Brain Heart Infusion Agar

A general purpose nutritious agar base. This medium was first used for the isolation of dental pathogens.

Code: KM1010

Typical formula	**(g/l)**
BHI solids	17.5
Sodium chloride	5.0
Disodium phosphate	2.5
Tryptose	10.0
Glucose	2.0
Agar	12.0

pH: 7.4 ± 0.2

Directions
Suspend 49 grams of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then swirl to mix. Sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47ºC then pour into petri dishes.

Description
The mixture of brain and heart infusions is particularly useful in the isolation of Actinomyces israeli and Histoplasma capsulatum. With the addition of 7% defibrinated blood the medium will support the growth of a wide range of fastidious organisms, the phosphate buffer will help neutralise the acids produced from the utilisation of glucose and thus maintain viability. The medium is not recommended for the determination of haemolytic reactions because of the glucose content.

Q.C. organisms:
S. aureus
E. coli

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: Surface, streaking out to single colonies.

Incubation: Time and temperature to suit specimen/organisms.

References
Roseburg. T., Epps, L. J. and Clarke, A. R. 1944. A study of the isolation, cultivation and pathogenicity of Actinomyces israeli recovered from the human mouth and from actinomycosis in man. J. inf. Dis., 74: 131-149.
Howell, E. 1948 Efficiency of methods of isolation of Histoplasma capsulatum. Pbl. Hlth. Rep. 63: 173-178. 3/108

Brilliant Green Agar (Original)

A selective media for the isolation of salmonellae from pathological specimens, dairy and food products.

Code: KM1012

Formulation	**(g/l)**
Beef extract	5.0
Yeast extract	3.0
Sodium di hydrogen orthophosphate	0.6
Sucrose	10.0
Brilliant green	0.0032
Peptone mix	10.0
Di sodium hydrogen orthophosphate	1.0
Lactose	10.0
Phenol red	0.09
Agar	12.5

pH: 6.9 +/- 0.2

Directions
Suspend by swirling 52.2g in 1 litre or the contents of the sachet in the stated volume of distilled or deionised water. Allow to stand for 15 minutes. Heat gently with occasional mixing until the medium is completely dissolved. DO NOT AUTOCLAVE. Cool to 50ºC, mix well and pour plates.

Description
In 1925 Kristensen et al. first showed the value of incorporating phenol red as an indicator in a brilliant green selective medium for Salmonella spp. A more recent survey, however, showed wide variations in plating efficiency between laboratories, and further comparative study showed the value of standardised techniques.

Method
Inoculate either directly from the specimen or after enrichment in Selenite F Broth, onto a dried plate of Brilliant Green Agar formulation. Incubate at 37ºC for 18-48 hours. Salmonella spp. appear as red colonies surrounded by a bright red halo but Shigella spp. are inhibited. If the latter are sought, XLD Agar should be used in parallel. Pseudomonas spp. grow as crenated, red colonies and Proteus spp. are either inhibited or grow as red non-swarming colonies. Most lactose and sucrose fermenting organisms are also inhibited, but occasionally grow as yellow/orange colonies.

References
Kristensen M, Lester V, Jurgens A. Br J Exp Pathol 1952; 6: 291-3.

BRILLIANT GREEN AGAR (MODIFIED)

A medium for the isolation of *Salmonella* other than *S.typhi*

Code: KM1013

Typical formula	**(g/l)**
Beef Extract	5.00
Peptone	10.00
Yeast Extract	3.00
Disodium Phosphate	1.00
Monosodium Phosphate	0.60
Lactose	10.00
Sucrose	10.00
Sodium Chloride	5.00
Phenol Rod	0.09
Brilliant Green	0.047
Agar	12.00

pH 6.9 +/- 0.2

Directions
Suspend 52g in 1000 ml of cold distilled water, allow to stand for 10 minutes and then heat to boiling with frequent agitation. Cool to 50°C, then pour into sterile petri dishes and dry the surface before inoculation. **Do not** re-melt or autoclave: overheating causes precipitation. Store plates away from light.

Description
Brilliant Green Agar Modified is suitable for the detection of *Salmonella* spp. in food and animal feed stuffs. Brilliant green inhibits Gram-positive bacteria and most Gram-negative bacteria. Sucrose and lactose are present as fermentable carbohydrates. In comparison with Kristensen medium, this medium is enriched with nutritive factors and is buffered with phosphate salts.

Method
For foodstuffs, ISO 6579 recommends the following procedure: In general, for the preparation of initial suspension add 25g of sample portion to 225ml of Buffered Peptone Water. If the required test portion is other than 25g, use the suitable quantity of Buffered Peptone Water to yield approximately 1/10 dilution (m/v). Incubate the initial suspension at 37°C for not less than 16 hours and not more than 20 hours. The selective enrichment broth's and the incubation temperature are different for different foodstuffs. ISO 6579 in the general guidance on methods for detection of *Salmonella* suggests the following procedure: Transfer 0,1ml of the pre-enriched culture to a tube containing 10ml of Rappaport Vassiliadis (RV) Broth and 10ml to a flask containing 100ml of Selenite Cystine Broth Incubate the inoculated RV Broth at 42°C for 24h Broth. Incubate the inoculated Selenite Cystine Broth at 37°C for 24 h and a further 24hrs. Using the culture obtained in the RV Broth inoculate by means of a 3mm loop a large-size petri dish or two 90mm petri dishes containing Brilliant Green Agar Modified. Proceed in the same way from the tubes of RV Broth, inoculating a second plating medium (e.g. Chromogenic Salmonella Agar), or other suitable selective *Salmonella* plating-out medium, has chosen by the laboratory. Using the cultures obtained in the Selenite Cystine Broth after 24 and 48 hours of incubation, repeat the procedure with the same two selective plating-out media. Invert the dishes and incubate at 37°C for 20-24h. Examine for the presence of typical colonies. On Brilliant Green Agar Modified, *Salmonella* grows with red colonies and the medium

