SIM MEDIUM

For the differentiation of members of the *Enterobacteriaceae*.

Code: KM3362

Typical formula	**(g/l)**
Tryptone	20.0
Peptone	6.0
Fe-ammonium Sulphate	0.2
Sodium Thiosulphate	0.2
Agar	3.5

pH 7.3 +/- 0.2

Directions
Suspend 30g in 1000ml of cold distilled water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description
SIM Medium is used for the differentiation of members of *Enterobacteriaceae* based on H_2S and indole production and motility.

Method
A small volume of the medium should be distributed into test tubes, and inoculated by stabbing with a needle that has rubbed the centre of a colony. Incubate at 37°C for 18-24 hours. Motility is shown by the medium becoming opaque starting from the point of inoculation; production of H_2S, by blackening of the medium; the production of indole, by the appearance of a pink colour in the tube following the addition of few drops of Kovacs' Reagent.

Quality assurance (37°C-24hrs)
E.coli ATCC 25922: motility +, indole +, H2S –
P.mirabilis ATCC 25933*:* motility +, indole -, H2S +

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

Reference
Edwards, P.R. & Ewing W.H. (1972) - Identification of *Enterobacteriaceae*. 3rd edition. Minneapolis: Burgess Publishing Company.

SIMMONS CITRATE AGAR

For the differentiation of Gram-negative bacteria on the basis of citrate utilisation

Code: KM1104

Typical formula	**(g/l)**
Ammonium Dihydrogen Phosphate	1.00
Dipotassium Phosphate	1.00
Sodium Chloride	5.00
Sodium Citrate	2.00
Magnesium Sulphate	0.20
Bromthymol Blue	0.08
Agar	15.00

pH 6.9 +/ 0.2

Directions
Suspend 24g in 1000ml of cold distilled water, heat to boiling with frequent agitation, distribute and autoclave at 121°C for 15 minutes. All glassware must be chemically clean and alkali free.

Description
Simmons Citrate Agar, modified with respect to Koser's modium by the addition of 1.5% agar and bromothymol blue as a pH indicator, is a medium whose only source of nitrogen is ammonium phosphate, and the only source of carbon is sodium citrate. Microorganisms that use citrate and ammonium salts as sole sources of carbon and nitrogen will grow on the medium with the production of an alkaline reaction, evidenced by a change in the colour of bromothymol blue, from green to blue. The medium differentiates between E.coli and *E.aerogenes*.

Method
Inoculate slants with a culture taking care to remove the microbial layer without accompanying traces of medium. Incubate at 37°C for 18 to 48 hours. Citrate-positive bacteria grow on the medium turning the indicator blue, whereas, citrate-negative bacteria do not grow.

Quality assurance (37°C-48hrs)

Citrate positive control
E.aereogenes ATCC 13048

Citrate negative control
E.coli ATCC 25922

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

References
Mac Faddin, J.F. (1976) - Biochemical Tests for Identification of Medical Bacteria. Baltimore: The William and Wilkins Company.
Simmons, J.S. (1926) - J. Infect. Dis. 39, 209-214.

SLANETZ & BARTLEY AGAR

A medium for the enumeration of enterococci in water and foodstuffs

Code: KM1105

Typical formula	**(g/l)**
Peptone mix	20.00
Yeast Extract	5.00
Glucose	2.00
Potassium Phosphate Monobasic	4.00
Sodium Azide	0.40
TTC	0.10
Agar	10.00

pH 7.2 +/- 0.1

Directions
Suspend 41.5g in 1000ml of cold distilled water, heat to boiling with frequent agitation, cool to approximately 50°C and pour into sterile plates. Do not overheat, and do not sterilise in the autoclave. Preserve away from light.

WARNING: the two media contain sodium azide. Read the section covering Hazard Precautions for media containing azide.

Description
Slanetz & Bartley Agar, originally described by Slanetz and Bartley, is a selective medium recommended for the isolation and enumeration of enterococci in water and foodstuffs, by the membrane filtration technique or by direct plating. The presence of sodium azide inhibits the development of all contaminating microorganisms, whilst the triphenyl-trazolium chloride (TTC) acts as an indicator. The microorganisms that reduce it grow with red colonies.

