This is a primary isolation medium capable of growing most clinically significant anaerobes. Starch and sodium bicarbonate act as detoxification agents. It contains promoting agents for Fusobacterium necrophorum, Propionibacterium acne, Bacteriodes fragilis, Eubacterium spp. and Veillionella spp. Pyruvate helps neutralise hydrogen peroxide.

Anaerobe Isolation agar + Kanamycin 75 (Code:KM1000N) for the selective isolation of Clostridium spp. and other anaerobes. Kanamycin is more inhibitory to anaerobic cocci. Metronidazole and Nalidixic acid for the isolation of actinomyces spp. from clinical material. The metronidazole will suppress the growth of most other anaerobes. Nalidixic acid for the isolation of non-sporing anaerobes from clinical material. Vancomycin for the isolation of Gram negative anaerobes from clinical material. When used with other blood agar bases, further enrichment of the medium with haemin and menadione is beneficial. When Neomycin 100 is added to egg yolk medium this supplement will allow the growth of clostridia whilst inhibiting other lecithinase producing organisms. When Neomycin 75 added to blood agar the resulting medium will allow the growth of clostridia, most bacteroides fragilis strains and some anaerobic cocci.

Quality specification:
Dehydrated medium: homogeneous, straw colored fine powder.
Ready to use medium: red due to addition of blood. The blood will darken (reduce) because of the presence of reducing agents.

QC Organism Results:
Bacteroides fragilis ATCC 25285 Growth
Clostridium perfringens ATCC 13124 Growth

Storage:
Dehydrated medium should be stored between 10 to 25ºC. Once opened, place the container in a dark, dry place. The dehydrated medium should not be used if there is lumps evident or if there is colour change.

Anaerobe Liquid Medium

Designed to give optimum growth of demanding anaerobes.

Code: KM1001

Formulation	**(g/l)**
Peptone mix	15.0
Yeast Extract	10.0
L-Cysteine HCl	0.5
Resazurin	0.001
Sodium bicarbonate	0.4
Haemin	0.005
Vitamin K	0.0005
Sodium thioglycollate	0.5
Sodium chloride	2.5
Agar	0.75

pH: 7.2 ± 0.2

Directions:
Suspend 30 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes, swirl to mix. Boil to dissolve the agar then dispense into screw cap containers. Sterilise by autoclaving at 121ºC for 15 minutes. Tighten the caps as soon as possible after autoclaving. **Do not reheat more than once**. Growth - indicators: The broth may become turbid or individual colonies may form suspended in the medium

Description:
The medium was designed to give optimum growth of anaerobes. It is rich in nutrients from the peptone content in the formulation. Vitamin K. haemin and L-cysteine are growth factors required by some anaerobes. L-cysteine together with sodium thioglycollate reduce the Eh of the medium and the agar content inhibits absorption of oxygen and convection currents. Resazurin is a redox indicator.

Q.C. organisms: B. fragilis

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

Inoculation: If used as a blood culture medium a minimum dilution of 1:10 should be used.

Incubation: 37ºC for 24-72 hours. Keep the container airtight.

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Microtrade
Bubble Check Agent

BFA15/BFA60 - Bubble Check agent is a proprietary anti-bubble additive, which prevents bubble formulation in solid, agar-based microbiological culture media without affecting the growth of human pathogens. By preventing bubble formation the bubble check agent eliminates the need for the flaming of plates and also prevents “splash” when using automated plate-pouring equipment.

LABORATORY REAGENT
NOT FOR THERAPEUTIC USE

Pack sizes available:

BFA15 - 15ml dropper bottle
BFA60 - 60ml dropper bottle

Storage and shelf life
Store at 2-8°C until the expiry date shown on the pack. **DO NOT FREEZE**.

Procedure

1. Add 1-4 drops of Bubble Check Agent to each litre of water before the addition and subsequent sterilisation of culture media.

2. Each laboratory should establish its own usage
Rate for different types of media. Viscosity’s may vary greatly depending on the various cells and nutrients.

Limitations

CAUTION. If a sheen appears on the medium surface, the levels in subsequent batches may be reduced. The sheen does not affect growth characteristics.

Do not add Bubble Check Agent to hot media as this will cause agglomeration of the drops and make dispersion impossible.

