

## TRIPLE SUGAR IRON (T.S.I.) AGAR (U.S.P.)

A medium for the differentiation of *Enterobacteriaceae*

**Code: KM1114**

<b>Typical formula</b>	<b>(g/l)</b>
Tryptone	10.00
Peptone	15.00
Lactose	10.00
Sucrose	10.00
Glucose	1.00
Ferrous Ammonium Sulphate	0.20
Sodium Chloride	5.00
Sodium Thiosulphate	0.20
Agar	14.00
Phenol Red	0.025

pH 7.4 +/- 0.2

### Directions

Suspend 65g in 1000ml of cold distilled water. Heat to boiling with frequent agitation, distribute into test tubes and sterilise by autoclaving for 15 minutes at 121°C. Allow solidifying in a slanting position so that a butt of 2 cm is formed.

### Description

Triple Sugar Iron Agar USP is prepared according to the formulation recommended by USP. The media is used for the differentiation of *Enterobacteriaceae* cultivated on selective or moderately selective media on the basis of lactose, glucose and sucrose fermentation, and the production of hydrogen sulphide and other gases. For details regarding the biochemical reactions involved and its working principle, see the technical sheet of Kligler Iron Agar; the only difference between the two media lies in the addition of sucrose as a third carbohydrate in Triple Sugar Iron Agar. The growth characteristics of *Enterobacteriaceae* on TSI media are slightly different from those on Kligler Iron Agar, because some non-lactose-fermenting bacteria may ferment sucrose, giving a different slope reaction.

### Method

To perform the test, inoculate a test tube of TSI Media by stabbing the butt with a needle, which has touched the surface of the centre of a colony, and streak the slope after stabbing. After 18-48 hours of incubation, examine for the following: only acid, or acid and gas in the butt; acid or alkaline or no change on the slope; H<sub>2</sub>S production. Acidification is shown by a yellow colour, gas production by the formation of small bubbles in the agar; H<sub>2</sub>S production by blackening of the medium.

### Quality assurance (37°C-3 days)

*E.coli* ATCC 25922: yellow slope and butt, gas positive, H<sub>2</sub>S negative

*S.typhimurium* ATCC 14028: red slope, yellow butt, gas and H<sub>2</sub>S positive

### Storage

Dehydrated medium: 15-30°C

User prepared tubes: 7 days at 2-8°C

### References

European Pharmacopoeia, 3<sup>rd</sup> ed. 2001 Supplement

ISO 6579:1993 - Microbiology - General guidance on methods for the detection of Salmonella. 1993-09-01

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

# Tryptone (Casein Hydrolysate)

PH1005

This material is a quality source of peptides produced by enzymatic hydrolysis of casein. It provides a highly concentrated profile of amino acids, peptides and protein fractions. 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.

## Description

<b>Appearance:</b>	A creamy white, homogenous, powder, which produces a light clear solution after autoclaving at 121°C for 15 minutes.
<b>Solubility:</b>	2% aqueous
<b>pH:</b>	6.7 - 7.0

## Chemical Characteristics (%) - typical data

$\alpha$ - Amino Nitrogen (AN)	4.5 - 7.0%
Total Nitrogen (TN)	12.0 - 13.5%
AN/TN	35 -40%
Moisture	< 5.0%
Ash	4.5 - 6.5%
Calcium	0.01 - 0.03%
Magnesium	0.01 - 0.03%
Sodium	2.0 - 3.5%
Phosphate	2.5 - 3.0%
Sulphate	0.01 - 0.03%
Potassium	0.05 - 0.08%

## Amino Acid Distribution (mg/g)

**Typical analysis:**

<b>AMINO ACID</b>	<b>Total</b>	<b>Free</b>	<b>%Free</b>
Alanine	28.0	9.2	33.0
Arginine	32.8	23.9	73.0
Aspartic acid	65.	6.5	10.0
Cysteine	3.3	-	-
Glutamic acid	182.0	14.6	8.0
Glycine	17.2	2.2	13.0
Histidine	24.6	7.1	29.0
Isoleucine	46.7	16.3	35.0
Leucine	79.3	49.2	62.0
Lysine	71.0	44.0	62.0
Methionine	25.9	15.0	58.0
Phenylalanine	43.4	43.4	100.0
Proline	93.5	1.9	2.0
Serine	51.9	14.5	28.0
Threonine	38.7	16.1	41.0
Tryptohan	10.5	10.5	100.0
Tyrosine	32.0	12.5	39.0
Valine	58.7	23.5	40.0
<b>Total</b>	<b>904.5</b>	<b>310.4</b>	<b>34.0</b>

<b>Microbiological content</b>
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Enterobacteriaceae:	< 10 per gram
Salmonella:	Absent in 25gram
Standard aerobic plate count:	< 5000 per gram
Thermophiles:	< 1000 per gram
Yeasts & Moulds:	<100 per gram

<b>Storage and Packaging</b>
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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.

## Tryptone Bile X-Gluc (TBX) Agar

A chromogenic medium for the detection of *E. coli* in foodstuff

**Code: KM1115XG**

### Typical formula (g/l)

Tryptone	20.0
Bile Salts No. 3	1.5
Agar	14.0
X-GLUC	75.0 mg

pH 7.2 ± 0.2

### Directions

Weigh 35.6g in 1000ml of cold distilled water. Heat to boiling with agitation until complete dissolution and autoclave at 121°C for 15 minutes. Cool to 50°C and distribute into sterile Petri dishes.