Method
For the enumeration of enterococci in water samples proceed as follows. Filter through a 0.45µm membrane, an appropriate volume of water (100-10-1-0.1-0.01ml) according to the degree of pollution expected. Transfer 10ml of medium to 60mm plates, pass a flame over the surface of the agar to eliminate any air bubbles. Leave to solidify and lay the filter membrane on the surface. After 48 hours of incubation at 37°C, count all the pink-dark red colonies, which can be considered to be enterococci. Confirm the colonies by transferring the membrane on a plate of Bile Aesculin Agar. If the colonies develop a brown or black halo they are confirmed as enterococci. For the enumeration of enterococci in foods proceed as follows: spread the homogenate and dilute with saline sample onto the surface of the agar medium. Incubate at 37°C for 48 hours. Count the pink-dark red colonies in the plates where there is growth of 15-150 colonies.

Quality assurance (37°C-24hrs)

Productivity control
E.faecalis ATCC 29212:good growth red colonies;

Selectivity control
S.pyogenes ATCC 19615: inhibited
E.coli ATCC 25922: inhibited

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

References
Burkwall, M . K. & Hartman P.A. (1964)- App. Microbiol. 12, 18.
Department of Health an Social Security (1969) - Report n. 71, 4th Ed., London. HMSO.
OMS (1965) - Normes Internationales pour l'Eau de Boisson, Deuxième édition .
Slantez L.W. & Bartley, C.H. (1957)- J. Bact., 74, 591.
Taylor, E.W. and N.P. Burman (1964) - J. App. Bact. 27, 294-303.

SODIUM BISELENITE

For use with Selenite Broth Base and Selenite Cystine Broth Base

Code: KM1101B

Final concentration is 4 g/litre.
Final pH 7.1 +/- 0.2

WARNING: Sodium Biselenite is very toxic by inhalation or if swallowed. Handle with great care.

Typical Analysis
Appearance..... white powder
Loss on drying..... <0,5 %
Assay (selenium)..... > 50 %
Impurities..... < 0.15 %

Directions
Dissolve 4 g of Sodium Biselenite in 1 litre of cold distilled water and add 19g of Selenite Broth Base or 19g of Selenite Cystine Broth Base . Warm until complete dissolution and distribute into sterile tubes or flasks.

SODIUM CHLORIDE
A bacteriological grade Sodium chloride

Code: SA550

Sodium chloride, which meets the requirements of EP, is used for a variety of applications in microbiology and other fields.

TYPICAL ANALYSIS
Aspect.....…….. white crystals
Loss on drying..... < 0,1 %
Assay …….…..... > 99.9 %
Heavy metals.....…….. < 5 ppm (Pb)

SODIUM PYRUVATE
(EC No. 204-024-4)
Case No. 2918 3000

Code: SA650

Typical Analysis:

Appearance……..white crystalline powder
Assay…… ($HClO_4$, titration) 99.5% +/- 0.5
Water content…… 0.1%:

Precautions:

Do not breathe dust.

Avoid contact with skin and eyes.

Wear suitable protective clothing, gloves and eye/face protection.

Protect from moisture by keeping container well sealed.

Salmonella Shigella (S.S.) AGAR

A medium for the selective isolation and differentiation of pathogenic enteric bacilli

Code: KM1100

Typical formula	**(g/l)**
Beef Extract	5.00
Peptone	5.00
Lactose	10.00
Bile Salts No. 3	8.50
Sodium Thiosulphate	8.50
Sodium Citrate	8.50
Ferric Citrate	1.00
Neutral Red	0.025
Agar	13.50
Brilliant Green	0.330mg

pH 7.0 +/- 0.2

Directions
Suspend 60g in 1000ml of cold distilled water, heat to boiling with frequent agitation then cool to 50°C and distribute into sterile Petri dishes. Do not overheat. Allow the surface of the plates to dry before inoculation.