ANTIBIOTIC AGAR A 1

A seed layer agar for microbiological assay of antibiotics

Code: KM8752

Typical formula (g/l)

Peptone	6.0
Tryptone	4.0
Yeast Extract	3.0
Beef Extract	1.5
Glucose	1.0
Agar	15.0

pH 6.5 +/- 0.1

Directions and Description

Suspend 80.5 g in 1000 ml of cold distilled water; heat to boiling and sterilise by autoclaving at 121°C for 15 minutes. The medium is prepared according to the specifications given in FDA, 21 CFR and in USP XXIV ed. It corresponds to Medium no. 1 of Grove and Randall: seed agar for microbiological assay of antibiotics and maintenance agar for test strains. It is used with *M.flavus* for the plate assay of bacitracin, with *Sarcina lutea* for the assay of chloramphenicol and with *S.aureus* for the assay of kanamycin sulphate, penicillin G, sodium methicillin and sodium oxacillin. It is also used as a base agar in the assay of the following antibiotics: chloramphenicol, kanamycin, colistin, methicillin, oxacillin, and vancomycin.

Storage

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

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ANTIBIOTIC BASE AGAR A 2

Base agar for the microbiological assay of antibiotics

Code: KM8502

Typical formula (g/l)

Peptone	6.0
Yeast Extract	3.0
Beef Extract	1.5
Agar	15.0

pH 6.6 +/- 0.1

Directions and Description

Suspend 25.5g in 1000ml of cold distilled water; heat to boiling and sterilise by autoclaving at 121°C for 15 minutes. The medium is prepared according to the specifications given in FDA, 21 CFR and in USP XXIV Ed. It corresponds to Medium no.2 of Grove and Randall: base agar for

the plate assay of antibiotics. It is used as the base agar in the microbiological assay of bacitracin and penicillin G

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

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ANTIBIOTIC BROTH A 3

Nutrient broth for the turbidimetric assay of antibiotics

Code: KM8652

Typical formula	**(g/l)**
Peptone	5.00
Yeast Extract	1.50
Beef Extract	1.50
Sodium Chloride	3.50
Glucose	1.00
Dipotassium Phosp	3.68
Monopotassium Phosp	1.32

pH 7.0 +/- 0.1

Directions and Description
Suspend 17.5g in 1000ml of cold distilled water; heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes. The medium in prepared according to the specifications given in FDA, 21 CFR and in USP XXIV Ed. It corresponds to Medium no. 3 of Grove and Randall: nutrient broth for the turbidimetric assay of penicillin and tetracycline with *Staphylococcus aureus*.

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

ANTIBIOTIC BASE AGAR

A base agar for the microbiological assay of antibiotics

Code: KM3501

Typical formula	**(g/l)**
Peptone	6.0
Yeast Extract	3.0
Beef Extract	1.5
Agar	15.0

pH 6.6 +/- 0.1

Directions
Suspend 25.5 g in 1000 ml of cold distilled water; heat to boiling and sterilise by autoclaving at 121°C for 15 minutes. The medium is prepared according to the specifications given in FDA, 21 CFR and in USP XXIV Ed. It corresponds to Medium no.2 of Grove and Randall: base agar for the plate assay of antibiotics. It is used as the base agar in the microbiological assay of bacitracin and penicillin G.

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

ANTIBIOTIC BROTH A 3

A Nutrient broth for the turbidimetric assay of antibiotics

Code: KM3651

Typical formula	**(g/l)**
Peptone	5.00
Yeast Extract	1.50
Beef Extract	1.50
Sodium Chloride	3.50
Glucose	1.00
Dipotassium Phosphate	3.68
Monopotassium Phosphate	1.32

pH 7.0 +/- 0.1

Directions
Suspend 17.5g in 1000 ml of cold distilled water; heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes. The medium in prepared according to the specifications given in FDA, 21 CFR and in USP XXIV Ed. It corresponds to Medium no. 3 of Grove and Randall: nutrient broth for the turbidimetric assay of penicillin and tetracycline with *Staphylococcus aureus*.

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

APT AGAR

For the cultivation and enumeration of heterofermentative lactobacilli

Code: KM8852

Typical formula	**(g/l)**
Tryptone	12.50
Yeast Extract	7.60
Glucose	10.00
Sodium Citrate	5.00
Sodium Chloride	5.00
Dipotassium Phosphate	6.00
Manganous Chloride	0.14
Magnesium Sulphate	0.80
Ferrous Sulphate	0.04
Sorbitan Mono.	0.20
Agar	15.00
Thiamine HCl	0.10 mg

APT BROTH

For the cultivation and enumeration of heterofermentative lactobacilli

Code: KM8902

Typical formula (g/l)	
Tryptone	12.50
Yeast Extract	7.60
Glucose	10.00
Sodium Citrate	5.00
Sodium Chloride	5.00
Dipotassium Phosphate	6.00
Manganous Chloride	0.14
Magnesium Sulphate	0.80
Ferrous Sulphate	0.04
Sorbitan Monoleate	0.20
Agar	15.00

pH 6.7 +/- 0.2

Directions
Suspend 46.2g of APT Broth or 61.29g of APT Agar in 1000 ml of cold distilled water; heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes. **Do not overheat**.