### Description

Chromogenic Tryptone Bile X-GLUC (TBX) Agar is a selective differential medium for the enumeration and immediate identification of *Escherichia coli* in foodstuff. It is prepared according to the formulation given by ISO 16649-2. The medium contains bile salts for the complete inhibition of Gram-positive bacteria and X-GLUC (5-bromo-4-chloro-3 indolyl  $\beta$ -D-glucuronide) for the detection of  $\beta$ -glucuronidase. Among the *Enterobacteriaceae* only *E. coli*, together with some strains of *Salmonella* and *Shigella*, is one of the few  $\beta$ -glucuronidase positive species, so cultivates on the plates with blue or green-blue colonies.  $\beta$ -glucuronidase negative bacteria grow with colourless colonies.

### Method

Transfer 1ml of the test sample into a sterile Petri dish if liquid, or 1ml of the initial suspension ( $10^{-1}$ ), in the case of other products. Inoculate 2 plates per dilution. Repeat the procedure with further decimal dilutions if necessary. Pour into each Petri dish about 15ml of TBX Agar pre-cooled to 47-50°C. Mix inoculum well with the medium. Invert the inoculated dishes and incubate at 44°C for 18-24 hours. In cases where stressed colonies are suspected incubate for 4 hours at 37°C before incubation at 44°. Do not incubate over 45°C. Count as presumptive all *E. coli* the blue or green-blue colonies. Report the results as UFC/g considering the "dilution factor". The medium can be inoculated even on the surface with the usual methods.

**Quality Assurance** (44°C - 24 hrs)

### Productivity control

*E.coli* ATCC 25922: growth, blue-green colonies

### Specificity control

*S.typhimurium* ATCC 14028: growth, colourless colonies

### Selectivity control

*E.faecalis* ATCC 19433: inhibited

### Storage

Dehydrated medium: 2-8°C

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

**Reference**

ISO 16649-2 (2001). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *E.coli*- part 2: colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid.

## TRYPTONE GLUCOSE EXTRACT AGAR

For the enumeration of microorganisms by the aerobic plate count in water, milk and dairy products.

**Code: KM1116**

<b>Typical formula</b>	<b>(g/l)</b>
Tryptone	5.0
Beef Extract	3.0
Glucose	1.0
Agar	15.0

pH 7.0 +/- 0.2

### **Directions**

Suspend 24g in 1000ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes.

### **Description**

Tryptone Glucose Extract Agar is a general-purpose medium recommended for the enumeration of microorganisms by the aerobic plate count in water, milk and dairy products. Tryptone Glucose Extract Agar is recommended by APHA, for the enumeration of microorganisms in water and foodstuff.

**Quality assurance** (30°C – 72hrs)

### Productivity control

*E.coli* ATCC 25922 : good growth

*S.aureus* ATCC 6538: good growth

*B.subtilis* ATCC 6633: good growth

### **Storage**

Dehydrated medium: 15-30°C

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

User prepared plates: 1month at 2-8°C

### **References**

APHA (1972) - Standard Methods for the Examination of Dairy Products, 13th Edition.

APHA (1985) - Compendium of Methods for the Microbiological Examination of Foods, 2nd Edition.

Bowers and Hucker (1935) - Tech. Bull. 228, N.Y. State Agr. Exp. Sta.

## TRYPTONE SOY AGAR

A general growth medium for isolation and cultivation of microorganisms

**Code: KM1117**

<b>Typical formula</b>	<b>(g/l)</b>
Tryptone	15.0
Soy Peptone	5.0
Sodium Chloride	5.0
Agar	12.0

pH 7.3 +/- 0.2

### **Directions**

Suspend 37g in 1000ml of cold distilled water, allow to stand for 10 minutes, swirl to mix, then sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C, mix well and pour plates.

### **Description**

Tryptone Soy Agar is a multipurpose medium, which support the growth of a wide variety of microorganisms. Because of the nutritional characteristics, absence of inhibitors, and the possibility of supplementation with several compounds, this medium is recommended for the isolation of many microorganisms, for maintaining stock cultures and for the preparation of vaccines. Tryptone Soy Agar may be supplemented with defibrinated animal blood (at concentrations between 5% and 15%) to provide a more nutritious medium for fastidious organisms and for the detection of their haemolytic pattern, or with antimicrobials to provide a selective medium for the isolation of specific microorganisms out of a mixed flora. In some cases (isolation of *Neisseria* and *Haemophilus*), blood is added to the medium and then heated to 80°C for 10 minutes to obtain a chocolate agar. If Tryptone Soy Agar is supplemented with 0.7g/l lecithin and 5g/l of Tween 80, the medium may be used as Microbial Content Test Agar for testing quaternary ammonium compounds. Tryptone Soy Agar is recommended by USP for aerobic plate count of pharmaceutical products.

**Quality assurance** (Tryptone Soy Agar, without blood) (30-35°C-48 hrs)

#### Productivity control

*S.aureus* ATCC 6538<sup>\*\*</sup>: growth

*E.coli* ATCC 8739<sup>\*\*</sup>: growth

*B.subtilis* ATCC 663<sup>\*\*3</sup>: growth

**Quality assurance** (Tryptone Soy Agar + 5% def. blood) (37°C-24 hrs)

#### Productivity control

*S.pyogenes* ATCC 19615<sup>\*</sup>: growth, β-haemolysis

*S.pneumoniae* ATCC 6305<sup>\*</sup>: growth, α-haemolysis

*S.aureus* ATCC 25923<sup>\*</sup>: growth

*E.coli* ATCC 25922<sup>\*</sup>: growth

#### CAMP Test:

*S.aureus* ATCC 25923<sup>\*</sup> or 33862

*S.agalactiae* ATCC 12386 : positive reaction (arrowhead area of clearing)

*S.pyogenes* ATCC 19615: negative reaction (no arrowhead formation)

\*NCCLS M22-A2 recommended strains.