Description
S.S. Agar is a selective and differential medium recommended for the isolation of *Salmonella* and *Shigella* from faeces and other materials, with a direct inoculation or after enrichment in liquid media. SS Agar clearly distinguishes non-lactose-fermenting enteric bacteria from lactose-fermenting ones; the growth of which is permitted by the inhibitors present. The sodium citrate, bile salts and brilliant green of SS Agar inhibit the growth of Gram-positive microorganisms and some non-pathogenic enteric bacteria. Lactose is present as a fermentable carbohydrate to differentiate lactose-fermenting from non-lactose-fermenting microorganisms. Neutral red is included as a pH indicator. When the medium becomes acid due to the fermentation of the lactose, the bile salts precipitate and the colonies take on the colour of the indicator. Sodium thiosulphate is added as a hydrogen sulfide source and ferric citrate as an indicator for hydrogen sulfide production. Some species of *Proteus* and *Salmonella* produce colonies with a black centre. This is due to the precipitation of iron sulphide, caused by the production of hydrogen sulphide from the sodium thiosulphate. The medium is highly selective and the R-strains of *Shigella* will not grow on the medium. For the isolation of *Shigella* the recommended plating media are Hektoen Enteric Agar and XLD Agar.

Method
In the isolation of *Salmonella* from faeces, it is recommended to use a combination of selective and differential media, with the aim of increasing the recovery of these microorganisms, especially if they are present in small numbers. Inoculate the medium with the selective enrichment culture in Selenite Broth or directly and heavily with the specimen. Spread the inoculum in order to obtain well-isolated colonies. Incubate for 18-24 hours at 37°C.

Quality assurance (37°C-24hrs)

Productivity control
S.typhimurium ATCC 14028*: growth, colonies colourless with black centres
S.flexneri ATCC 12022*: growth, colourless colonies

Selectivity control
E.coli ATCC 25922*: poor growth, pink to rose-red colonies with precipitate
E.faecalis ATCC 29212*: inhibited
*NCCLS M22-A2 recommended strains.

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

References
Isenberg, H.D., Kominos, S. & Siegel, M. (1969). Appl. Micriobiol. 18, 656-659.
NCCLS document M22-A2, 1996. Quality Assurance for Commercially prepared Microbiological Culture Media-2nd ed.; Approved Standard.
Rahaman, M.N., Hug, I. & Pajan Dey, C. (1975) J. Inf, Dis., 131, 700-703.
Taylor & Harris (1965) - Am. J. Clin. Path., 3s, 476.
Yoshikawa, M. (1972) J. Inf. Dis., 125, 310-312.

STAA AGAR BASE

A basal medium for the isolation of *Brochothrix thermosphacta* from meat products.

Code: KM3792

Typical formula	**(g/l)**
Peptone	20.00
Yeast Extract	2.00
Dipotassium Phosphate	1.00
Magnesium Sulphate	1.00
Cycloheximide	0.05
Agar	10.50
Bromocresol Purple	0.02

Directions
Dissolve 17.3g in 500ml of cold distilled water and add 7.5g of glycerol. Heat to boiling with frequent agitation and sterilise by autoclaving at 115°C for 15 minutes. Cool to 50°C and add the contents of one vial of STAA Supplement reconstituted with 5ml of sterile distilled water. Mix well and distribute into sterile petri dishes.

WARNING: the powder medium STAA Agar Base contains cycloheximide. Handle with care, avoiding contact with skin and eyes. The selective supplement contains thallous acetate. **Do not** inhale; avoid contact with skin and eyes. Handle the product wearing gloves and using eye protective gear.

Description
STAA Agar Base, supplemented with streptomycin and thallous acetate, is used for the isolation of *B. thermosphacta* from meat products. The medium is prepared according to Gardner's typical formulation. *B. thermosphacta* is a Gram-positive bacillus that is responsible for the spoilage of meats and meat products stored aerobically or vacuum packed. The medium contains cycloheximide to inhibit the growth of yeasts, streptomycin sulphate to inhibit the growth of Gram-negative and Gram-positive bacteria and thallous acetate, which inhibits the growth of yeasts as well as many aerobic and facultative anaerobic bacteria.

Method
Prepare tenfold dilutions of the test sample in Peptone Water. Transfer 0.1ml volumes to the agar plate and streak to get well-isolated colonies. Incubate aerobically at 22°C for 48 hours. *B.thermosphacta* grows as straw-coloured colonies about 1mm in diameter, oxidase negative.