Description
Evans and Niven studied APT Agar and APT Broth for the cultivation of heterofermentative lactobacilli that produce greening of cured meat products. These formulae are also used for the conservation and preparation of the *Lactobacillus viridescens* ATCC 12706 strain inoculums, which is the test organism in the microbiological assay of thiamine according to the method described by Deibel et al. A.P.H.A. recommends the use of APT Agar for the detection of lactobacilli in foodstuffs.

Method
The technique suggested is the standard plate count; the details change according to the material to be tested.

Material to be tested	Diluent	Incubation
Sauerkraut	distilled water	32°C for 3 days
Fruit juices	distilled water	32°C for 6 days
Salted canned meat	phosphate buffer	21°C for 6 days

For the detection of H_2O_2 producing strains, APT Agar may be prepared with MnO_2: suspend 20 g of MnO_2 in 200 ml of APT Broth, distribute 10ml in tubes and sterilise by autoclaving at 121°C for 15 minutes. To 100ml of APT Agar add 10ml of MnO_2 suspension. Prepare the plates with a 15ml base layer of APT Agar without MnO_2, leave the medium to solidify then add 15 ml of a surface layer of APT Agar with MnO_2. Inoculate the sample and the sample dilutions onto the surface of the medium. H_2O_2 producing lactobacilli grow with colonies surrounded by a transparent halo.

As these media are non-selective and permit the growth of contaminants, the presumptive diagnosis of the presence of lactobacilli should be confirmed by microscopic and biochemical examinations.
APHA moreover, recommends an artificial pollution test to confirm the diagnosis of bacterial greening of canned meats. Transfer a few colonies from the APT Agar plates to APT Broth tubes and incubate at 32°C for 24 hrs. Prepare a moist sterile chamber (Petri dish with filter paper imbued with sterile water) and put a slice of the test material in this chamber under aseptic conditions. Inoculate the surface with a loopful of Broth culture in APT Broth; incubate at 32°C for 24 hours and observe whether the meat has greened. If it occurs and if an uninoculated control specimen is found to be unchanged, the diagnosis is confirmed. The presence of greening due to exceeding nitrites is to be distinguished from the bacterial greening by carrying out identification tests and assays of nitrites with the standard reagents.

User quality assurance (APT Agar + MnO_2 : 30°C-5 days)

Productivity control
L.viridescens ATCC 12706: growth, colonies with transparent halo
L.brevis ATCC 14869: growth, colonies with transparent halo
L.sakie ATCC 215521: growth, colonies without transparent halo
L.mesenteroides DSM 20241: growth, colonies without transparent halo
P.damnosus ATCC 29358: growth, colonies without transparent halo

Storage
Dehydrated media: 15-30°C
User prepared plates and tubes: up to 7 days at 2-8°C

References

• APHA (1966) - Recommended Methods for the Microbiological Examination of Foods. 2nd. edition.
• D'Aubert S. (1963) Ann. Microbiol., 8, 189
• Deibel, RH., Evans, J.B. & Niven, C.P. Jr. (1957) - J. Bact., 74, 818-821.
• Niven, C.F. Jr. & Evans, J.B. (1957) - J. Bact., 73, 758-759.
ciation, Leatherhead, U.K.

ASPARAGINE ENRICHMENT BROTH

A selective medium for the detection of *Pseudomonas aeruginosa*

Code: KM9512

Typical formula	**(g/l)**
DL-Asparagine	3.0
Dipotassium Phosphate	1.0
Magnesium Sulphate	0.5

pH 7.0+/- 0.2

Directions
Suspend 4.5g in 1000ml of cold distilled water: heat to dissolve, distribute and sterilise at 121 °C for 15 minutes. Inoculate not more than 1ml sample in 10 ml of medium or use a multiple strength medium.

Description
Asparagine Enrichment Broth is recommended by APHA as a selective medium for the detection of *Pseudomonas aeruginosa* in pool and bathing waters.

Method
Collect the specimens to be tested in bottles containing sodium thiosulfate (about 100mg per litre of specimen) in order to neutralise the possible residual chlorine. Inoculate five 10ml, five 1ml and five 0.1ml samples into Asparagine Enrichment Broth. Examine for the presence of *P. aeruginosa* according to the most probable number method, counting as positive all the tubes where presence of a green-blue fluorescent pigment (under long-wave ultraviolet light), and turbidity are observed after incubation at 35°C for 48 hours. The count obtained in this way is to be considered as presumptive.

User quality assurance (37°C-24 hrs)

Productivity control
P.aeruginosa ATCC 27853: good growth

Selectivity control
E.coli ATCC 25922: partially inhibited

Reference
APHA (1985) - Standard Methods for the Examination of Water and Wastewater, 16th edition, pp. 978-980.