### **Storage**

Dehydrated medium: 15-30°C

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

**References**

Bailey, W.R. & Scott, E.G. (1974) - Diagnostic Microbiology, 4th edition. St. Louis: C.V. Mosby Company.

Buttiaux, R., Beerens, H. & Tacquet, A. (1969) - Manuel de Techniques Bacteriologiques. Ed. Med. Flammarion.

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

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

## TRYPTONE SOY BLOOD AGAR BASE

An improved TSA for the preparation of blood agar plates

**Code: KM8512**

<b>Typical formula</b>	<b>(g/l)</b>
Pancreatic Digest of Casein	14.5
Soy Peptone	5.0
Sodium Chloride	5.0
Agar	14.0
Growth Factors	1.5

pH 7.3 +/- 0.2

### **Directions**

Suspend 40g in 1000ml of cold distilled water, heat to boiling with frequent agitation, distribute and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add 5-7% of defibrinated sheep, horse or rabbit blood. Distribute into sterile petri dishes.

### **Description**

Tryptone Soy Blood Agar Base is prepared with especially selected Pancreatic Digest of Casein, Soy Peptone and Agar in order to improve the beta haemolytic reaction of streptococci. The medium is supplemented with growth factors to achieve a bigger and faster growth of fastidious microorganisms. Tryptone Soy Blood Agar Base is especially formulated, to be used with sheep, horse or rabbit blood for the preparation of TSA Blood Agar Plates.

### **Method**

Firmly roll the swab over one sixth of the blood agar plate. Use a sterile wire loop to cross hatch the remainder of the plate. Stab the agar several times with the wire loop to obtain subsurface growth and to permit the detection of both streptolysine O and S. Incubate for 18-24 hours in an aerobic atmosphere, or with 5-10% CO<sub>2</sub>. The appearance of streptococcal and non-streptococcal colonies, on Sheep Blood Agar plates, is summarised below. The colonies of Group A streptococci typically are about 0.5mm in diameter, transparent or translucent, and domed, having a smooth surface and an entire edge. They are surrounded by a well-defined zone of complete haemolysis, usually two or three times the diameter of the colony. The appearance of surface or subsurface beta-haemolytic group C and group G streptococcal colonies do not differ sufficiently from that of group A colonies to be of any value in identification. Group B streptococcal colonies may be somewhat larger than group A colonies, but both are smooth with entire edges. The group B colonies are surrounded by a much smaller zone of complete haemolysis and some strains do not lyse the blood at all. Group D streptococcal colonies (*S.bovis*) are somewhat larger than other streptococcal colonies, they are less opaque, raised, and grey to grey-white. Group F streptococci (*S.anginosus*) generally form miniature colonies. Zones of haemolysis similar in size of those produced by group A streptococci surround these minute colonies. The viridans streptococcal colonies vary in size from pinpoint to a size equal to, or

larger than, that of group A streptococci. The colonies are usually smaller than those of the pneumococci. They may appear mucoid or translucent or glossy and non-translucent. The colonies may be surrounded by a small zone of alpha-haemolysis (partial destruction of red blood cells) or have no zone of haemolysis. Under anaerobic incubation, viridans streptococci are usually non-haemolytic. Pneumococcal colonies are round with entire edges, mucoid, and about 1mm in diameter. When the culture has been incubated in CO<sub>2</sub> incubators, the colonies are surrounded by a fairly large zone of alpha-haemolysis. Microscopic examination of the colonies is a useful aid in differentiating the pneumococci from the viridans streptococci. The colonies of *Aerococcus*, *Gemella* and *Pediococcus* species are similar to those of viridans streptococci. The colonies of *Enterococcus*, *Lactococcus* and *Leuconostoc* are larger than those of streptococci. The colonies are raised and white or grey-white. *E.faecalis* and *E.durans* may exhibit beta-haemolytic zones when cultivated on plates prepared with horse blood. Most *Enterococcus*, *Lactococcus* and *Leuconostoc* colonies are non-haemolytic initially on blood agar plates and develop a weak alpha-haemolysis after 48-72 hours of incubation. Staphylococci colonies are yellow or white with or without the beta-haemolysis zone. *Listeria* colonies are surrounded by a small beta-haemolytic zone. Consult appropriate references for processing the specimens and for the limitations of the procedures involving the use of animal blood.

### **Quality assurance**

(Tryptone Soy Agar Blood Agar + 5% def. sheep blood) (37°C-24 hrs)

#### Productivity control

*S.pyogenes* ATCC 19615\*: growth, β-haemolysis  
*S.pneumoniae* ATCC 6305\*: growth, α-haemolysis  
*S.aureus* ATCC 25923\*: growth  
*E.coli* ATCC 25922\*: growth

#### CAMP Test:

*S.aureus* ATCC 25923\* or 33862  
*S.agalactiae* ATCC 12386 : positive reaction (arrowhead area of clearing)  
*S.pyogenes* ATCC 19615: negative reaction (no arrowhead formation)  
\*NCCLS M22-A2 recommended strains.