Quality assurance (22°C-48hrs)

Productivity control
B.thermosphacta ATCC 11509: growth

Selectivity control
E.coli ATCC 25922: partially inhibited

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

Reference
Gardner, G.A. (1966) J. Appl. Bacteriol. 29, 455 - 460

STAPHYLOCOCCUS SELECTIVE AGAR

For the isolation and enumeration of staphylococci from clinical and non-clinical specimens

Code: KM1106

Typical formula	**(g/l)**
Tryptone	10.0
Yeast Extract	2.0
Gelatin	30.0
Lactose	2.0
Mannitol	10.0
Sodium Chloride	75.0
Dipotassium Phosphate	5.0
Agar	15.0

pH: 7.0 +/- 0.2

Directions
Suspend 149g in 1000ml of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Test mannitol fermentation with bromocresol purple, and gelatin hydrolysis with a saturated aqueous solution of ammonium sulphate.

Description
Staphylococcus Sel. Agar is a selective medium for the isolation of staphylococci from clinical and non-clinical materials. Staphylococci Sel. Agar described by Chapman for the primary isolation of staphylococci, is prepared according to the FDA recommended formula. FDA-BAM recommends Staphylococcus Sel. Agar for the examination of foods in a suspected staphylococcal food poisoning outbreak. The presence in the medium of large quantities of sodium chloride inhibits the growth of Gram-negative microorganisms and the majority of Gram-positive microorganisms, making the medium selective for staphylococci because of their considerable tolerance to high sodium chloride levels. Mannitol is present as a fermentable carbohydrate: *S. aureus* ferments mannitol, with acidification of the medium around the colonies. The gelatin is incorporated to test the gelatin-hydrolysing properties of staphylococci. On Staphylococci 110 Medium plates, *S.aureus* grow with orange-yellow colonies, while *S.epidermidis* and *S.saprophyticus* grow with un-pigmented colonies.

Method
Blend 20g of food with sterile 80ml 0.2 M NaCl solution for 3 minutes at high speed. Prepare decimal dilutions with Butterfield's buffer. Place 0.1ml portion of each dilution onto prepared Staphylococcus Sel. Agar plates and spread with a sterile bent glass rod. Incubate plates at 35°C for 48 hours. Count plates at the dilution having 30-300 well-distributed colonies. Note any variation in type or amount of pigment or other morphological characteristics produced by colonies. Transfer minimum 2 colonies of each type to Nutrient Agar slant. Test for enterotoxigenicity and /or for other relevant assays (coagulase, thermonuclease etc.).
To the remaining area, add a drop of bromocresol purple: if a colour change takes place from purple to yellow, the test is positive for mannitol

fermentation. Then add 5ml of saturated ammonium sulphate solution to the surface of the medium and incubate at 37°C for 10 minutes. In the presence of ammonium sulphate, the gelatin forms a milky precipitate; the formation of a transparent zone around the colonies or the areas from which the colonies have been removed, therefore indicates that hydrolysis of the gelatin has taken place. The table below indicates the growth characteristics of some microorganisms on Staphylococcus Sel. Agar, and the results of the above mentioned tests.

Microorganisms	Growth	Coagulase	Mannitol Fermentation	Gelatin Liquefaction	Thermonuclese
S.aureus	+	+	+	+	+
S.*epidemidis*	+	-	*-(+)*	+	-
E.faecalis	+/-	-	+/-	-	-
E.coli	-	-	-	-	-

Quality assurance (37°C-48hrs)

Productivity Control
S.aureus ATCC 25923: growth
S.epidermidis ATCC: 12228 growth

Selectivity control
P.mirabilis ATCC 12453: partially inhibited

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

References
Chapman, G.H. (1946), J. Bact., 51, 409-410
FDA (1995), Bacteriological Analytical Manual, 8th ed, revision 1, 1998. Published and distributed by AOAC International.

STREPTOCOCCUS SELECTIVE AGAR

For the selective isolation and enumeration of streptococci

Code: KM8872

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	4.0
L-Cystine	0.7
Sodium Sulphite	0.2
Glucose	5.5
Sodium Azide	0.2
Agar	15.0
Crystal Violet	0.2mg

STREPTOCOCCUS SELECTIVE BROTH

Selective enrichment liquid medium for streptococci

Code: KM8882

Typical formula	**(g/l)**
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	4.0
L-Cystine	0.7
Sodium Sulphite	0.2
Glucose	5.5
Sodium Azide	0.2
Crystal Violet	0.2mg

pH 7.4 +/- 0.2

Directions
Suspend 45.6g of Streptococcus Selective Agar or 30.6g of Streptococcus Selective Broth in 1000ml of cold distilled water. Heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 118°C for 15 minutes. **Do not** overheat these media.

