VIOLET RED BILE GLUCOSE AGAR (V.R.B.G.A.)

A medium for the enumeration of *Enterobacteriaceae* in foodstuff

Code: KM1124

Typical formula (g/l)

Peptone	7.00
Yeast Extract	3.00
Sodium Chloride	5.00
Bile Salts No.3	1.50
Glucose	10.00
Neutral Red	0.030
Crystal Violet	0.002
Agar	12.00

pH 7.4 +/- 0.2

Directions

Suspend 38.5g in 1000ml of cold distilled water. Heat to boiling with agitation and boil for 10 minutes. Cool to 45ºC, mix well and dispense into tubes or bottles

Description

Violet Red Bile Glucose Agar (VRBGA) can be used for: -enumeration of *Enterobacteriaceae* in foodstuffs with MPN technique without resuscitation (EE Broth Mossel + VRBGA) – see EE Broth Mossel technical sheet. (Recommended when the number of *Enterobacteriaceae* is expected to be in the range 1 to 100/ml or g) -enumeration of *Enterobacteriaceae* in foodstuff with plate count technique without resuscitation (recommended when the number of *Enterobacteriaceae* is expected to be greater than 100/ml or g) -enumeration of *Enterobacteriaceae* in foodstuffs with pre-enrichment (Buffered peptone Water + EE Broth Mossel + VRBGA). (Presence/Absence Test)

Methods

<u>Enumeration of Enterobacteriaceae in foodstuffs with plate count technique without resuscitation:</u>

Take two sterile petri dishes and transfer by means of a sterile pipette to each dish 1ml of the test sample, if the product is liquid, or 1ml of the initial suspension in the case of other products. Take two other sterile petri dishes and transfer by means of a other sterile pipette to each dish 1ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products. If necessary repeat the procedure with the further dilutions using a fresh sterile pipette for each decimal dilution.
Pour about 15ml of VRBGA cooled to 45°C into each petri dish.
Carefully mix the inoculum with the medium by rotating the plates and allow to solidify on a cool horizontal surface. Pour about 10 to 15ml of VRBGA cooled to approximately 45°C on the surface of inoculated medium to prevent spreading growth and to obtain semi-anaerobic conditions. Allow to solidify. Invert the prepared petri dishes and place them in the incubator at 37 °C for 24 hours. Do not stack the dishes more than six high. Stacks of the dishes should be separated from one

each other and from the walls and the top of the incubator. Count the colonies on the plates containing less than 150 typical pink to violet-red colonies (with or without precipitation halo) of diameter 0.5mm or more. Select five typical colonies for biochemical confirmation (oxidase test, glucose fermentation).

Enumeration of Enterobacteriaceae in foodstuffs with pre-enrichment:

Prepare the test sample using the pre-enrichment Buffered Peptone Water 1g of test sample+10ml of liquid medium. Incubate the initial suspension at 37°C for not less than 16 hrs and not more than 20 hrs. Transfer 1ml of the culture to 10ml of EE Broth Mossel and incubate at 37°C for 18-24 hrs. Streak the incubated EE Broth Mossel onto the surface of VRBGA and incubate the plate for 24 hrs at 37°C. From each of the incubated plates on which typical pink or red-violet colonies with or without halo, choose five colonies for biochemical confirmation (oxidase test, glucose fermentation).

Quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, purplish red colonies
S.typhimurium ATCC 14028*:* growth, purplish red colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

Storage
Dehydrated medium: 15-30°C
User prepared flasks or plates: 5 days at 2-8°C

References
ISO 7402:1993 -Microbiology- general guidance for the enumeration of *Enterobacteriaceae* without resuscitation - MPN technique and colony count technique.
ISO 8523 : 1991 - Microbiology- general guidance for the detection of *Enterobacteriaceae* with pre-enrichment.