### **Storage**

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

### **References**

Isenberg , H. D. Schoenknecht, F. D., Von Gravenitz, A. (1979) Cumitech no. 9 ASM, Washington D. C.  
Balows, A., Hausler, W.J., Herrmann, K.L., Isenberg H.D. and Shadomy, H.J. (ed) (1991) Manual of Clinical Microbiology, 5 th edition, ASM, Washington D.C.

## TRYPTONE SOY BROTH (USP)

A multipurpose medium for the growth of a wide variety of microorganisms

**Code: KM1118**

<b>Typical formula</b>	<b>(g/l)</b>
Tryptone	17.0
Soy Peptone	3.0
Sodium Chloride	5.0
Dipotassium Phosphate	2.5
Glucose	2.5

pH 7.3 + 0.2

### **Directions**

Suspend 30g in 1000ml of cold distilled water, swirl to mix and warm if necessary to completely dissolve. Distribute into tubes or flasks and sterilise by autoclaving at 121°C for 15 minutes. **Do not** exceed temperature.

### **Description**

Tryptone Soy Broth USP is multipurpose medium that supports the growth of a wide variety of microorganisms including the fastidious ones. Because of their nutritional characteristics, absence of inhibitors, and the possibility of supplementation with several compounds, this liquid medium is useful for the cultivation of fastidious microorganisms for maintaining stock cultures and for the biotechnological preparation. Tryptone Soy Broth is used for the cultivation of aerobic and facultative anaerobic microorganisms, including some fungi, and with agar added at concentrations of 0.1-0.2 %, for the cultivations of anaerobic bacteria. Tryptone Soy Broth is used in food bacteriology as the basal medium to which a variety of selective compounds may be added for selective enrichment of *E.coli* O157. Tryptone Soy Broth is used also for blood cultures and, together with Tryptone Soy Agar, in biphasic cultures according to Castaneda technique. Tryptone Soy Broth is recommended by USP for the sterility test at 22-25°C of pharmaceutical products.

**Quality assurance** (30-35°C-24 hrs)

#### Productivity control

*S.aureus* ATCC 6538\*\* : growth

*E.coli* ATCC 8739\*\* : growth

*B.subtilis* ATCC 6633\*\* : growth

*S.aureus* ATCC 25923\* : growth

*E.coli* ATCC 25922\* : growth

\*NCCLS M22-A2 recommended strains.

### **Storage**

Dehydrated medium: 15-30°C

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

### **References**

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

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

## TRYPTONE SOY BROTH MODIFIED

A selective liquid medium for the enrichment of *E.coli* O157

**Code: KM1118M**

<b>Typical formula</b>	<b>(g/l)</b>
Casein Hydrolysate	17.0
Soy Peptone	3.0
Sodium Chloride	5.0
Dipotassium Hydrogen Phosphate	2.5
Glucose	2.5
Bile Salts No. 3	1.5

pH 7.3 ± 0.2

### **Directions**

Suspend 31.5g in 1000ml of cold distilled water; heat to boiling distribute 225ml into flasks of suitable capacity and sterilise by autoclaving at 121°C for 15 minutes. Cool to approximately 45-50°C and to each 225ml flask add, under aseptic conditions, 2.25ml of Novobiocin supplement, reconstituted with 5ml of sterile distilled water. Final concentrations: 4.5 mg/225 ml – 20 mg/litre.

### **Description**

Tryptone Soy Broth Modified with Novobiocin is recommended by ISO/DIS 16654 for the preparation of initial suspension of the sample and for the enrichment procedure for the detection of *E.coli* O157 in foodstuffs.

### **Method**

Add 25g of sample to 225ml of m-TSB+N warmed to 41 +/- 1°C. Incubate at 41 +/- 1°C for 6 hours and subsequently for a further 12-18 hours. Perform the immuno-magnetic separation on the enriched culture after 6 and 12-18 hours of incubation. Transfer 50µl of resuspended immuno-magnetic particles to a plate of Tellurite Cefixime MacConkey Sorbitol Agar and to a second isolation medium (e.g. Chromogenic *E.coli* O157 Agar). Streak out the particles and incubate the plates at 35 or 37°C for 18-24 hours. Select 5 typical colonies and perform the latex agglutination test for confirmation.

**Quality assurance** (41°C-24 hrs)

#### Productivity Control

*E.coli* O157 NCTC 12900: growth

#### Selectivity control

*S.aureus* ATCC 25923: inhibited

### **Storage**

Dehydrated medium: 10-30°C

User prepared flasks with novobiocin: use the same day of preparation

User prepared flasks without novobiocin: 1 month at 2-8°C

## TRYPTONE WATER

A liquid medium for the production of indole by microorganisms

**Code: KM1119**

### Typical formula (g/l)

Tryptone	10.0
Sodium Chloride	5.0

pH 7.5 +/- 0.2

### Directions

Weigh 15g in 1000ml of cold distilled water. Mix well to dissolve the liquid medium, distribute into final containers and sterilise at 121°C for 15 minutes.

### Description

Tryptone Water is recommended for the detection of indole production by microorganisms, especially *Enterobacteriaceae*, because of its high content of tryptophan. The formulation meets the requirement of Tryptone Water recommended by ISO 7251. Organisms possessing the enzyme tryptophanase degrade the tryptophan to produce indole and other metabolic performance. The indole production can be determined by the production of a red-violet colour complex upon application of Kovacs Reagent. If present, indole reacts with the aldehyde group of p-dimethyleminobenzaldehyde with the development of a red-violet colour.