AZIDE BLOOD AGAR BASE

A medium for the isolation of streptococci and staphylococci

Code: KM3002

Typical formula	**(g/l)**
Tryptose	10.0
Beet Extract	3.0
Sodium chloride	5.0
Sodium Azide	0.2
Agar	15.0

pH 7.2 +/-0.2

Directions
Suspend 33.2g in 1000 ml of cold distilled water. Heat to boiling and sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and, under aseptic conditions; add 5% sterile defibrinated blood.

WARNING: Hazardous precautions for media containing azide.

Description
Azide Blood Agar Base is recommended for the selective isolation of streptococci and staphylococci from faeces, water, foodstuffs and other specimens grossly contaminated with Gram-negative flora. The review by Hartman et al. lists more than forty types of selective media for streptococci with sodium azide base; this substance has a bacteriostatic effect on a number of bacterial species, particularly Gram-negative ones (probably by blocking the metalloporphyrinic enzymatic systems: catalase, cytochrome C oxidase). It inhibits the swarming of *Proteus* spp., which does not interfere with the phenomenon of haemolysis, and allows the development of some Gram-positive species, particularly streptococci, staphylococci and some anaerobes. The material under examination can be plated onto the surface, or included into the agar mass. The latter method is preferable: it is noticed that streptolysin O is inactivated by oxygen and that some strains of streptococci grow better when oxygen presence is reduced. Several authors recommend anaerobic incubation, or the preparation of a double set of plates, one for anaerobic and the other for aerobic incubation. The typical appearance of haemolysis in Azide Blood Agar plates is as follows: α-haemolysis: greenish-brown ring, sometimes surrounded by a light zone; under the microscope the red cells appear discoloured but intact. β-haemolysis: transparent red ring. The diagnosis of streptococci is confirmed with standard microscopic, biochemical and serological examination.

User quality assurance (37°C-24 h)

Productivity control
S.pyogenes ATCC 19615: growth, beta haemolysis
S.pneumoniae ATCC 6305: growth alpha haemolysis
S.aureus ATCC 25923: growth

Selectivity control
P.mirabilis ATCC 12453: partially inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: up to 7 days at 2-8°C

References
Hartman. P.A. Beinbold, G.W. & Saraswat D.S. (1966) - Adv. Appl. Micr. 8, 253-289.
Moody, M.D. (1972) - Old and new techniques for rapid identification of group A streptococci.

AZIDE DEXTROSE BROTH

A selective medium for the detection of enterococci in water and sewage

Code: KM3052

Typical formula	**(g/l)**
Peptone mix	15.0
Beef Extract	4.5
Glucose	7.5
Sodium Chloride	7.5
Sodium Azide	0.2

pH 7.2 +/- 0.2

Directions
Suspend 34.7g of Azide Dextrose Broth in 1000 ml of cold distilled water. Heat gently to dissolve, distribute into 10ml tubes and sterilise by autoclaving at 121°C for 15 minutes. For inocula of more than 1ml per 10ml medium prepare Azide Dextrose Broth at double or multiple concentration.

WARNING: Hazardous precautions for media containing azide.

Description
Azide Dextrose Broth is a selective medium for the detection of enterococci in water and sewage; its composition is in accordance with WHO specifications.

Method
The enumeration of enterococci is carried out in tubes, according to the method of the most probable number (MPN); vary the amount of inoculum (in multiples or fractions of 1ml) depending on the type of specimen preparing at least five tubes of each dilution. As a liquid dilution, use a phosphate buffer or else a 0.5% aqueous peptone solution *(w/v).* Incubate at 35°C for 24 hours, and observe if growth has occurred; if not, continue incubation for a further 24 hours. Calculate the result using the appropriate tables, and express it as the most probable presumptive number. Confirm the presumptive result with a subculture in Ethyl Violet Azide Broth.

User quality assurance (37°C-2hrs)

Productivity control
E.faecalis ATCC 19433: good growth

Selectivity control
E.coli ATCC 25922: inhibited

Storage
Dehydrated media: 15-30°C
User prepared tubes: 1 month at 2-8°C

AZIDE MALTOSE AGAR

Selective medium for the isolation and enumeration of enterococci

Code: KM8072

Typical formula	**(g/l)**
Peptone mix	10.00
Yeast Extract	10.00
Sodium Chloride	5.00
Na Glycerophosphate	10.00
Maltose	20.00
Lactose	1.00
Agar	15.00
Sodium Azide	400 mg
Bromocresol Purple	15 mg

pH 7.2 +/- 0.2

Directions
Suspend 71.4g in 1000ml of cold distilled water, heat to boiling, and boil for five minutes, cool in a water bath to 50°C and aseptically add 10ml of TTC 1% Solution. Mix well and pour into sterile 55mm Petri dishes for water analysis, or into flasks for poured plated technique. The medium without TTC can be autoclaved at 121°C for 10 minutes.