Description
Streptococcus Selective Agar and Broth are recommended for isolation and enumeration of streptococci from mixed flora. Crystal violet and sodium azide make the medium selective for streptococci. Coliforms, *Proteus*, *Pseudomonas* and *Bacillus* are completely inhibited; staphylococci rarely grow on these media with characteristics, which can be easily recognised. Streptococcus Selective Agar is used for the enumeration of streptococci including group A beta-haemolytic strains. Streptococcus Selective Broth is used to isolate sodium azide-resistant streptococci.
The streptococci once isolated have to be transferred to blood agar for a first classification in alpha-beta-and non- haemolytic, and to Todd Hewitt Broth for serological identification.

Quality assurance (37°C-24hrs)

Productivity control
E.faecalis ATCC 29212: growth

Selectivity control
E.coli ATCC 25922: inhibited

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

STUART TRANSPORT MEDIUM

For the preservation of *Neisseria* spp. and other fastidious microorganisms.

Code: KM1107

Typical formula	**(g/l)**
Sodium Glycerophosphate	10.00
Sodium Thioglycollate	1.00
Calcium Chloride	0.10
Agar	3.40
Methylene Blue	0.002

pH 7.3 +/- 0.2

Directions
Suspend 14.4g in 1000ml of cold distilled water, heat to boiling with frequent agitation and distribute into small screw-capped vials filling each vial almost to the top. Sterilise by autoclaving with caps loose, at 121°C for 10 minutes, then screw caps on tightly, and cool vials rapidly.

Description
Stuart Transport Medium, prepared according to the Stuart formula, is a semi-solid, highly reducing, non-nutritive medium used for the transport of specimens of clinical source to the laboratory. Stuart's preparation was designed to preserve *Neisseria gonorrohoeae* and *Trichomonas vaginalis* without permitting their proliferation. The medium has been shown to be suitable for transporting *Haemophilus influenzae, Streptococcus pyogenes*, *Corynebacterium diphteriae*, *Enterobacteriaceae* and bacteria of the respiratory tract. These microorganisms can live on Stuart Transport Medium for not more than 24 hours, whilst, more resistant microorganisms stay alive for up to 72 hours. However, as a general rule it is better to subject all specimens to microbiological analysis as soon as possible.

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. Keep the test tubes in a refrigerator until dispatch.

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

Reference
Stuart, R.D., Toshach Sheila, R. & Patsula, T.M. (1954) - Canad. J. Pub. Hlth. 45, 73-83.

BACTERIOLOGICAL SUCROSE

Code: CH1010

A bacteriological grade sucrose for use in microbiological culture media as a fermentable carbohydrate.

Typical Analysis

Appearance.....……white crystals
Solubility in water.....complete
Loss on drying.....0.2 - 0.7%
Granulometry.....…...0.1-1.1 mm

SUGAR FREE AGAR

A medium for the enumeration of mesophilic aerobic Gram-negative bacteria not related to food production process.

Code: KM1108

Typical formula	**(g/l)**
Gelatin Peptone	7.5
Tryptone	7.5
Sodium Chloride	5.0
Agar	14.0

Directions
Suspend 34g of Sugar Free Agar in 1000ml of cold distilled water, heat to boiling with frequent agitation. Subdivide in 100ml aliquots and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45°C and to each 100ml aliquot add the contents of one vial of Penicillin G 500 IU Selective Supplement. Mix well and distribute into inoculated petri dishes.

Description
The complete Sugar Free Agar with Penicillin is used for the enumeration of mesophilic aerobic Gram-negative bacteria not related to the production process of the food: *Pseudomonas*, *Flavobacterium, Alkaligenes, Aeromonas, Xantomonas, Acinetobacter, Enterobacteriaceae*, etc.

Method
Pipette 1ml of each food dilution into sterile petri dishes. Add 20ml of Sugar Free Agar with Penicillin cooled to 45°C and mix the inoculum well with the melted medium. Incubate at 30°C for 72 hours. Count all well formed colonies. Do not consider the pinpointed ones.