### Method

Inoculate a tube containing 5ml of Tryptone Water and incubate at 37°C for 24 +/- 3 hours. Incubation at 44°C for 24 hours is advisable for detecting indole production in the confirmation test for faecal coliforms or *E.coli*. After incubation add 1ml of Kovacs Reagent. The formation of a red-violet ring indicates a positive reaction. A yellow-brown ring indicates a negative reaction.

**Quality assurance** (37°C-24hrs)

#### Indole positive control

*E.coli* ATCC 25922

#### Indole negative control

*K.pneumoniae* ATCC 23357

### Storage

Dehydrated medium: 15-30°C

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

### References

ISO 7251. Microbiology-General Guidance for enumeration of *E. coli* - Most Probable Number Technique. 1993-12-15

Mackenzie, E.F.W., Taylor, E.W. & Gilbert. W.E. (1948) J. Gen. Microbiol., 2, 197.

## TRYPTOSE AGAR

A general purpose agar

**Code: KM3452**

### Typical formula (g/l)

Tryptose	20.00
Glucose	1.00
Sodium Chloride	5.00
Thiamine HCl	0.005
Agar	15.00

## TRYPTOSE BROTH

A general purpose broth

**Code: KM3462**

### Typical formula (g/l)

Tryptose	20.00
Glucose	1.00
Sodium Chloride	5.00
Thiamine HCl	0.005

pH 7.2 +/- 0.2

### Directions

Suspend 41g of Tryptose Agar or 26g of Tryptose Broth in 1000ml of cold distilled water. Heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes.

### Description

Tryptose Agar and Tryptose Broth are prepared according to the APHA formulations for the cultivation of *Brucella* spp. (Tryptose Vitamin B Media), and they correspond to media indicated by the WHO as Tryptose Media. Tryptose Agar can be used as it is, or as a base for the preparation of enriched, selective and diagnostic media. The World Health Organisation recommends the preparation of the following media:

1. Serum glucosate medium, for *Brucella* cultures and phagic lysis tests: to each 95ml of Tryptose Agar add 5ml of serum-glucosate solution, sterilised and cooled to 50°C. Mix thoroughly, pour into sterile petri dishes and incubate before use to confirm sterility. Serum-glucosate solution: dissolve 10g of glucose in 50ml of bovine or equine serum free from anti-brucella antibodies and inactivated by heating for 30 minutes at 56°C and sterilised by filtration.
2. Tween glucosate medium can substitute serum glucosate in the absence of serum. Dissolve Tryptose Agar in one litre of water containing 5ml of Tween 40, distribute and autoclave: cool to 50°C and aseptically add the glucosate solution sterilised by filtration (20% solution: 5ml to 95ml of medium); mix thoroughly and pour into sterile dishes; incubate before use to check sterility.

3. Glycerine glucosate medium (for dissociation studies): dissolve Tryptose Agar in one litre of water containing 20ml of glycerine; proceed as with Tween glucosate medium.
4. Selective medium (for isolation from samples containing mixed flora). To Tryptose Agar (or glucosate media indicated in points 1,2 and 3 above) autoclaved and cooled to 50°C, add 100mcg/ml cycloheximide, 26 units/ml bacitracin, 6 U.I/ml polymyxin B; the further addition of crystal violet (1.25 mcg/ml) is only recommended in special cases because the stain is toxic for some *Brucella* species. Mix thoroughly and distribute into sterile plates.
5. Diagnostic media (to study sensitivity to basic thionin and fuchsin): prepare two 0.1% aqueous solutions of basic thionin and fuchsin, store in dark glass bottles with screw-caps and renew every three months; add stains to Tryptose Agar (or to serum glucosate medium), sterilised and cooled to 50°C in the following proportions: Thionin 1:25,000 (40 mcg/ml), 1:50,000 (20 mcg/ml), 1:100,000 (10 mcg/ml). Basic fuchsin 1:50,000, 1:100,000. Mix thoroughly and distribute into sterile petri dishes.

Tryptose Broth is recommended for blood, cerebrospinal fluid, exudate and bone cultures and specimens. Tryptose Broth can also be used in association with Tryptose Agar in the biphasic Castaneda culture technique.

**Quality assurance** (37°C-24 hrs)

Productivity control

*S.aureus* ATCC 25923: growth

*E.coli* ATCC 25922: growth

**Storage**

Dehydrated medium: 15-30°C

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

**References**

APHA (1963), Diagnostic Procedures and Reagents. Fourth edition

## TRYPTOSE PHOSPHATE BROTH

This is a versatile buffered medium, nutritionally rich for the cultivation of fastidious micro-organisms and, as a supplement for cell culture.

**Code: KM1120**

Typical formula	(g/l)
Tryptose	20.0
Glucose (Dextrose) Anhydrous	2.0
Sodium Chloride	5.0
Disodium Hyd. Phosphate AR	2.5

pH :  $7.3 \pm 0.2$

### Directions

Suspend 29.5 grams of powder in 1 litre of deionised water; heat to dissolve, distribute into final containers. Sterilise by autoclaving at 121°C for 15 minutes.

**Storage:** Capped containers - up to 3 months at 15-20°C in a dark area.

**Inoculation:** For blood culture work dilute sample at least 1:10 in broth.

**Incubation:** Dependent on application.

## TSC AGAR BASE

A basal medium to be used with D-cycloserine for the enumeration of *C.perfringens* in foodstuffs.

