GIOLITTI CANTONI BROTH

A liquid enrichment medium for the enumeration of coagulase positive staphylococci in foodstuffs

Code: KM3162

Typical formula	**(g/l)**
Tryptone	10.0
Beef Extract	5.0
Yeast Extract	5.0
Lithium Chloride	5.0
D-Mannitol	2.0
Sodium Chloride	5.0
Glycine	1.2
Sodium Pyruvate	3.0

pH 6.9 +/- 0.2.

Directions
Suspend 54.2 g (or 108.4g in the case of double strength medium) in 900 ml of cold distilled water. If requested by the laboratory technique, add 1ml (2ml in the case of double strength medium) of Tween 80. Heat to dissolve, dispense in quantities of 10ml into tubes with dimensions of 16 x 160mm in the case of single strength medium and into tubes with dimensions of 20 x 200mm in the case of double-strength medium. Sterilise by autoclaving at 121°C for 15 minutes. Cool and add to each tube of single strength medium 0.1ml of a filter sterilised Potassium Tellurite 1% solution and 0.2ml to a double-strength medium. Inoculate 1ml of specimen and cover the surface of the medium with a 30mm layer of sterile plain agar or paraffin oil.

Description
Giolitti Cantoni Broth, prepared according to the formula of Giolitti and Cantoni, is an enrichment liquid medium used for the enumeration of coagulase positive staphylococci in foodstuffs with MPN technique, mainly when low numbers are suspected. Gram-positive bacteria are inhibited by the potassium tellurite in the medium, and Gram-negative bacteria by the lithium chloride. The presence of glycine, sodium pyruvate and mannitol, together with the anaerobic incubation conditions, contribute to the more likely and more luxuriant development of staphylococci compared to other micro-organisms in the specimen.

Method
To prepare a test sample the initial suspension and the dilutions should be in accordance with the specific International Standard dealing with the product concerning. ISO 6887 recommending the use of peptone salt (see Maximum Recovery Diluent as a general diluent for food and animal feeding stuffs. Add 10ml of test sample, if liquid, or 10ml of the primary dilution of other products, to each of three tubes containing 10ml of double strength complete Giolitti Cantoni Broth. Add 1ml of test sample, if liquid, or 1ml of the primary dilution of other products, to each of three tubes containing 10ml of single-strength complete Giolitti Cantoni Broth. Proceed in the same manner for each of the subsequent dilutions. Carefully mix the inoculum with the liquid medium, avoiding the introduction of air. Carefully pour a plug of agar (20g/l sterile solution) cooled between 44 and 47°C, onto the top of the medium in each inoculated tube and allow it to solidify to form a seal. Incubate the inoculated tubes at 37°C for 24 +/- 2 hours. After removing the plug of agar subculture 0.01ml of the blackened cultures to a plate of Baird Parker Agar. Incubate

the remainder of inoculated tubes for further 24 +/- 2hrs and subculture all tubes in the same way (developing or not a black precipitate after 48 h). For the incubation and interpretation method of the subcultures see the technical sheets of Baird Parker Agar Base. The result expression will be done using MPN table.

Quality assurance (37°C-24hrs)

Productivity control
S.aureus ATCC 25923: growth

Selectivity control
S.epidermidis ATCC 12228: partially inhibited
E.coli ATCC 25922: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared tubes (complete medium): use the same day of preparation

References
Giolitti, G . & Cantoni C. (1966) J. Appl. Bact., 29, 395.
ISO 6888-3 Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase positive staphylococci (*S.aureus* and other species) – part 3: MPN technique for low number

GN BROTH (HAJNA)

A liquid medium for the enrichment of Gram-negative micro-organisms

Code: KM3242

Typical formula	**(g/l)**
Tryptose	20.0
Sodium Chloride	5.0
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.5
Sodium Citrate	5.0
Sodium Desoxycholate	0.5
Mannitol	2.0
Dextrose	1.0

pH 7.0 +/- 0.1

Directions
Suspend 39g in 1000 ml of cold distilled water. Heat to dissolve, distribute into tubes and autoclave at 121°C for 15 minutes. Avoid excessive heating of the medium.

Description
GN Broth is prepared according to the formula proposed by Hajna. The medium is recommended for the enrichment of Gram-negative microorganisms from clinical, industrial and environmental samples. The presence of the phosphate buffer and two fermentable carbohydrates, with a mannitol concentration twice that of glucose, allows for a poor growth of *Proteus* and *Pseudomonas* in samples where *Salmonella* is present in the first two hours of incubation. The medium is particularly recommended for the promotion of *Shigella* growth.

Method
Faeces samples must be inoculated in GN Broth and in Selenite Broth; urine should be inoculated in a ratio of 5 ml to 10 ml broth and blood in a ratio of 1 ml to 10 ml broth. Incubate at 35°C for 18-24 hours, but if, after sixth hours a growth is present, streak XLD Agar and Hektoen Enteric Agar plates. For the isolation of Gram-negative micro-organisms from blood, incubation must be prolonged for 7 days before disposing of the tubes.