changes from pink to red. Some strains of lactose and sucrose fermenting *Enterobacteriaceae*, grow with yellow colonies. If growth is slight, or if no typical colonies are observed, re-incubate at 37°C for further18-24hrs. Any typical or suspected colony should be subjected to a biochemical and serological confirmation using a pure subculture in a nutrient agar plate. Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of beta-galactosidase, VP reaction, indole detection. Serological confirmation includes the detection of the presence of *Salmonella* O-, Vi and H antigens by slide agglutination test.
Biochemical confirmation could be substituted with the rapid test.

User quality assurance (37°C-24hrs)

Productivity control
S.enteritidis ATCC 13076: growth, red-purple colonies, surrounded by a diffused red halo.
S.typhimurium ATCC 14028: growth, red-purple colonies, surrounded by a diffused red halo.

Selectivity control
S.aureus ATCC 25923: inhibited
E.faecalis ATCC 29212: inhibited

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

References
Edel, W. and Kampelmacher, E.H. (1968) Bull. WHO, 39 (3), 487.
ISO 6579 Microbiology - General guidance on methods for the detection of *Salmonella*. 1993-09-01

BRILLIANT GREEN BILE 2% BROTH

Used for the detection and confirmation of coliforms in water, sewage, dairy products and foodstuffs.

Code: KM1015

Typical Formula (g/l)

Ox Bile	20.00
Lactose	10.00
Peptone	10.00
Brilliant Green	0.0133

pH 7.2 +/- 0.2

Directions

Suspend 40g in 1000ml of cold distilled water; heal to dissolve, distribute into fermentation tubes and sterilise by autoclaving at 115°C for15 minutes. Do not exceed time and temperature of sterilisation.

Description

Brilliant Green Bile Broth 2% is a selective medium recommended for the detection and confirmation of coliform and heat - tolerant coliform bacteria and *E.coli* in water, sewage, dairy products and foodstuffs. The presence of the brilliant green suppresses the growth of the anaerobic lactose-fermenting bacteria *(Clostridium pertringens)* without obtaining false positives when incubated at 44°C. The brilliant green and bile salts inhibit the growth of Gram-positive microorganisms. It is recommended by ISO 4831 for the confirmatory test of coliform bacteria in foodstuffs and by ISO 5541/2 as primary inoculation liquid medium for the detection of coliform bacteria in milk and milk products by MPN method. FDA Bacteriological Analytical Manual reports the use of Brilliant Green Bile Broth 2% for the confirmed test of coliforms. It is recommended by APHA for the confirmatory test of faecal coliform bacteria by incubating at 44°C. Brilliant Green Bile Broth 2% is used together with Peptone Water at 44°C for the Mackenzie test.

Method

For the enumeration of coliform bacteria with the most probable number (MPN) technique proceed as following: Prepare the test sample and the decimal dilution in accordance with the specific laboratory method using Maximum Recovery Diluent or other suitable diluent. Take three tubes of double-strength Lauryl Peptone Broth and by means of a sterile pipette transfer to each tube 10ml of the test sample, if liquid or 10ml of the initial suspension in the case of other products. Than take three tubes of single-strength Lauryl Peptone Broth and by means of a sterile pipette transfer to each tube 1 ml of the test sample, if liquid or 10ml of the initial suspension in the case of other products. Repeat the inoculation of the single strength and the double strength liquid medium for each further decimal dilution, using a fresh pipette for each dilution. Incubate the tubes of double-strength Lauryl Peptone Broth at 30 or 37°C for 24 +/- 2 hours. Incubate the tubes of single-strength Lauryl Peptone Broth at 30 or 37°C for 24 +/- 2 hours and for further 24 hours if neither gas nor opacity is observed after 24 hours. From each of the incubated tubes with double-strength Lauryl Peptone Broth inoculate with a loop a tube of Brilliant Green Bile Broth (confirmation medium) and incubate at 30 or 37°C for 24 +/- 2 hours or, if gas formation is not observed, for 48 +/- 2 hours. Carry out the same procedure for the incubated single-strength Lauryl Peptone Broth showing gas formation or opacity. For each dilution of incubated confirmation liquid medium count the total number of tubes in which gas formation is observed. Express the results as the Most Probable Number of coliforms

on the basis of gas production in the Brilliant Green Bile Broth 2% tubes after 48 hours incubation.

For the enumeration of faecal coliform bacteria with the most probable number technique proceed as following: From each of the positive tubes of MacConkey Broth, inoculate, with a loop, a tube of Brilliant Green Bile Broth 2% and a tube of Peptone Water and incubate at 44 °C. Observe for gas production in Brilliant Green Bile Broth 2%, after 24 and 48 hours of incubation and test the indole production in Peptone Water after 24 hour of incubation. The MacConkey Broth tubes, which are positive to gas production in Brilliant Green Bile Broth 2% and are indole positive must be considered positive for faecal coliforms.