WARNING: Hazard Precautions for media containing azide

Description
Azide Maltose Agar, prepared according to the formula of Kenner, Clark and Kabler, is a selective medium used for the isolation and enumeration of enterococci in faeces, milk water and other materials by plate pouring or membrane filtration techniques. Azide Maltose Agar is selective for the following Group D and Group Q species: Group D enterococci (*E. faecalis, E.. faecalis* subsp. *liquefaciens, E. faecalis* subsp. *zymogenes, E. faecium, E*. *bovis, E. equinus) Group Q: S.avium* Also S. *mitis* and *S.salivarius* grow on Azide Maltose Agar with pink-red colonies due to reduction of the TTC. After 48 hours at 35°C a scarce growth is observed on the medium of colourless colonies of *Lactobacillus plantarum* and *Pediococcus cerevisiae*; streptococci not of group D (*S. cremoris, S.lactis, S.pyogenes, S. termophilus*) are completely inhibited. As are other lactic acid bacteria (*Leuconostoc mesenteroides, L.lactis, L. acidophilus)* and the coliforms.

Methods
Membrane Filtration Method
Distribute 4-5ml of medium into 55mm dishes. Filter samples through a sterile membrane to give 20 to 200 colonies on the membrane surface. Use volume of 100, 10, 1, 0,1 or 0,01, depending on the degree of pollution. Transfer the membrane to the agar, invert the plates and incubate at 35°C for 48 hours. Count all red or pink colonies eventually with the aid of a low power (10 to 15 magnifications) microscope. Calculate the number of enterococci and report as faecal streptococci per 100 ml.

Plate Count Method

Prepare dilutions of the sample to give a count of 30-300 colonies.
Transfer 1ml of sample dilution in duplicate into sterile petri dishes
Pour 20ml of liquefied and cooled to 45-47°C medium to each plate.
Mix the medium well with the sample and solidify the agar as quickly as possible. Incubate the inverted plates at 35°C for 48 hours. Count all red or pink colonies eventually with the aid of a low power (10 to 15 magnifications) microscope. Calculate the number of enterococci and report as faecal streptococci per 100ml.

For confirmation of colonies a serological or biochemical test is recommended. For biochemical confirmation, transfer 5-10 typical colonies to Brain Heart Infusion Broth and incubate for 18-24 hours at 35°C. Use the broth culture for a Gram stain and a catalase test, a subculture into Brain Heart Infusion Broth (incubate at 45°C), and into the same medium with bile salts. The diagnosis of faecal streptococci is given by a negative catalase reaction, development in BHI incubated at 45°C, and in BHI with bile salts after 72 hours at 35°C.

Precautions

Azide Maltose Agar is not specific for the identification of Group D streptococci and a serological confirmation must be carried out.
The pH of the medium should not fall below 7.0 as it may become inhibitory towards enterococci.

User quality assurance (37°C-24 hrs)

Productivity control

E.faecalis ATCC 19433: good growth, red colonies

Selectivity control

E.coli ATCC 25922: inhibited

Storage

Dehydrated medium: 15-30°C
User prepared plates and flasks: 30 days at 2-8°C

References

APHA (1985). Standard Methods for Examinations of Water and Waste-water, 16 th edition
FDA (1976), Bacteriological Analytical Manual for Foods, 4th edition
Hartman, P.A., Reinbold G.W., & Saraswat, D.S. (1966) Adv. App, Micr., 8, 253
Kenner, B.A., Clark, H.F. & Kabler, P.W. (1961). App. Microbiol., 9,15.

AZIDE VIOLET BLOOD AGAR BASE

A highly selective medium for the isolation of streptococci

Code: KM3102

Typical Formula	**(g/l)**
Tryptose	10.00
Beef Extract	3.00
Sodium Chloride	5.00
Sodium Azide	0.20
Crystal Violet	0.002
Agar	15.00

Directions
Suspend 33.2g in 1000 ml of cold distilled water, heat to boiling distribute and sterilise by autoclaving at 121°C for 15 minutes. To prepare blood agar plates cool to about 50°C, aseptically add 5%(v/v) of sterile defibrinated blood, mix well and pour into sterile petri dishes.

WARNING: Hazardous precautions for media containing azide.

Description
Azide Violet Blood Agar Base, with sodium azide and crystal violet, is highly selective for streptococci, with Gram-negative organisms and staphylococci being inhibited. Azide Violet Blood Agar Base is recommended for the isolation of enterococci in urine and is suggested by Mossel for the enumeration of faecal streptococci in foodstuffs.