Quality assurance (37°C-24hrs)

Productivity control
E.coli ATCC 25922: growth

Selectivity control
E.faecalis ATCC 19433: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared flasks (medium base): 3 months at 2-8°C

Reference
Manuel Swiss des Denrées Alimentaires. 5° edition, deuxième volume, Chap. 56. 1988

SABOURAUD DEXTROSE AGAR

A medium for the enumeration and cultivation of fungi.

Code KM1096

Typical formula	**(g/l)**
D(+)-Glucose	40.0
Casein peptone	5.0
Meat peptone	5.0
Agar	12.0

pH 5.6 ± 0.2

Directions
Suspend 62g in 1 litre distilled water and bring to the boil with frequent stirring. Distribute into final containers and sterilise by autoclaving at 121° C for 15 minutes. **Do not overheat** as the gel strength of the medium may diminish if recommended sterilisation time or temperature is exceeded. Medium's acidity may partially hydrolyse the agar.

Description
Sabouraud Dextrose Agar is a modification of the classical Sabouraud medium for the cultivation of fungi. This new formula helps maintain the fungi's cultural appearance and thus allows a reliable cultivation and differentiation.

Its selectivity is due to a low pH and a high glucose concentration, which together with incubation at a relatively low temperature (25-30°C) favours the growth of fungi while discouraging that of bacteria. Besides, the peptone's composition has been studied to provide the fungi with all their nitrogenated nutrient requirements.

Since the Sabouraud medium's strong acid reaction partially hydrolyses the agar, only the necessary amount should be prepared and never re-melted. Any overheating will considerably diminish its gelling capacity. Should a higher selectivity be required, a variety of inhibitors may be added after sterilisation, while the medium is still molten. It can even be made differential by adding indicator agents.

Sabouraud Dextrose Agar with Chloramphenicol

Recommended for the isolation of non-pathogenic and pathogenic fungi.

Code: KM1096C

Typical formula	**(g/l)**
Peptone	10.0
Glucose	40.0
Chloramphenical	0.05
Agar	15.0

pH 5.6 +/- 0.2

Directions
Weigh 65.0 grams of dehydrated culture medium to 1 litre of distilled water until evenly dispersed. Heat with repeated stirring and boil for one minute to dissolve completely. Distribute and autoclave at 121°C for 15 minutes. The medium contains 0.05g/l chloramphenicol, which is inhibitory to a wide range of Gram negative and Gram-positive bacteria.

Description
This medium is recommended for the isolation of non-pathogenic and pathogenic fungi. Sabouraud dextrose agar can be used as a base for preparing selective media, by the addition of antibiotics such as cycloheximide, streptomycin, chloramphenicol, novobiocin and gentamicin. The combination of cycloheximide and chloramphenicol inhibits many pathogenic fungi.

Q.C.: Dehydrated medium: homogeneous, light beige fine powder.
Ready to use medium: light amber coloured agar, slightly opalescent.

Organism Results
Escherichia coli ATCC 25922 No growth
Staphylococcus aureus ATCC 25923 No growth
Aspergillus niger ATCC 16404 Woolly and black mycelium
Candida albicans ATCC 10231 Off-white creamy colonies

Storage:
Dehydrated medium should be stored between 10 to 25°C. Once opened, place the container in a dark, dry place. The dehydrated medium should not be used if there is any lump or if the colour has changed from the original.

SABOURAUD LIQUID MEDIUM (U.S.P.)

A liquid medium for the cultivation of yeasts, moulds and acidophilic bacteria.

Code: KM1097

Typical formula (g/l)

Tryptone	5.0
Meat Peptone	5.0
Glucose	20.0

pH 5.7 +/- 0.1

Directions

Suspend 30g in 1000ml of cold distilled water; heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.

Description

Sabouraud Liquid Medium is used for the cultivation of yeasts, moulds and acidophilic bacteria. The meat and casein peptones in the medium provide the ingredients necessary for growth of yeasts and moulds. The acid pH inhibits the growth of bacteria, with the exception of those, which are acidophilic. The addition of 4% HCl increases the inhibitory action of the medium on bacterial contamination. Once inoculated, Sabouraud Liquid Medium should be protected from light and incubated at 25-30°C for 1-4 weeks, or longer. Examine for growth by comparing turbidity to an un-inoculated control. Subculture onto an appropriate agar medium when growth is seen. Identification of fungi is performed by observing various aspects of colony morphology, characteristic microscopic structures, rate of growth and media, which support the organisms growth and source of specimen. Yeasts are identified by performing various biochemical tests.