For the detection of coliform bacteria in milk and milk products, with MPN technique, proceed as following:

1. Take 3 tube of double strength Brilliant Green Bile Broth 2% and transfer 10ml of the liquid test sample or 10ml of the primary dilution
2. Take 3 tube of single strength Brilliant Green Bile Broth 2% and transfer 1 ml of the liquid test sample or 1 ml of the primary dilution.
3. For each of the dilutions 10-1 or 10-2 (according to the circumstances) take three tubes of single strength Brilliant Green Bile Broth 2% and transfer 1ml of the respective dilution into each of these tubes.
4. Incubate the tubes of double strength broth at 30°C for 24 hours.
5. Incubate the tubes of single strength broth at 30°C for 48 hours.
6. From each of the incubated tubes of double strength broth inoculate with a loop a tube of single strength broth and incubate at 30°C for 48 hours.
7. From each of the inoculated tubes showing production of gas in the Durham tubes streak a loopful on Levine EMB Agar and incubate at 30°C for 18-24 hours. Consider as characteristic growth those colonies that are metallic, red/pink and mucoid in appearance (confirmatory test)
8. Record for each dilution the number of confirmed positive tubes.

User quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: growth, gas production
C.freundii ATCC 43864: growth, gas production

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

Storage
Dehydrated medium: 15-30°C
User prepared plates: 1 month at 2-8°C in the dark

References
APHA (1980). Standard Methods for the Examination of Water and Wastewater. 15th edition
Mackenzie, E.F.W., Taylor, E.W. and Gilbert, W.E. (1948), J. Gen. Microbiol., 2,197
FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.
ISO 4833 Microbiology-General guidance for the enumeration of coliforms – Most probable number technique. 1991-03-01 & ISO 5541-2 Milk and Milk products-Enumeration of coliforms – Part 2 Most probable number technique at 30°C . 1986-12-01

BRILLIANT GREEN BILE 2% BROTH with MUG

A liquid medium for the detection of coliforms and *E.coli*

Code: KM3662

Typical formula (g/l)

Ox Bile	20.00
Lactose	10.00
Peptone	10.00
Brilliant Green	0.0133
MUG	0.100

pH 7.2 +/- 0.2

Directions
Suspend 40g in 1000 ml of cold distilled water; heal to dissolve, distribute into fermentation tubes and sterilise by autoclaving at 121°C for15 minutes. Do not exceed time and temperature of sterilisation. With inocula greater than 1ml for 10ml of medium, use multiple strength medium.

Description
Brilliant Green Bile 2% Broth MUG is used for the detection of *E. coli,* with the MPN procedure, in water and foods. Incubate the inoculated tubes for 18-24 hours and observe periodically for the development of fluorescence using a Wood's lamp. Refer to the various compendia for the test being performed and to technical sheet of Brilliant Green Bile 2% Broth for the details of the procedure for the detection and confirmation of coliforms.

User quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: growth, gas production, fluorescent under Wood's lamp
C.freundii ATCC 43864: growth, gas production, not fluorescent under Wood's lamp

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

Storage
Dehydrated medium: 15-30°C
User prepared plates: 1 month at 2-8°C in the dark

BROMCRESOL PURPLE BASE BROTH

A liquid culture medium with indicator to carry out sugar fermentation studies.

Code: KM2031

Typical formula	**(g/ltr)**
Gelatine peptone	10.00
Sodium chloride	5.00
Bromocresol purple	0.02

pH 6.8 ±0.2

Directions
Dissolve 15g of powder into 1 litre of distilled water. Add substrate to assay in the desired concentration and distribute into containers provided with Durham's tubes. Sterilise in the autoclave at 121°C for 10 minutes. Heat up the autoclave before putting in the tubes to avoid sugar caramelisation. Addition of some sugars may require a pH adjustment. To study the fermentation of some sugars like Glucose, Maltose, Mannitol and Sucrose it is advisable to add them at a 10g/l concentration.

Description
Bromcresol Purple Base Broth is the liquid version suitable to determine, helped with Durham's tubes, gas production on enterobacteria sensitive to phenol red.
In the ADSA=MICRO formulation, meat extract has been removed because it was unnecessary and moreover it provides little concentrations of fermentable sugars that could change the results. Some bacteria may ferment the carbohydrates in the medium, and then it changes to yellow, and if they produce gas, it is retained in the Durham's tube.

References
ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.

BROMOCRESOL PURPLE LACTOSE AGAR

A medium for the enumeration of spores of mesophilic and thermophilic *Bacillus*

Code: KM1201

Typical formula	**(g/l)**
Peptone mix	7.50
Lactose	8.50
Bromocresol Purple	0.025
Agar	12.00

pH 6.8 ± 0.2

Directions
Suspend 28g in 1000ml of cold distilled water; heat to boiling with agitation and sterilise in the autoclave at 121°C for 15 minutes.

Description
Bromocresol Purple Agar is used for the enumeration of spores of *Bacillus* responsible for flat sour (especially *B. stearothermophilus*) in sugar, sweet desserts, spices, aromatic preparation and other foodstuffs.

Method
- Destroy the vegetative cells by heating the sample.
- Inoculate the plates with 1 ml of the sample or of its tenfold dilutions and pour 15 ml of medium into petri dishes
- Incubate at 30°C for 5 days to enumerate *Bacillus* spores.
- Incubate at 55°C for 5 days to enumerate thermophilic *Bacillus* spores. Pour several drops of sterile paraffin oil in the lid of the plate as a tight seal.
- Bacteria acidifying the medium will grow with yellow colonies, bacteria not metabolising the glucose will grow with blue colonies.