User quality assurance (37°C-24hrs)

Productivity control
S.pyogenes ATCC 19615: growth, beta haemolysis
S.pneumoniae ATCC 6305: growth alpha haemolysis

Selectivity control
S.aureus ATCC 25923: inhibited
E.coli ATCC 25922: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: up to 24 hours at 2-8°C

Reference
Mossel, D.A.A., Diepen, H.M.S De Bruin, A.S. (1967). J. Appl. Bact. 20, 265-272.

A1 BROTH

A liquid medium for the detection of faecal coliforms.

Code: KM8012

Typical formula (g/l)

Tryptone	20.0
Lactose	5.0
Salicin	0.5
Sodium Chloride	5.0
Triton X-100	1.0

pH 6.9 +/- 0.1

Directions
Suspend 32.5g in 1000ml of cold distilled water. Gently heat to dissolve completely and distribute 9 ml into test tubes with an inverted Durham tube. Sterilise by autoclaving at 121°C for 15 minutes. If required prepare multi-strength broth weighing the appropriate quantity of powdered medium.

Description
A1 Broth, is used for the detection of faecal coliforms in foods, treated wastewater and seawater bays as a most probable number (MPN) method.

Method
1ml of multiple dilutions of sample (3 or 5 replicates per dilution) is added to test tubes containing 10 ml of A1 Broth. After incubation at 37°C for 3 hours and at 44.5°C for 21 hours, tubes with gas accumulation in the Durham tubes are scored positive for faecal coliforms and, those with no gas as negative. A MPN table is consulted to determine the most probable number of faecal coliforms. From the positive tubes, subculture 0.1 ml to 10 ml of Peptone Tryptone Water. After incubation at 44°C for 18-24 hours, add 0.5 ml of Kovacs' Reagent. The tubes, which develop a red-ring are considered positive for *E.coli.*

Quality Assurance (44 °C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, gas production

Selectivity control
E.faecalis ATCC 19433: partially inhibited, no gas production

Storage
Dehydrated medium: 15-30°C
User prepared tubes: up to 7 days in the dark at 2-8°C

Reference
• Andrew, Presnell, (1972) App. Microb. 23: 521.

Acetate Differential Agar

For the differentiation of *Shigella* species from *Escherichia coli* and for the differentiation of non-fermenting Gram-negative bacteria.

Code: KM6802

Typical formula	**(g/l)**
Sodium Acetate	2.0
Sodium chloride	5.0
Magnesium Sulphate	0.2
Dipotassium phosphate	1.0
Agar Agar	20.0
Bromothymol Blue	0.08
Ammonium dihyd. phos.	1.0

pH 6.8 ± 0.2

Directions
Weigh 29.3 grams of powder and add to 1 litre of deionised water (conductivity <10ms). Gently heat and bring to boiling. Distribute into tubes to produce a 1cm butt and 30cm slant. Autoclave for 15 minutes at 121°C (equivalent heat process). Cool tubes in slanted position.

Description
Used for the differentiation of *Shigella* species from *Escherichia coli* and also for the differentiation of non-fermenting Gram-negative bacteria. Bacteria that can utilise acetate as the sole carbon source turn the medium blue.

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 7 days at 2-8°C

References
•Simmons, J.S. (1926) - J. Infect. Dis. 39, 209-214.

Acid Hydrolysed Casein

This is a premium quality source of peptides produced by acid hydrolysis of casein. It is recommended for use as a microbiological nutrient in media (for toxin production), and fermentation's. It can also be used in nutritional studies on tryptophan and in vaccine production where high free amino acid content is required.

Code: PH1007A

Description
A light creamy beige, free-flowing homogenous, spray-dried powder, which produces a light clear solution after autoclaving at 121°C for 15 minutes.

Solubility: 2% aqueous

pH: 6.6 ± 0.2

Chemical Characteristics (%) - typical data

α - Amino Nitrogen (AN)	5.7%
Total Nitrogen (TN)	8.1%
AN/TN	70%
Free Amino Acids	69.0%
Moisture	2.6%
Ash	38%
Calcium	0.02%
Magnesium	0.01%
Sodium	3.70%
Phosphate	1.62%
Sulphate	0.04%
Potassium	0.07%

Amino Acid Distribution (mg/g) - typical data

AMINO ACID	**Total**	**Free**	**%Free**
Alanine	23.3	19.8	85.0
Arginine	20.6	17.4	84.0
Aspartic acid	44.8	38.	85.0
Cystine	1.1	-	-
Glutamic acid	129.0	93.6	73.0
Glycine	15.1	10.9	72.0
Histidine	26.6	11.2	42.0
Isoleucine	28.8	14.5	50.0
Leucine	48.2	33.7	70.0
Lysine	47.3	31.5	67.0
Methionine	15.	13.4	89.0
Phenylalanine	22.8	15.3	67.0
Proline	52.2	34.3	66.0
Serine	45.9	27.	59.0
Threonine	21.1	16.5	78.0

Tryptohan	<0.5	<0.5	-
Tyrosine	12.0	5.0	42.0
Valine	36.3	23.4	64.0
Total	**590.1**	**405.5**	**69.0**

Molecular Weight Distribution - (%)

<200 D	60.3	500-1000 D	7.8
200-500 D	31.0	>1000 D	0.8

Storage and Packaging

Store in a cool dry place away from the direct sunlight. Stored in this way in the original packaging, the product has a shelf life of 4 years from date of manufacture.