**Code: KM4582**

Typical formula	(g/l)
Tryptose	15.0
Soy Peptone	5.0
Yeast Extract	5.0
Sodium Metabisulphite	1.0
Ferric Ammonium Citrate	1.0
Agar	13.0

### Add supplement:

D-Cycloserine                      200 mg (sufficient for 500ml of medium)

pH 7.6 +/- 0.2

### Directions

Suspend 20g of TSC Agar Base in 500 ml of cold distilled water, heat to boiling and autoclave at 121 °C for 15 minutes. Add the contents of one vial of D-Cycloserine supplement. reconstituted with 5 ml of sterile distilled water. Mix well and pour into sterile Petri dishes.

### Description

*C. perfringens* causes gastro enteric disorders characterized by abdominal pain and diarrhoea generally without fever or vomiting. The illness is usually proved by ingestion of reheated meats, such as meat pies, stews, gravies, etc, contaminated by soil or faeces. The food poisoning can be diagnosed using quantitative anaerobic cultures to test foods and faeces. The minimum infective dose is 10<sup>5</sup> cells/g of food.

The complete medium TSC Agar, prepared without Egg Yolk Emulsion, meets the requirements given by ISO 7937

### Method

For the enumeration of *C. perfringens*, ISO 7937 recommends the following technique:

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning. ISO 6887 recommends the use of peptone salt (see Maximum Recovery Diluent) as general diluent for foods and animal feeding stuffs.
2. Transfer by means of sterile pipettes 1 ml of the test sample (if liquid) or 1 ml of the initial suspension and 1 ml of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
3. Pour 15 – 20 ml of Egg Yolk free TSC Agar into each dish and mix well with the inoculum.
4. When the medium has solidified add an over layer of 10 ml of the same TSC Agar.
5. Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37°C for 20 hours. Longer incubation may result in excess blackening along the bottom rim of the plates.

- Count the black colonies on the plates containing between 15 and 150 characteristic colonies. If parts of the plates are completely blackened count the colonies at the next higher dilution even their number may be less than 15.

To confirm the presence of *C. perfringens* the following tests are recommended:

- reduction of nitrate to nitrite (+)
- motility test (-)
- gelatin liquefaction (+)

**Quality assurance** (37°C - 24hr - Anaer.)

Productivity control

With Supplement: *C.perfringens* ATCC 13124: growth, black green colonies

Without Supplement: *C.sporogenes* ATCC 19404: growth, black green colonies

Selectivity control

With Antimicrobial Supplement : *E.coli* ATCC 25922 : inhibited

Specificity control

Without Antimicrobial Supplement : *E.coli* ATCC 25922 : growth, white colonies

**Storage**

Dehydrated medium: 15-30°C

User prepared medium base in flasks: 1month at 2-8°C

**References**

ISO 7937 (1985) Microbiology – General guidance for enumeration of *Clostridium perfringens*- Colony count technique.

Haushild, A.H.W. & Hilaheimer, A. (1974). App. Microbiol. 27, 78

Harmon, S.M., Kautter, O.A. & Peeler, J.T. (1971). App. Microbiol., 22,688

Shehidi, SA. & Ferguson, AR. (1971). App. Microbiol., 21, 500-606

## TCBS AGAR

A selective plating medium for the isolation of *Vibrio* spp.

**Code: KM1109**

Typical formula	(g/l)
Tryptone	10.00
Yeast Extract	5.00
Sodium Thiosulphate	10.00
Sodium Citrate	10.00
Sodium Chloride	10.00
Oxgall	8.00
Sucrose	20.00
Ferric Citrate	1.00
Thymol Blue	0.04
Bromothymol Blue	0.04
Agar	14.00

pH 8.6 +/- 0.2

### Directions

Suspend 88g in 1000ml of cold distilled water; heat to boiling with frequent agitation, cool to approximately 50°C and pour into sterile petri dishes. **Do not** autoclave.

### Description

TCBS Agar is recommended by ISO 8914 and by AOAC for the isolation of *Vibrio parahaemolyticus* and *Vibrio cholerae*. The medium because of its alkaline pH, salt content and presence of bile salts inhibits the growth of most microorganisms except for the halophiles. Enteric bacteria do not grow on TCBS Agar. The rare colonies of some strains of *Proteus* and enterococci are easily distinguished by their reduced size and absence of colour. Sucrose serves as a fermentable carbohydrate that, with the help of bromothymol blue and thymol blue indicators, allows the differentiation of those vibrios, which utilise sucrose.

### Method

For the isolation of *V.cholerae* from faeces and from other materials, streak the surface of the plates directly with the material under examination and with the enriched sample in Alkaline Peptone Water, and incubate for 18-24 hours at 35-37°C. Cultures grown on TCBS Agar should be examined immediately after removal from the incubator as yellow colonies of sucrose fermenting vibrios may revert to a green colour when left at room temperature. *V.cholerae* and its biotype Eltor, after 18-24 hours of incubation, grow on TCBS Agar forming yellow colonies (fermentation of sucrose). *Vibrio parahaemolyticus* does not ferment sucrose and grow on TCBS Agar with blue-green colonies. *Vibrio alginolyticus* ferments sucrose and grows with yellow colonies. The suspected colonies are submitted to oxidase test to string test with sodium desoxycholate and transferred to a Kligler Iron Agar. After subculture on Tryptic Soy Agar the agglutination tests with specific antisera are carried out.