User quality assurance

Productivity control
B.stearothermophilus ATCC 10149: growth, yellow colonies (55°C-72 h)
B.subtilis ATCC 6633: growth, yellow colonies (55°C-72 h)

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

Reference
National Canners Association (1993) Bacterial Standards for Sugar.

BRUCELLA BROTH

For the cultivation of *Brucella* and other fastidious and non-fastidious microorganisms.

Code: KM3742

Typical formula	**(g/l)**
Peptone	20.0
Yeast Extract	2.0
Glucose	1.0
Sodium Chloride	5.0
Sodium Bisulphite	0.1
Sodium Phosphate	3.0

pH 7.0 +/- 0.1

Directions
Suspend 31.1g in 1000 ml of cold distilled water. Heat to dissolve, distribute and sterilise by autoclaving at 121 °C for 15 minutes.

Description
Brucella Broth is a high nutritive solid medium used for the cultivation of *Brucella* and other fastidious and non-fastidious microorganisms. Brucella Broth is used as basal medium for the isolation of Campylobacter and other fastidious microorganisms.

Method
Inoculate the medium with purified cultures or other type of inoculum. For the cultivation of Brucella incubate the inoculated tubes for up to 7 days at 37°C in an aerobic atmosphere with carbon dioxide. For the cultivation of other microorganisms, incubate at the required optimum temperature in a suitable atmosphere for the time necessary to develop growth.

Storage
Dehydrated medium: 15-30°C

BRUCELLA MEDIUM BASE

A medium used for the cultivation of *Brucella*, *Campylobacter* and other microorganisms

Code: KM1014

Typical formula	**(g/l)**
Peptone	10.0
Beef Extract	5.0
Glucose	10.0
Sodium Chloride	5.0
Agar	15.0

pH 7.5 +/- 0.2

Directions
Suspend 45g in 1000 ml of cold distilled water. Heat to boiling and autoclave at 121°C for 15 minutes. Cool to 50°C and add 5 % of horse serum, inactivated by heating at 56°C for 80 minutes. To obtain a selective medium, various antibiotics and dyes can be added: the addition of 10mg cycloheximide, 2500 I.U. bacitracin, 600 IU. polymixin B to 100 ml of medium is recommended. In addition, as suggested by Renoux, ethyl violet at a final concentration of 1:800,000 can be added. Brucella Medium Base may be used for the preparation of Campylobacter selective plating media by adding the suitable supplements: Defibrinated or lysed blood, Campylobacter Growth Supplement, Skirrow Supplement or Blaser Wang Supplement.

Description
Brucella Medium Base can be used to prepare the glucose serum antibiotics medium described by Jones and Brinley Morgan, and is recommended by the WHO for the selective isolation of *Brucella,* including fastidious strains, and *Brucella abortus* type II, which is very difficult to grow on common media. *Brucella* grows on the medium, with incubation in a 10% CO_2 atmosphere at 37°C, after 3 days. However, examination of the plates is recommended on the fourth day, when the colonies have a diameter of 2-3mm. Cultures considered negative after four days of incubation should be re-examined on the eighth and tenth day, and then eliminated. Examined in indirect sunlight, the colonies appear translucent, with a slightly amber tinge. To check that the colonies are *Brucella,* a specific antiserum agglutination test is suggested. The WHO recommends the use of thionine and basic fuchsin resistance tests to differentiate *Brucella melitensis, Brucella abortus* and *Brucella suis.* Prepare 0.1 % solution of the dyes in distilled water and boil in water baths for an hour; add the dyes (final concentrations from 1:25,000 to 1:100,000) to the medium (with added serum) and pour into plates. Optimal working concentrations must be established using standard fuchsin and thionine. Dry the dishes with covers off by incubation at 37°C for 1-2 hours. Divide the dishes into four squares, and inoculate each quadrant with a different microbial suspension, tracing five streaks, without reloading the loop. In addition, inoculate all the suspensions to be examined onto plates without the addition of dyes. Incubate in CO_2 atmosphere for four days. *Brucella abortus* grows in the presence of fuchsin and does not grow in the presence of thionine. *Brucella melitensis* grows in the presence of dyes. *Brucella suis* only grows in the presence of thionine. Brucella Medium Base is recommended as a base medium for the preparation of the selective medium for the isolation of Campylobacter jejuni.

Storage
Dehydrated medium: 15-30°C

References
Kuzdas, C.D. & Morse, E.V. (1953) - J. Bact., 56, 502.
Jones, Lois M. & Brinley Morgan, W.J. (1958) - Bull. Wld. Hlth. Org., 19, 200.
Renoux, G. (1954) - Ann. Inst. Pasteur, 87, 25.

BRYANT BURKEY BROTH BASE (WITH RESAZURIN)

A liquid medium for the enumeration of spores of lactate fermenting clostridia

Code: KM3291

Typical formula	**(g/l)**
Tryptone	15.0
Yeast Extract	5.0
Beef Extract	7.5
Sodium Acetate	5.0
Cysteine HCl	0.5
Resazurin	2.5 mg

pH 5.9 ± 0.1

Directions
Suspend 33 g in 1000 ml of cold distilled water; add 10 g of 50% sodium lactate solution. Heat to dissolve stirring constantly. Distribute 10ml in 16x160mm tubes. Autoclave at 121°C for 15 minutes.

Description
Bryant Burkey Broth Base (with Resazurin) is recommended for the enumeration of spores of lactate fermenting clostridia in milk and dairy products, especially *Clostridium tyrobutyricum* responsible for the browning of cheeses.