Quality assurance (25°C up to 7 days)

Productivity Control

C.albicans ATCC 10231: growth

T.mentagrophytes ATCC 9533: growth

Storage

Dehydrated medium: 15-30°C

User prepared tubes: 3 months at 2-8°C

References

The Pharmacopoeia of the U.S.A. XVII 1965.

SABOURAUD MALTOSE AGAR

For the isolation, cultivation and maintenance of yeasts and moulds

Code: KM1098

Typical formula (g/l)

Peptone mix	10.0
Maltose	40.0
Agar	15.0

pH 5.6 +/- 0.2

Directions

Suspend 65g in 1000ml of cold distilled water, heat to boiling stirring until complete dissolution. Sterilise by autoclaving at 121°C for 15 minutes. Do not exceed boiling and sterilisation time and temperature.

Description

Sabouraud Maltose Agar differs from Sabouraud Dextrose Agar in that maltose takes the place of glucose. Sabouraud Maltose Agar does not contain selective agents, and the inhibition of bacteria is exclusively due to its acid pH. The medium provides an excellent base for the cultivation of yeasts and moulds, particularly those that cause skin lesions. Glucose is replaced by maltose because the latter carbohydrate is especially suitable to fulfil the nutritional requirements of fungi. As the medium has the same characteristics as Sabouraud Dextrose Agar, refer to that section for further details.

Quality assurance (25°C up to 7 days)

Productivity Control

C.albicans ATCC 10231: growth

T.mentagrophytes ATCC 9533: growth

Storage

Dehydrated medium: 15-30°C

User prepared plates: 1 month at 2-8°C

SELENITE BROTH BASE

A liqud enrichment medium base, for the detection of *Salmonella* spp. in clinical specimens.

Code: KM1101A

Typical formula	**(g/l)**
Tryptone	5.0
Lactose	4.0
Sodium Phosphate Bibasic	10.0

pH 7.1 +/- 0.2

Directions
Dissolve 4g of Sodium Biselenite in 1 litre of cold distilled water and then add 19g of Selenite Broth Base. Warm until complete dissolution and distribute into sterile tubes. Selenite Broth Base is prepared to minimise any possible risk of teratogenicity to laboratory workers. Sodium biselenite must be added as solution to Selenite Broth Base.

Description
Selenite Broth is recommended for the selective enrichment of Salmonella Spp. in clinical specimens. The medium is prepared in accordance with the formula described by Leifson and recommended by the APHA. Sodium Selenite possesses a high level of toxicity at a neutral pH for *Escherichia coli,* but not for the major part of microorganisms belonging to the *Salmonella* group. A buffer system is present in the medium, which tends to minimise the alkalinising effects induced by the reduction of sodium selenite. These alkalinising effects would notably diminish the selective properties of the medium. The acids produced by the microorganisms from lactose also contribute by neutralising the alkaline reactions of the medium. Selenite Broth is toxic for *Salmonella cholerae-suis* and for *Salmonella abotus-ovis.* The preliminary enrichment of samples to be examined in Selenite Broth followed by inoculation onto plates of one or more selective media considerably increases the number of positive results with regard to the isolation of *Salmonella.*

Method
For faeces examination, inoculate test tubes containing 8-10ml of medium with 1g of faeces, or 1ml of faecal suspension. It is common practice to emulsify 2-3g of solid specimen to 15ml of saline solution and then withdraw approximately 1ml of the supernatant and inoculate 10ml of Selenite Broth. For the examination of urine samples, Selenite Broth must be used at double concentration dispensed in amounts of 5 - 7.5ml for each test tube, and inoculated with an equal volume of sample. After a vigorous emulsification of the inoculum, incubate for 18-24 hours at 35-37° then streak onto two plates chosen between the following plating media: Bismuth Sulphite Agar, MacConkey Agar, Desoxycholate Citrate Agar, SS Agar, XLD Agar, Hektoen Enteric Agar. The plating media should be chosen as a combination of greater and little inhibition. For the isolation of *Salmonella,* including *Salmonella typhi,* it is advisable to use as plating medium Bismuth Sulphite Agar, and to inoculate two plates, the first by streaking the inoculum on the surface and the second with a poured plate technique. Selenite Broth, after the growth of *Salmonella,* presents a pink colour.