Available in 500g, 5kg, 10kg & 25kg sizes, alternative quantities available upon request. The packaging used is either plastic containers or fibreboard drums (with tamper seal) or corrugated cardboard boxes, depending upon pack size and customer preference.

m-AEROMONAS SELECTIVE AGAR BASE
AEROMONAS STARCH DNA AGAR BASE

Base media and selective supplement for the isolation of *Aeromonas*

Code: KM3191 m-Aeromonas selective agar base
Code: KM8202 Aeromonas starch DNA agar base

Typical formula

Aeromonas Selective Agar Base (g/l)	
Tryptose	5.00
Yeast Extract	2.00
Dextrin	11.40
Sodium Chloride	3.00
Potassium Chloride	2.00
Magnesium Sulphate	0.10
Ferric Chloride	0.06
Sodium Desoxycholate	0.10
Bromothymol Blue	0.08
Agar	13.00

Aeromonas Selective Agar Base (g/l)	
Tryptone	15.00
Soy Peptone	5.00
Sodium Chloride	5.00
DNA	2.00
Maize Starch	10.00
Agar	15.00

pH: 8.0 +/- 0.2

Directions
Suspend 18.35g of m-Aeromonas Selective Agar Base or 26g of Aeromonas Starch DNA Agar Base in 500 ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and, under aseptic conditions, add the contents of one vial of Aeromonas Selective Supplement-Ampicillin reconstituted with 5ml of sterile distilled water. Mix well and distribute Aeromonas Selective Agar into sterile 55mm dishes, and Aeromonas Starch DNA Agar into 90 mm Petri dishes.

Description
The significance of *Aeromonas* species as human pathogens is getting increasing attention (Holmberg and Farmer); many investigators have reported that the aquatic environment can be considered the biggest source of infection, Buchanan and Palumbo implicated *Aermonas* as potential food-poisoning agent. **m-Aeromonas Selective Agar Base** supplemented with ampicillin, corresponds to the medium described by Havelaar, During and Versteegh. It is used for the detection of *Aeromonas* in water and other liquid samples by means of a membrane filtration procedure. Appropriate volumes or decimal dilutions of the samples are filtered using membrane filters 0.45µm pore size, and the filters are transferred onto the plates. After 24 hours of incubation at 30°C in aerobic conditions, *Aeromonas* colonies show a visible yellow colour (dextrin fermentation). The detection of dextrin fermentation is considered

by Havelaar to be highly specific and until now no dextrin negative *Aeromonas* strains have been found. Confirm the presumptive detection with standard biochemical tests. The use of ampicillin suppresses adequately the background flora without having any decrease in the *Aeromonas* recovery. Strains sensitive to 10 mg/l of ampicillin appear to occur at a frequency of 1% or less (Havelaar et al.).

Aeromonas Starch DNA Agar is a modification of Palumbo's et al. formulation and consists of Tryptone Soy Agar, starch, DNA and ampicillin. The complete medium allows the selective growth of *Aeromonas* spp. and the presumptive differentiation of *A. hydrophila* by means of DNA and starch hydrolysis. Aeromonas Starch DNA Agar is useful for the isolation and enumeration of *Aeromonas* spp. from food and clinical samples. The undiluted or diluted sample is surface plated and incubated at 30°C for 24 hours in aerobic conditions. After incubation, check for a clearing halo around the colonies (starch hydrolysis).

Pick suspected colonies up for further biochemical tests. Flood the plates with HCl 1 N solution. If the colonies are DNAse positive, a new clearing halo occurs on an opaque background. Amylase positive and DNAse positive cultures must be subjected to biochemical characterisation for a complete identification.