Quality assurance (37°C-24 hrs)

Productivity control
S.typhimurium ATCC 14028: growth on subculture
S.sonnei ATCC 9290: growth on subculture
E.coli ATCC 25922: growth on subculture

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

Reference
Hajna, A.A. (1955 Pub. Hlth. Lab. 13, 83.

G.C. SELECTIVE AGAR BASE

A General purposes medium suitable for the preparation of chocolate media.

Code: KM1034

Typical formula	**(g/l)**
Peptone mix	15.0
Corn Starch	1.0
Dipotassium Phosphate	4.0
Monopotassium Phosphate	1.0
Sodium Chloride	5.0
Agar	12.0

pH 7.2 +/- 0.2

Can be supplemented with:

VCN Supplement (vial contents for 500 ml of medium)
VCNT Supplement (vial contents for 500 ml of medium)
Vitex/Restoring Fluid (vial contents for 500 ml of medium)
Haemophilus Supplement (vial contents for 500 ml of medium)

Directions
Suspend 38 g in 1000 ml of distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Cool to 50°C and proceed as following: Chocolate Agar Enriched. To the sterilised medium cooled to 50°C, aseptically add 5-10% of defibrinated horse blood and heat in a water bath, heat-controlled to 80°C for 15 minutes. Cool to 50°C and add to 500 ml of chocolated medium, one vial of Vitex reconstituted with 5 ml of Restoring Fluid.

Neisseria selective media
To 250 ml of GC Medium Base, prepared at double concentration, add 250ml of 2% haemoglobin solution, autoclaved and cooled to 50°C. Additionally, add one vial of VCN Supplement, reconstituted with 5ml of sterile distilled water and the contents of one vial of Vitex reconstituted with the Restoring Fluid. For the examination of specimens of rectal origin, VCNT Supplement, instead of VCN, may be added to the basal medium in order to inhibit the overgrowth and swarming of *Proteus* spp.

Haemophilus Selective Medium
To the sterilised medium cooled to 50°C, aseptically add 5-10% of defibrinated horse blood and heat in a water bath, heat-controlled to 80°C for 15 minutes. Cool to 50°C and add to 500 ml of chocolated medium, one vial of Vitex reconstituted with 5 ml of Restoring Fluid and the contents of one vial of Haemophilus Selective Supplement reconstituted with 5ml of sterile distilled water.

Description
With added enrichment and selective supplements GC Medium Base is suitable for the isolation and cultivation of gonococci, meningococci and *Haemophilus* spp.
GC Medium Base can be used for he preparation of chocolate agar enriched for the isolation of *Haemophilus* spp. For the selective isolation of *H. influenzae,* from specimens contaminated with upper respiratory tract microbial flora, the Haemophilus containing bacitracin, vancomycin and clindamycin, should be added to chocolate agar enriched.

GC Medium Base is used for the preparation of Thayer Martin Agar (without trimethoprim) and Modified Thayer Martin Agar (without trimethoprim), for the isolation of *Neisseria* spp. Final concentration of antimicrobics: vancomycin 3 mcg/ml, colistin 7.5 mcg/ml, nystatin 0.0125 U.I/ml, (trimethoprim 5 mcg/ml).

Method

In septicaemia and meningitis, *N. meningitidis* can be isolated from the blood, from the cerebrospinal fluid, from the nasopharynx, and less frequently from other sites.

In the case of acute female blennorrhagic disturbances, the specimens for microbiological examination must be collected with a sterile cotton swab, chiefly from the cervix and secondarily from the rectum, from the urethra and from the nasopharynx. In the case of males, *N.gonorrhoeae* must be sought in the purulent material of urethral elimination and in the rectum. If not treated immediately, the specimens must be preserved in a transport medium such as the Amies Transport Medium. For the primary isolation of *Neisseria spp.,* after microscopic examination of the specimens, the use of Thayer Martin Medium is recommended. Ideally, specimens from sterile sites as joint fluids, skin lesion and conjunctiva should be inoculated onto both selective (Thayer Martin Medium) and non selective medium (Chocolate Agar Enriched) Incubate the inoculated plates at 37°C in 5% CO_2 enriched atmosphere with increased humidity conditions, for up to 72 hours, examining plates at 24hr intervals. If no growth is observed after 72 hrs the cultures should be reported as “no growth”. Colonies of *N.gonorrhoeae* vary in diameter from 0.5 to 1mm owing to the formation of different colony types. When examining cultures of *N.gonorrhoeae,* remember that AHU strains may produce atypically small colonies (0.25 mm*). N.meningitidis* colonies are usually larger (1 to 2 mm) and flatter than those of *N.gonorrhoeae.* Colonies of encapsulated serogroup A and C strains may be mucoid. Among the *Neisseria* and related species, colonies of *N.lactamica, N.cinerea, N.polysaccharea*,, *N. kochii* and *N.denitrificans* are similar in size, appearance, and consistency to those of *N.gonorrhoeae* and *N.meningitides.* Colonies of *B.catharralis* and the saccharolytic species *N.subflava, N.sicca*, are usually 1 to 3mm in diameter, opaque and vary in colour from greyish pink (*B.catharralis)* to yellow *(N.sublfava).* Specimens containing *Haemophilus* spp should be as fresh as possible of other microorganisms because of the likelihood that colonies of other microorganisms will obscure the presence of *Haemophilus* spp. The specimens submitted to *Haemophilus* detection are blood, body fluids, ocular specimens, respiratory specimens, and genital specimens. When specimens are suspected to be contaminated by a high number of competitive microorganisms, they should be inoculated onto both selective (Chocolate Agar Bacitracin or Chocolated GC Medium supplemented with Vitex and Haemophilus Selective Supplement) and non-selective medium (Chocolate Agar Enriched).