Method
1. Cool the tubes to 25°C after autoclaving or regenerate the anaerobic conditions by heating if the medium is pink for more than 1/3 of its height under surface.
2. Inoculate 1 ml of the tenfold dilutions of the sample.
3. Pour paraffin at 58-60°C, previously sterilised at 121°C for 20 minutes.
4. Heat the tubes at 75°C for 15 minutes to destroy vegetative cells.
5. Cool in an ice-bath to solidify the paraffin.
6. Incubate at 37°C for 7 days.

Tubes with growth and gas are to be considered as positive.

User quality assurance (48 h-37°C)

Productivity control
C.tyrobutyricum: growth

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 1 month at 2-8°C (regenerate at 100°C for 20 min before use)

References
Bryant, M.P., Burkey, L.A. (1956) J. Bact., 71, 43-46
Bergère, J.L., Gronet, P., Hermier, P. Moquot, A. (1968) Ann. Inst. Pasteur Lille, 19, 41-54

BUFFERED PEPTONE WATER

A non-selective pre-enrichment liquid medium for *Salmonella* detection in foodstuffs and water.

Code: KM1016

Typical formula	**(g/l)**
Peptone	10.00
Sodium Chloride	5.00
Disodium Phosphate anhydrous	3.50
Monopotassium Phosphate	1.50

pH 7.2 +/- 0.2

Directions
Suspend 20g in 1000ml of cold distilled water; heat to dissolve, distribute into flasks of suitable capacity and sterilise in the autoclave at 121°C for 20 minutes.

Description
Buffered Peptone Water is a non-selective enrichment broth, recommended for the pre-enrichment of *Salmonella* in foodstuffs by ISO 6579 and for the non-selective pre-enrichment of *Enterobacteriaceae* by ISO 8523. It is also suitable for the preparation of stock suspensions of powdered milk, concentrated milk, yoghurt and other dairy products.

Method
For the analysis of wate*r,* Harvey recommends the preparation of 25ml aliquots of the medium at double concentration, inoculated with an equal volume of water and incubated for 15 hours at 37°C. The procedure recommended by ISO/DIS 6579:2000, is the following: Add 25g of sample portion to 225ml of Buffered Peptone Water. If the required test portion is other than 25g, use the suitable quantity of Buffered Peptone Water to yield approximately 1/10 dilution (m/v). Incubate the initial suspension at 37°C for not less than 16 hours and not more than 20 hours. Transfer 0,1ml of the pre-enriched culture to a tube containing 10ml of Rappaport Vassiliadis Soy (RVS) Broth and 1ml to a flask containing 10ml of Mueller Kauffmann Broth (MKB) Incubate the inoculated RVS Broth at 41,5°C +/- 1°C for 24 h +/- 3 hrs Incubate the inoculated MKB at 37°C +/- 1 for 24 h +/- 3. Using the culture obtained in the RVS 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 other suitable selective *Salmonella* plating-out medium chosen by the laboratory). Using the cultures obtained in MKB after 24 hours of incubation, repeat the procedure with the same two selective plating-out media. Invert the dishes and incubate at 37°C for 24 hrs. +/- 3 h. 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.

User quality assurance (48 h-37°C)

Productivity control
S.enteritidis ATCC 13076: growth
S.typhimurium ATCC 14028: growth

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

References
Harvey R.W.S. and Price T.H. (1977) J. App. Bact. 43, 145-148.
ISO/DIS 6579 Microbiology of food and animal feed stuffs Horizontal method for the detection of Salmonella spp. 2000.
ISO 8523: 1991 - Microbiology- general guidance for the detection of *Enterobacteriaceae* with pre-enrichment 1991-10-01

Buffered Salt Peptone Solution

A sample preparation liquid medium

Code: KM3952

Typical formula	**(g/l)**
Potassiun Dihydrogen Phosphate	3.60
Disodium Phosphate Anhydrous	5.76
Sodium Chloride	4.30
Tryptone	1.00

pH 7.0 +/- 0.1

Directions
Suspend 14.6g in 1000ml of cold distilled water. Heat to dissolve, distribute into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes. Before sterilisation surface-active agents or inactivators of antimicrobial agents may be added, such as Polysorbate 80, 1g/ltr to 10g/ltr. A typical neutralising fluid is supplemented before sterilisation with: Polysorbate 80 30g/ltr, Lecithin 3g/ltr, histidine HCl 1g/ltr.

Description
Buffered salt peptone solution is prepared according to the formulation recommended by European Pharmacopoeia. It is used as a universal diluent for sample preparation used in the microbiological examination of non-sterile products. Refer to EP 3rd edition for details of the procedures of sampling and sample preparation.

Storage
Dehydrated medium: 15-30°C
User prepared tubes: 3 months at 2-8°C

Bacillus Cereus Medium (P.R.E.P.)

For the enumeration of Bacillus cereus in foods

Code: KM1005

Typical formula	**(g/l)**
Beef Extract	1.0
Peptone	10.0
D-Mannitol	10.0
Sodium chloride	10.0
Phenol red	0.025
Agar	15.0

pH: 7.2 ± 0.2

Directions
Weigh 46 grams of powder, disperse in 900ml of deionised water. Allow to stand for 10 minutes, swirl to mix then sterilise by autoclaving at 121ºC for 15 minutes. Cool to 47ºC and aseptically add 100 mls of egg yolk emulsion and 2 vials of Polymixin Selective supplement.

Q.C. organisms:
B. cereus
E. coli (inhibition)

Storage: up to 7 days at 2-8ºC in the dark.

Inoculation: Surface, spreading or streaking for single colonies.