**Quality assurance** (37°C-24hrs)

#### Productivity control

*V.fluvialis* NCTC 11212: growth, yellow colonies

#### Selectivity control

*E.coli* ATCC 25922: inhibited

**Storage**

Dehydrated medium: 15-30°C

User prepared plates: 14 days at 2-8°C

**References**

AOAC (1995) - Official Method of Analysis, 16th Ed.

ISO 8914 - Microbiology - General Guidance for the detection of *Vibrio parahaemolyticus*. 1990-12-01

FDA (1995) Bacteriological Analytical Manual, 8<sup>th</sup> ed. Revision A, 1998. Published by AOAC International.

MacCormack, W.M., et al., E.J. (1974) -. J. Inf. Dis., 129, 497-500.

## TERGITOL 7 AGAR BASE

A medium for the detection of coliforms by membrane filtration technique in water analysis.

**Code: KM4023**

<b>Typical formula</b>	<b>(g/)</b>
Bacteriological peptone.....	10.00
Meat extract .....	5.00
Lactose .....	20.00
Yeast extract .....	6.00
Bromothymol blue .....	0.05
Agar .....	13.00
Tergitol 7.....	0.10

pH 7.2 ± 0.2

### **Directions:**

Suspend 54g in 950ml-distilled water and heat till boiling to completely dissolve, then sterilise by autoclaving at 121° C for 15 minutes. Cool to 45-50°C. Add 50ml of TTC 0.05% solution, filter sterilised. Mix well and pour into sterile petri dishes.

### **Description:**

This medium is a selective medium for the enumeration of coliforms; it is particularly suitable for the membrane filtration technique with water specimens. The medium contains Tergitol 7 as a selective agent, bromothymol blue as the lactose fermentation indicator and moreover triphenyl tetrazolium chloride (TTC) which may be reduced to formazan.

### **Method:**

1. Filter the specimen to be analysed through two membranes.
2. Place membranes upon two Tergitol & Agar plates.
3. Incubate a plate at 37°C for 24 hours (total coliforms) and the other at 44°C for 16-24 hours (faecal coliforms).

### **Q.C Results:**

Violet colonies: TTC reduction

Yellow colonies: absence of TTC reduction

Yellow halo: lactose fermentation

Blue halo: no lactose fermentation

The yellow colonies with deep yellow halo after incubation at 44°C should be identified as faecal coliform bacteria.

## **Tetrathionate Broth Base (A.P.H.A.)**

A selective enrichment broth for the growth of *Salmonella typhi* and other *Salmonella* spp. from faeces, foods etc.

**Code: KM1110**

<b>Typical formula</b>	<b>(g/l)</b>
Balanced Peptone	5.0
Bile Salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

pH: 8.4 ± 0.2

### **Directions**

Weigh 46 grams of powder and add to 1 litre of deionised water. Bring to the boil with frequent swirling to fully dissolve the medium. Cool to 45°C and add 20ml of iodine solution prepared as indicated below. Mix well before dispensing into bottles and continue swirling whilst dispensing to avoid the calcium carbonate sedimentation. For the best results the medium should be used the same day as prepared. Iodine solution: Dissolve 5g of potassium iodide and 6g of iodine crystals in 20mls of distilled water.

### **Description**

This formulation is recommended by the American Public Health Association for use in the examination of dairy products and foods for salmonellae. Organisms, which reduce tetrathionate, such as salmonellae, proliferate in the medium, whilst most enteric organisms are inhibited. Certain members of the *Proteus* group will also reduce tetrathionate thereby impairing the performance of the medium in some cases. To overcome this, Novobiocin may be added to the medium at a level of 40 microgram/ml before addition of the iodine. Gram-positive organisms are inhibited by the inclusion of bile salts.

**Q.C. organisms:** *Salmonella* sp., *E. coli* (inhibition)

**Storage:** Capped containers up to 7 days at 4°C in the dark.

**Inoculation:** Add 1 part of sample suspension or inoculated pre-enrichment medium to 9 parts of Tetrathionate Broth.

**Incubation:** 12-24 hours at 37°C.

**Subculture:** Onto Brilliant Green Agar (Modified), XLD Agar, Hektoen Enteric Medium or some other *Salmonella* selective media.

### **References:**

Standard methods for the Examination of Dairy products, 10th Edition. APHA, 1953.

## Thioglycollate Medium (Brewer)

This is the original formula by Brewer a clear medium for the cultivation of anaerobes

**Code: KM1112**

Typical formula	(g/l)
Beef Extract	1.0
Yeast Extract	2.0
Balanced Peptone	5.0
Dextrose	5.0
Sodium chloride	5.0
Sod. thioglycollate	1.1
Methylene blue	0.002
Agar	1.0

pH: 7.2 ± 0.2

### Directions

Weigh 20g of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then bring to the boil with gentle agitation to dissolve the solids. Distribute into screw top containers leaving minimal headspace. Sterilise by autoclaving at 121°C for 15 minutes. Tighten caps as soon as possible after autoclaving.

### Description

The agar makes the medium viscous slowing down the permeation of oxygen and any convection currents. Sodium thioglycollate acts as a reducing agent and also neutralises the bacteriostatic properties of mercurial compounds. Methylene blue is a redox indicator, which is colourless at low Eh but turns blue on exposure to oxygen.

### Appearance

Straw coloured, translucent, viscous liquid, which may have a blue surface due to contact with oxygen. If the medium has a diffuse blue tinge it should not be used until the oxygen has been driven off by holding in a boiling water bath for 5 minutes.