Incubate the plates streaked with the specimens at 35-37°C in humid atmosphere enriched with 5-10% CO_2, for 48-72 hours. *Haemophilus* colonies tend to be small and translucent and exude a mouse nest odour owing to their production of indole from tryptophan. Encapsulated strains of *H.influenzae* form glistening colonies that are somewhat larger after overnight incubation than non-encapsulated strains. The most part of *Haemophilus* spp. grows within 24 hours of incubation. *H.aegyptius* requires 2-4 days*, H.ducrey* prefers the incubation at 33°C and requires, sometimes 9 days. The differentiation between the species is obtained with X and V Factors requirement test, porphyrin test and other biochemical tests.

Quality assurance (Chocolate Agar 37°C-24 hrs, CO_2)

Productivity control
N.gonorrohoeae ATCC 43069 or 43070: growth
H.influenzae ATCC 10211: growth

Quality assurance (Neisseria Selective Media, 37°C-24 /48 h, CO_2)

Productivity control
N.gonorrohoeae ATCC 43069 or 43070: growth

Selectivity control
S.epidermidis ATCC 12228: partially inhibited
P.mirabilis ATCC 43071: partially inhibited (to be used with trimethoprim containing media)

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

References
Chapin C.K. and G.V. Doern (1983) J. Clin. Microbiol. 17, 1163-1165.
Kellogg D.S., Holmes, K.K., Hill G.A. (1976) Laboratory Diagnosis of Gonorrhea. Cumitech 4, American Society for Microbiology. Washington, D.C.
Manual of Clinical Microbiology, American Society for Microbiology, 7th edition, 1999, 586-615
Martin, J.E., Armstrong J.H., Smith P.B. (1974) App. Microbiol. 27, 802-805.
Memor, Recom.to use the same medium, MTM in both plates and bottles for the GC. Cul. Scr. Prog., CDC, Atlanta, GA, 1975.
Seth, A. (1970) Brit. J. Vener, Dis. 46, 201-202
Thayer J.D. and Martin J.E. (1966) Public Health Reports. 81, 559-562.
U.S. Pub. Hlth. Service, CDC, Veneral Dis. Br.: Criteria and Techniques for the Diagnosis of Gonorrhea, 1971.

GELATINE POWDER
A bacteriological grade of gelatine

Code: GA1015

DESCRIPTION
Gelatine is used as a solidifying agent in culture media for microbiology and for studying the gelatinolytic activity of bacteria. Gelatine is free from fermentable carbohydrates and preservatives, and is very soluble in water giving a clear, colourless solution.

TYPICAL ANALYSIS
Gel strength ..... 250 - 280 Bloom
Viscosity ..... < 55 mp
Isoelectric point ..... 8 - 9
Loss on drying ..... < 13 %
Ash ..... < 2 %
Proteins..... > 86 %

GELATINE PEPTONE
A peptone obtained by enzymatic hydrolysis of gelatine

Code: PH1038

DESCRIPTION
Gelatine Peptone is a pancreatic digest of gelatine with a low cystine and tryptophan content. It is also free of carbohydrates. This peptone is suitable for preparing media for microorganisms that are not particularly fastidious in their nutritional requirements.

TYPICAL ANALYSIS
Proteins .....> 88 %
Amino Nitrogen (AN) ..... 2.0 – 3.3 %
Total Nitrogen (TN) ..... > 16 %
Ash ..... < 6.5 %
Sodium Chloride ..... < 4 %
pH (sol. 6 %) ..... 6.6 – 7.5
Loss on drying ..... < 6 %

GELATINE PEPTONE AGAR

Used for the cultivation of non-fastidious bacteria

Code: KM6102

Typical formula	**(g/l)**
Gelatin Peptone	5.0
Agar	15.0

pH 7.0 +/- 0.1

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

Description
The medium contains a pancreatic hydrolysate of gelatin with characteristics of low fertility as regard to microbial growth, and with low cystine and tryptophan contents. Bacteria, which are not particularly fastidious in their nutritional requirement, grow on Gelatine Peptone Agar. The medium is suitable for the microbial plate count of ice cream and ice-cream related products.

Quality assurance (37°C-24hrs)
E.coli ATCC 25922: growth
L.bulgaricus: very poor growth

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