Incubation: 30ºC aerobically for 24-48 hours.

References
Mossel, D. A. A., Koopman, M. J. and Jongerius, E. 1967. Enumeration of Bacillus cereus in foods. Appl. Microbiol. 15: 650-653. Thatcher, F. S., Clarke, D. S. 1978 Micro-organisms in foods. Volume 1 second edition. University of Toronto.
BS5763 Part 1L:1994. ISO7932:1993 3/100

BAIRD PARKER MEDIUM

A medium base used for the detection of coagulase-positive staphylococci.

Code: KM1006

Typical formula	**(g/l)**
Tryptone	10.00
Beef Extract	7.50
Yeast Extract	1.00
Sodium Pyruvate	10.00
Glycine	12.00
Lithium Chloride	5.00
Agar	20.00

Supplement with Egg Yolk Tellurite Emulsion 100ml – Code: KMS006

pH 6.8 +/- 0.2

Directions
Suspend 65.5g in 1000 ml of cold distilled water; heat to boiling and autoclave at 121°C for 15 minutes. Cool to 50°C and, using aseptic conditions, add 50 ml of Egg Yolk Tellurite Emulsion; mix well and pour into sterile petri dishes.

Description
Baird Parker Agar Base is a selective and diagnostic medium recommended by ISO 6881 and by FDA for the isolation and enumeration of coagulase-positive staphylococci in foodstuffs. It is also recommended by USP and EP for the isolation of *S.aureus* in non-sterile pharmaceutical products. Lithium chloride and potassium tellurite inhibit contaminating flora, glycine and sodium pyruvate facilitate the development of staphylococci. Some yeasts, fungi and bacilli also grow, but these are easily distinguishable by their morphology and by the grey colour of the colonies. ISO 6881 recommends the addition of 5 mg/l of sulphamezathine to the medium after sterilisation to inhibit the development of *Proteus* spp.

Method
ISO 6881-1 recommends the following procedure: Prepare the sample suspension and further decimal dilutions with Maximum Recovery Diluent. Transfer by means of a sterile pipette 0,1 ml of the test sample if liquid or 0,1 of the initial suspension in the case of other products, to each of two agar plates. Repeat the procedure for further decimal dilutions if necessary. Carefully spread the inoculum as quickly as possible over the surface of the agar plate. Allow the plates to dry. Invert the plates and incubate them for 24 +/- 2hrs at 37°C and re-incubate for a further 24 +/- 2hrs. After incubation for 24hrs mark on the bottom of the plates the positions of any typical colonies. Re-incubate, and then mark any new typical colonies. Also mark any atypical colonies present. Enumerate those plates that contain at maximum 300 colonies with 150 typical and/or atypical colonies at two successive dilutions. One of the plates shall contain at least 15 colonies. Select for confirmation five characteristic colonies if there are only characteristics colonies, or five non-characteristic colonies if there are only non-characteristic colonies, or five characteristic and five non-characteristic colonies if both types are present, from each plate. Typical *S .aureus* are black or grey, shining and convex colonies (1-1.5 mm in diameter after 24 h of incubation, 1,5-2,5 mm in diameter after 48 hours), surrounded by a zone of clearing of the medium. After at least 24hrs an opalescent ring immediately in contact with the colonies may appear in this clear zone. Atypical colonies may present one of the following morphologies: Shining black colonies with or without a narrow white edge; the clear zone is absent or barely visible and the opalescent ring is absent or hardly visible. Grey colonies free of clear zones. Bacteria belonging to genera other than *Staphylococcus* may grow with colonies similar to staphylococci. Microscopic examination of Gram stain will enable the distinction of other genera from staphylococci. For confirmation carry out coagulase test using Coagulase Plasma EDTA according to the procedure reported by technical sheet. Baird Parker recommends enrichment in Tryptone Soy Broth with 10%

NaCl or Giolitti-Cantoni Broth to test material containing less than five staphylococci per gram. AOAC recommends a technique for coagulase-positive staphylococci detection in foodstuffs, which involve enrichment in Tryptone Soy Broth with 1% Sodium Chloride for 48 hours, then a transfer to Baird-Parker Medium and catalase and coagulase tests.

User quality assurance (37°C-48 hrs)

Productivity control
S.aureus ATCC 6538: good growth, black colonies with clear halo
S.aureus ATCC 25923: good growth, black colonies with clear halo

Selectivity control
E.coli ATCC 25922: inhibited

Specificity control
S.epidermidis ATCC 12222: partially inhibited, black colonies without halo

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

References
Baird-Parker, A.C. (1962) J. Appl. Bact., 25, 12-19.
Eurpean Pharmacopoeia, 3rd ed. 2001 Supplement
ISO 6888-1. Microbiology of foods and animal feeding stuffs Horizontal method for the enumeration of coagulase positive staphylococci, part 1: technique using Baird Parker Agar Medium . 1998.
FDA (1995) Bacteriological Analytical Manual, 8th ed. Revision A, 1998. Published by AOAC International.
U.S. Pharmacopoeia 24, NF 19 (2000)

BRAIN HEART INFUSION BROTH

A general purpose liquid medium in powder and ready to use, for the cultivation of fastidious and non-fastidious microorganisms.