**Do not** reheat more than once.

**Q.C. organisms:** *C. perfringens*

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

**Inoculation:** Ensure adequate dispersal of the inoculum in the broth.

**Incubation:** At 37°C for 24-72 hours.

**Growth Indicators:** A diffuse turbidity or individual colonies.

### References

Brewer, J. H. 1940. Clear liquid mediums for the culture of anaerobes. J. Amer. Med. Ass. 115: 598-600.

## TODD HEWITT BROTH

A liquid medium for the cultivation of streptococci prior to serological grouping and for the production of antigenic streptococcal haemolysin.

**Code: KM1113**

<b>Typical formula</b>	<b>(g/l)</b>
Beef Heart Infusion	10.0
Tryptone	20.0
Glucose	2.0
Sodium Chloride	2.0
Sodium Bicarbonate	2.0
Disodium Phosphate	0.4

pH 7.8 +/- 0.2

### **Directions**

Suspend 36.4g in 1000ml of cold distilled water, heat to dissolve, distribute into containers and sterilise by autoclaving at 115°C for 20 minutes.

### **Description**

Todd Hewitt Broth is a modification of the original formula of Todd and Hewitt proposed by Updyke and Nickel. Todd Hewitt Broth is used for the cultivation of streptococci prior to serological grouping and for the production of antigenic streptococcal haemolysin. The medium is also used for the cultivation of pathogenic microorganisms, for blood cultures and for the cultivation of  $\beta$ -haemolytic streptococci for MIC testing. In the presence of some peptones, many group A streptococci produce proteolytic enzymes which digest protein M. For the grouping of streptococci it is necessary that the cellular content of this protein is maintained, therefore, it is necessary to use a culture medium with peptones which prevent the formation of these proteases. As well as supporting excellent growth of group A streptococci, Todd Hewitt Broth favours the production and maintenance of protein M and hinders the formation of proteases. Serological identification of streptococci is achieved by testing a cellular extract with antibodies of known specificity. The cellular extract may be obtained from 30ml of Todd Hewitt Broth inoculated with pure cultures and incubated for 18-24 hours at 37°C.

### **Method**

Inoculate the tube directly with the specimen swab. Express excess fluid from the swab while removing from the liquid medium. Incubate at 35-37°C in 5-10% CO<sub>2</sub> for 2- 5 hours prior to use in fluorescent antibody procedures pre incubate for 24 hours if extraction procedures for serological typing are to be performed. Follow serological and extraction procedures, as directed by the manufacture of the kit.

**Quality assurance** (30-35°C 3 days)

### Productivity control

*S.pyogens* ATCC 19615: growth

*S.pneumoniae* ATCC 6303: growth

**Storage**

Dehydrated medium: 15-30°C

User prepared tubes or flasks: 1 month at 2-8°C

**References**

Focklam, R.R. (1974) - In: Manual of Clinical Microbiology, 2nd edition, ed. Lenette, E.M., Spaulding, E.H. Truant, J.P., ch. 8, 96-108, Washington: American Society for Microbiology.

Todd, E.W. & Hewitt, L.F. (1932) - J. Path. Bact., 35, 973-974.

# TOMATO JUICE AGAR

A solid medium for the cultivation and enumeration of Lactobacillus species.

FORMULATION	g/l
Tomato extract	20.0
Peptone	10.0
Milk peptone	10.0
Agar	13.0

Code:	KM5279
Colour:	Red
Appearance:	Homogeneous hygroscopic powder
pH before autoclaving:	6.1 (approx.) at 20 °C

## Direction:

Suspend 53g in one litre of distilled water and boil to dissolve the medium completely. Sterilise by autoclaving at 121°C for 15 minutes. If it is desired to adjust the reaction to pH 5.1 cool to 55°C and add Lactic Acid Solution to the agar in the necessary (approx. 1 ml) quantity.

## Warning:

Once acidified with lactic acid, the medium should not be re-heated.

## Storage conditions:

Store the dehydrated medium below 25 °C and use before the expiry date on the label. Store the prepared medium at 2-8 °C.

## Quality Control:

Positive control - Lactobacillus acidophilus

**References:** Kulp and White (1932) Science 76: 17

## TRICHOMONAS BROTH

A basal medium for the detection of *Trichomonas*

**Code: KM5312**

### Typical Formula (g/l)

Tryptone	20.00
Liver Extract	12.00
Cystine HCl	1.60
Maltose	1.10
Agar	1.00
Methylene Blue	0.003

pH 6.5 +/- 0.2

### Directions

Suspend 35.7g in 950ml of Ringer solution. Heat to boiling and autoclave at 121°C for 15 minutes. Cool and add 50ml of sterile horse serum. Distribute into sterile test tubes and store in the dark at 20°C. Just before use, aseptically add 1mg/ml of streptomycin, 1000 I.U./ml of penicillin and 50mg/l of chloramphenicol.

### Description

Trichomonas Broth is a base medium to which various selective agents can be added for the isolation of microorganisms belonging to the genus *Trichomonas*. The antibiotics must be aseptically added to the sterilised medium just before use.

### Storage

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

### References

Kean, S.H. and Day, E. (1954) - Am. J. Obst. and Gynec., 68:1510 - 1518  
Kupferberg A.B. (1955) - International Rec. Med. and Gen Practice Clinics, 168:709 - 717.  
Williams, M.H. (1950) - Am. J. Obst. and Gynec., 60:224 - 225.