Quality assurance (37°C - 24hrs, subculture on TSA)

Productivity Control
S.typhimurium ATCC 14028*: growth

Selectivity control
E.coli ATCC 25922*: partially or completely inhibited
*NCCLS M22-A2 recommended strains

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

References
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SELENITE CYSTINE BROTH BASE

An enrichment liquid medium base for the detection of *Salmonella* spp. in foodstuff

Code: KM1102A

Typical formula	**(g/l)**
Tryptone	5.0
Lactose	4.0
Sodium Phosphate Bibasic	10.0
L-Cystine	0.01

pH 7.0 +/- 0.2

Directions
Dissolve 4g of Sodium Biselenite in 1 litre of cold distilled water and then add 19g of Selenite Cystine Broth Base. Warm until complete dissolution and distribute into sterile tubes or flasks.

WARNING: Supplement this medium with sodium Biselenite, which is very toxic and must be handled with great care.

Description
Selenite Cystine Broth Base is an enrichment liquid medium for the isolation of *Salmonella* spp. from foods and other specimens. The medium complies with the requirements of USP XXIV. It is recommended by USP as a selective enrichment broth for the detection of *Salmonella* in pharmaceutical products (Microbial Limit Tests), by ISO 6579:1993.in the general guidance on methods for the detection of *Salmonella* in foodstuffs and by FDA BAM for the detection of *Salmonella* in food other than raw flesh foods, highly contaminated foods and animal feeds; for these foods FDA BAM recommends Tetrathionate Broth.

Method
In general, for the preparation of initial suspension add 25g sample portion to 225ml of Buffered Peptone Water or Lactose Broth. If the required test portion is other than 25g, use a suitable quantity of pre-enrichment broth to yield approximately 1/10 dilution (m/v). Incubate the initial suspension at 37°C for not less than 16 hours, but not more than 20 hours. Transfer 1ml or 10ml to a tube or to a flask containing 10 or 100ml of Selenite Cystine. For the second selective enrichment broth refer to the literature or to specific norms. Incubate the inoculated Selenite Cystine Broth Base at 37°C for 24hrs and, if required by specific standards, for a further 24hrs. Subculture from the two enrichment broths to any combination of greater or lesser inhibitory plating out selective agar for *Salmonella*. USP XXIV recommends the use of Brilliant Green Agar, XLD Agar and Bismuth Sulphite Agar. FDA BAM recommends Hecktoen Enteric Agar, XLD Agar and Bismuth Sulphite Agar.
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.

Quality assurance (37°C-24 hrs, subculture on TSA)

Productivity Control
S.typhimurium ATCC 14028*: growth

Selectivity control
E.coli ATCC 25922*: partially or completely inhibited
*NCCLS M22-A2 recommended strains

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

References
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U.S. Pharmacopoeia 24, NF 19 (2000)

SENSITIVTY TEST AGAR (S.T.A.)

A medium formulated for antibiotic susceptibility testing by the Joan Stokes technique.

Code: KM1103

Typical formula	**(g/l)**
Peptone-Infusion Solids	21.5
Starch	0.6
Sodium chloride	5.0
Disodium citrate	1.0
Adenine sulphate	0.01
Guanine hydrochloride	0.01
Uracil	0.01
Xanthine	0.01
Aneurine hydrochloride	0.01
Uridine	0.1
Agar	12.0

pH: 7.4 ± 0.2

Description
S.T.A. is inhibitor-free, very rich and includes various nucleotides to enable fastidious organisms to be tested. It is necessary to add lysed or chocolated blood for some organisms. 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 for 15 minutes at 121ûC. To prepare blood agar cool to 45ºC and add 7% lysed blood or 6% defibrinated blood according to preference. Mix well then pour plates.

Q.C. organisms: *S. aureus* NCTC 6571, *E. coli* NCTC 10418 (antibiotic sensitivity zones)

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

Inoculation method: Surface, according to technique.

Incubation: 37ºC, atmosphere to suit organisms metabollic requirements.

Interpretation: There are no defined zone sizes as in Mueller Hinton, but all antibiotics should give adequate zone sizes when compared to controls using standard organisms, e.g. S. aureus NCTC 6571, E. coli NCTC 10418, Ps. aeruginosa NCTC 10662.

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