Code: KM1011

Typical formula	**(g/l)**
BHI Solids	12.2
Peptone mixture	15.0
Glucose	2.0
Sodium Chloride	5.0
Disodium Phosphate	2.5

pH 7.4 +/- 0.2

Directions
Suspend 37g of BHI broth in 1000ml of cold distilled water. Heat to boiling, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
Brain Heart infusion Broth is used for the cultivation of a wide variety of microorganisms, including fastidious organisms, moulds and yeasts. With the addition of penicillin (20 I.U./ml) and streptomycin (40 I.U/ml) the media become selective for pathogenic fungi, the growth of bacteria and many saprophytic fungi, being retarded and/or inhibited. The addition of paraminobenzoic acid, which neutralises sulphonamides facilitates the isolation of microorganisms from patients undergoing chemotherapeutic treatment. Brain Heart Infusion Broth is especially recommended for the cultivation of streptococci, pneumococci and meningococci, and for the preparation of Castaneda biphasic blood-culture bottles. With the addition of 10% horse blood and the agar concentration of 2%, BHI Broth is recommended for the preparation of the culture of *S.aureus* to be used for coagulase test.

User quality assurance (24 h -37°C)

Productivity control
S.aureus ATCC 25923: growth
E.coli ATCC 25922: growth

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

Reference
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.

BIGGY (NICKERSON) CANDIDA AGAR

Used for the isolation of *Candida* from clinical specimens

Code: KM3802

Typical formula	**(g/l)**
Yeast Extract	1.0
Bi-Ammonium Citrate	5.0
Sodium Sulphite	3.0
Glucose	10.0
Glycine	10.0
Agar	15.0

pH 6.8 +/- 0.2

Directions
Suspend 44g in 1000 ml of cold distilled water; heat with frequent agitation and boil for not more than one minute. Cool to about 50°C, swirl to disperse the insoluble matter and pour into plates. Do not overheat or autoclave.

Description
The medium, which has a composition unfavourable to the development of schizomycetes, permits the isolation of *Candida* spp. from clinical specimens (mucous secretions, skin or nail fragments) containing mixed flora. Candida Agar contains a bismuth indicator, which turns black in the presence of hydrogen sulphide: according to the sulphite reducing capacity of the species being examined, the colonies appear more or less black. *C. albicans* grows on the medium with metallic black colonies, non-pathogenic candida with brown colonies. According to Nickerson, some yeasts (among those *C. albicans, C. krusei, C. tropicaiis)* also develop sulphite-reducing enzymes, which spread in the culture medium: the colonies of these species are surrounded by a black zone, sometimes with a metallic sheen.

Method
Inoculate the material being examined onto the surface of the medium and incubate at 37°C for 48-72 hours; the development of black colonies permits a presumptive diagnosis of the genus. Obviously, this diagnosis must be confirmed with at least a microscopic test (fresh preparation cleared with lactophenol methyl blue: branched pseudomycelium budding yeast cells, presence of chlamydospores, absence of ascospores) and the species must be identified with currently used morphological, physiological or immunological tests.

User quality assurance (48 h-37°C)

Productivity control
C.albicans ATCC 60193: growth, brown metallic colonies

Selectivity control
E.coli ATCC 25922: inhibited

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

Reference
Nickerson V.J. (1953) J. Inf. Dis., 93, 43-56.

BILE AESCULIN AGAR

For the confirmation of enterococci with bile tolerance and aesculin tests

Code: KM8172

Typical Formula	**(g/l)**
Beef Extract	3.0
Peptone	5.0
Oxbile	40.0
Ferric Ammonium Citrate	0.5
Aesculin	1.0
Agar	12.0

pH: 6.4 +/- 0.2

Directions
Suspend 61.5g in 1000ml of cold distilled water. Heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
Bile Aesculin Agar is used for the confirmation of enterococci with bile tolerance and aesculin tests. It is recommended by ISO FDIS 7899-2. Enterococci hydrolyse aesculin to form aesculetin and glucose; aesculetin combines with ferric ammonium citrate to form black or dark brown complex. Oxbile inhibits the Gram-positive bacteria other than enterococci.

Method
For the confirmation of the colonies cultivated on Slanetz & Bartley Agar during the MF technique for liquid samples, transfer the membrane after 48hrs of incubation at 37°C onto a plate of Bile Aesculin Agar. Incubate at 37°C for 4 hours. If the colonies develop a dark brown or black halo, they are confirmed as enterococci. Bile Aesculin Agar may be inoculated also with 4-5 colonies cultivated on any plating medium and incubated at 37°C for 18 hours. The result is positive for bile tolerance and aesculin hydrolysis if blackening of the medium occurs.

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

Productivity control
E.faecalis ATCC 29212*: good growth with blackening of medium around the colonies

Selectivity control
S.pyogenes ATCC 19615*: partially 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
ISO/FDIS 7899-2: 2000 – Qualitée de l'eau - Recherche et dénombrement des streptocoques fécaux. Partie 2_ méthod par filtration sur membranes
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.

BILE AESCULIN AZIDE AGAR

Code: KM3141

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

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
Fe-Ammonium Citrate	0.50
Sodium Azide	0.25
Agar	13.00

BILE AESCULIN AZIDE BROTH

Code: KM3142

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

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
Fe-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 1000ml 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 48 hrs)

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.

BILE PRODUCTS

Bile Salts No. 3

Code: BP1025

A superior bile salt, comprising mainly sodium cholate and sodium desoxycholate. It is used as a selective agent in culture for the isolation and differentiation of enteric organisms. This small percentage of bile is highly active, allowing maximum selection of organisms of enteric origin at relatively low concentrations.