User quality assurance (30°C-24 hrs)

Productivity control
A.hydrophila ATCC 7965: good growth

Selectivity control
E.coli ATCC 25922: inhibited

Storage
Dehydrated media: 15-30°C
User prepared plates: up to 7 days at 2-8°C

References

• Buchanan, R.L., Palumbo, S.A. (1985) J. Food Saf. **7**, 15-29
• Havelaar, A.H., During, M., Versteegh, J.F.M. (1987) J. App. Bact. **62**, 279-287
• Holmberg, S.C., Farmer, J.J. (1984) Rev. Inf. Dis. **6**, 633-639
• Palumbo, S.A. et al. (1985) App. Environ. Microbiol. **50**, 1027- 1030

AESCULIN BILE AZIDE AGAR

A selective medium with sodium azide for the isolation and differentiation of Group D streptococci

Code: KM3141

Typical formula	**(g/l)**
Tryptone	17.00
Peptone	3.00
Yeast Extract	5.00
Oxgall	10.00
Sodium Chloride	5.00
Sodium Citrate	1.00
Aesculin	1.00
Ferric Ammonium Citrate	0.50
Sodium Azide	0.25
Agar	13.00

AESCULIN BILE AZIDE BROTH

A selective liquid medium with sodium azide for the isolation and differentiation of Group D streptococci

Code: KM3142

Typical formula	**(g/l)**
Tryptone	17.00
Peptone	3.00
Yeast Extract	5.00
Oxgall	10.00
Sodium Chloride	5.00
Sodium Citrate	1.00
Aesculin	1.00
Ferric Ammonium Citrate	0.50
Sodium Azide	0.25

pH 7.1 +/- 0.1

Directions
Suspend 55.7g of agar or 42.7g of broth in 1000 ml of cold distilled water. Heat to boiling with frequent agitation the agar medium or heat to dissolve the liquid medium. Distribute and sterilise by autoclaving at 121° C for 15 minutes. Do not exceed sterilisation time and temperature.

Description
Aesculin Bile Azide Agar and Aesculin Bile Azide Broth are selective and diagnostic media for the detection of group D streptococci. These microorganisms grow in the presence of sodium azide and bile salts and are additionally capable of hydrolysing aesculin to glucose and aglycone 6-7 dihydroxycumarin: The aglycone reacts with the iron salts in the medium, giving them a black colouring. Aesculin Bile Azide Agar can be used with 8 mcg/ml of vancomycin for the screening of Vancomycin Resistant Enterococci.

Method
Prepare tenfold dilutions of the sample with peptone water. Within 3 hours from the sample preparation, spread 0.1 ml of the inoculum onto the plates. Incubate at 35°C or at 42°C for 18-24 hours (the higher incubation temperature increases the selectivity of the medium). Colonies of enterococci on Aesculin Bile Azide Agar (after 24 hours of incubation at 37°C) have the following characteristic appearance: Convex colonies, translucent or whitish, 1-2 mm diameter, dark brown or black surrounding zone; incubation should not be prolonged beyond 24 hours, because extensive blackening of the medium makes reading difficult. Using a sterile inoculating loop, inoculate broth with well-isolated colonies. Incubate at 37°C for 18-24 hours and observe for growth and blackening.

User quality assurance (37°C-24 and 48hrs)

Productivity control
E.faecalis ATCC 29212*:
Aesculin Bile Azide Agar: good growth with blackening of medium around the colonies
Aesculin Bile Azide Broth: the liquid medium turns black

Selectivity control
S.pyogenes ATCC 19615*: partially inhibited
E.coli ATCC 25922*: inhibited
*NCCLS M22-A2 recommended strains.

Storage
Dehydrated media: 15-30°C
User prepared plates: up to 7 days at 2-8°C
User prepared tubes and flasks: 1 month at 2-8°C

References
Isenberg, H.D., Goldberg, D. & Sampson, J. (1970) Appl. Micr., 20, 433-436.
Jensen Bette, J. (1996) Lab.Med. 27, 53
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.
Swan, A. (1954) J. Clin. Path, 7, 160-163.
Williams, R.E.O. & Hirch, A. (1950). J. Hyg (Camb.), 48, 504.

BACTERIOLOGICAL AGAR

Code: GA1002

A high clarity agar with good gelling properties and a low concentration of metal ions. This agar is suitable for all bacteriological purposes including sensitivity testing and ready pour plates. A firm gel is achieved at working concentrations of 1.0 to 1.5%. No significant precipitation is experienced upon reheating or prolonged holding at 65ºC.

Typical Analysis

Gel strength (Nikan)	700-1100g/m2
Colourimetry (1.5% soln at 65ºC)	> 0.28 at 340nm > 0.02 at 525nm
Melting point	> 85ºC
Setting point	32-35ºC
PH	6.5-7.3
Moisture	< 10%
Total ash	< 3%
Calcium	< 0.02%
Magnesium	< 0.02%
Sodium chloride	< 1.0%
Iron	< 0.01%
Insoluble ash	< 0.1%
Sulphate	1.5%

Microbiology

Salmonella	Absent
TVC	< 103/g
Spores	< 2/g