Typical Analysis
Appearance white powder
Solubility in water at 2% total
Clarity clear
pH of a 2% solution 8.0 ± 0.5

Sodium Desoxycholate

Code: BP1026

This is a specific bile acid, derived from deconjugated bile salts. Results show that desoxycholic acid had the most inhibitory effect on bacterial growth, and that this could be enhanced by the removal of magnesium ions by chelating with sodium citrate.

Typical Analysis
Appearance white powder
pH of 2% solution 8.3 ± 0.5
Solubility in water at 2% total
Moisture <5%
Heavy metals <20 ppm
Sodium cholate <2%

Special (Bacteriological) Bile

Code: BP1018S

Typical Analysis
Appearance: Straw beige coloured free-flowing powder
Solubility:Total dissolution in <1 minute at 20°C (2% solution)
pH: 7.5 ± 0.5
Total Bile Acids: 65.1%
Loss on drying: 2.5%

Ox Bile

Code: BP1012

Typical Analysis
Appearance: Light Buff free-flowing powder
Solubility: Total dissolution in < 1 minute at 20°C (2% solution)
pH: 8.0 ± 0.5

Cholic Acid	62.6%
Loss in drying	4.7%

Sodium Taurocholate

Code: BP1954

Typical Analysis
Appearance: Fine hydroscopic free-flowing powder
Solubility: Soluble in water and alcohol
Total Bile Acids: Not less than 65%
Acid values: Not more than 145
Loss on drying: 2.9%

BLOOD AGAR BASE No. 2

An improved blood agar base for the cultivation of fastidious and non-fastidious microorganisms.

Code: KM1008

Typical formula	**(g/l)**
Peptone	15.0
Liver Extract	2.5
Yeast Extract	5.0
Sodium Chloride	5.0
Agar	12.0

pH 7.4 +/- 0.1

Directions
Suspend 39.5 g in 1000 ml of cold distilled water, heat to boiling and autoclave at 121 °C for 15 minutes. Cool to 45-50°C and add 5-7% sterile defibrinated blood.

Description
Blood Agar Base No. 2 is a modification of Blood Agar Base studied in order to obtain a superior growth of fastidious microorganisms. The peptones present in the medium allow achieve accurate haemolytic zones and an increase in the size of the colonies.

User quality assurance (Blood Agar Base no. 2 + 5% def. blood) (37°C-24hrs)

Productivity control
S.pyogenes ATCC 19615: good growth, β haemolysis
S.pneumoniae ATCC 6305: good growth, α haemolysis
S.aureus ATCC 25923: good growth
E.coli ATCC 25922: growth

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

Reference
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.

BLOOD AGAR BASE (I)

A basal medium for the preparation of blood agar plates

Code: KM1007

Typical formula	**(g/l)**
Beef Extract	10.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	12.0

pH 7.4 +/- 0.2

Directions
Suspend 37 g in 1000 ml of cold distilled water, heat to boiling and autoclave at 121ºC for 15 minutes. Cool to about 50°C, aseptically add 5% (v/v) of sterile defibrinated blood; mix well and pour into sterile petri dishes. Incubate at 37°for at least 24 hours before use, to test the sterility.

Description
Blood Agar Base is a multipurpose medium suitable for the growth of pathogenic and non-pathogenic microorganisms. With defibrinated animal blood the medium is suitable for the preparation of blood agar plates for the cultivation of streptococci, staphylococci, pneumococci and for the determination of their haemolytic activity. With blood agar horse *H.influenzae* develops a beta haemolytic halo and mimics *S.pyogenes.* With serum or other enrichments Blood Agar Base, is suitable for the cultivation of fastidious microorganisms.
Without any addition the medium is used as a rich nutrient medium.

User quality assurance
(Blood Agar Base + 5% def. blood)(37°C-24hrs)

Productivity control
S.pyogenes ATCC 19615*: good growth, β haemolysis
S.pneumoniae ATCC 6305*: good growth, α haemolysis
S.aureus ATCC 25923*: good growth
E.coli ATCC 25922*: growth
*NCCLS M22-A2 recommended strains.

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

Reference
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.

Bordetella Transport Medium

A transport medium for the preservation of nasal-pharyngeal swabs for the isolation of *B.pertussis*.

Code: KM4945

Typical formula	**(g/l)**
Beef Extract	5.00
Peptone	5.00
Starch	5.00
Charcoal	5.00
Sodium Chloide	2.50
Nicotinic Acid	0.500 mg
Agar	6.00
Cefalexin	0.04
Amfotericin B	0.005

pH 7.4 +/- 0.2

Description
Bordetella Selective Agar is a very nutritious medium for the isolation of *B.pertussis* from nasal-pharyngeal secretions. Sutcliffe and Abbott found cefalexin to be superior penicillin in recovering stressed cells and in suppressing unwanted nasal-pharyngeal flora. Only *P. aeruginosa* and some fungi may grow on the plates. Bordetella Transport Medium is used for the preservation of nasal-pharyngeal swabs for the isolation of *B.pertussis*.

Method
Collect per nasal swabs in the early stage of the illness and place in tubes of Bordetella Transport Medium. Inoculate the swab onto the plating medium Bordetella Selective Agar. Replace the swab in the original transport medium and hold at room temperature. If necessary use the swab to inoculate another Bordetella Selective Agar plate. Incubate at 35°C up to six days in a moist atmosphere and examine after 2 days incubation and twice daily thereafter. Look for small, shiny, greyish-white, round convex colonies. Suspicious colonies should be Gram-stained. Confirm the identification with direct fluorescent antibody (DFA) test.

Reference
Suttcliffe, E.M. Abbott, J.D. (1979) B.M.J. ii 732-733