VOGEL JOHNSON AGAR

For the isolation of *Staphylococcus aureus*

Code: KM3921

Typical formula	**(g/l)**
Tryptone	10.00
Yeast Extract	5.00
Mannitol	10.00
Dipotassium Phosphate	5.00
Lithium Chloride	5.00
Glycine	10.00
Phenol Red	0.025
Agar	18.00

pH 7.2 +/- 0.2

Directions
Suspend 61g in 1000ml of cold distilled water. Heat to boiling and sterilise by autoclaving at 121°C for 10 minutes. Cool to 50°C and aseptically add 20ml of Potassium Tellurite 1% Solution (code 42211501). The medium complete with tellurite, cannot be heated. A less selective medium may be prepared by adding 10ml of 1% Potassium Tellurite Solution.

Description
Vogel Johnson Agar is a selective medium for the isolation of *S. aureus* from foodstuffs, clinical specimens or other materials contaminated with these microorganisms. It is recommended by USP for the detection of *S.aureus* in pharmaceutical products (Microbial Limit Test). The medium is prepared according to the Vogel and Johnson modification of the Tellurite Glycine Agar of Zebovitz, Evans and Niven and meets the requirements given by USP. Compared with the original formulation of Vogel and Johnson Agar has a greater mannitol content and uses phenol red as a pH indicator to differentiate staphylococci which ferment mannitol. The selective agents in the medium, glycine, lithium chloride and potassium tellurite, inhibit the growth of almost all microorganisms with the exception of *S. aureus*. In addition, the potassium tellurite forms a black precipitate inside the colonies when reduced by staphylococci to tellurium. Mannitol fermentation is indicated by a yellow zone around the colonies.

Method
Inoculate the medium by streaking the specimen or the enriched culture on the surface of the plate and incubate at 37°C for 48 hours. *S. aureus* grows on Vogel Johnson Agar with large black colonies surrounded by yellow zones. *S.epidermidis* grows poorly with black colonies, without changing the colour of the indicator. After about 18 hours *Proteus* also grows on the medium with black colonies, changing the colour of the medium to brown. However, after 48 hours of incubation the pH of the medium inverts to alkaline, with the development of a purple colour.

Quality assurance (37°C-48 hrs)

Productivity Control
S.aureus ATCC 25923: growth, black colonies with yellow halo

Selectivity control
S.epidermidis ATCC 12228 poor growth, black colonies
P.mirabilis ATCC 12453: partially inhibited

Storage
Dehydrated medium: 15-30°C
User prepared plates: 5 days at 2-8°C

References
USP XXIV Ed. (2000).
Vogel, R.A. & Johnson M. (1960) - Public Health Lab., 18, 131.
Zebovits, E., Evans, J.B. & Niven, C.F.jr. (1955) - J. Bact., 70, 686-690.

VIOLET RED BILE AGAR

Used for the isolation and enumeration of coliforms in foodstuffs.

Code: KM1123

Typical formula	**(g/l)**
Peptone	7.00
Yeast Extract	3.00
Bile Salts No.3	1.50
Lactose	10.00
Sodium Chloride	5.00
Neutral Red	0.03
Crystal Violet	0.002
Agar	12.00

pH 7.4 + 0.2

Directions
Suspend 38.5g in 1000ml of cold distilled water. Heat to boiling and boil for about 2 minutes, cooling in a water bath to approximately 45°C and transfer to inoculated petri dishes. After solidification, add a covering layer of medium to prevent surface microbial growth.

Description
Violet Red Bile Agar is a selective and differential medium recommended by the ISO 4832 for the isolation and enumeration of coliforms in foodstuffs. Violet Red Bile Agar contains Bile Salts No.3 and crystal violet, which inhibit the growth of Gram-positive bacteria; the neutral red permits differentiation of lactose-fermenting from non-lactose-fermenting microorganisms. Lactose fermentation causes acidification of the medium, with a consequent colour change of the indicator to violet-red and a precipitation of the bile salts.

Method
Prepare the test sample and the decimal dilutions in accordance with the specific Laboratory method using Maximum Recovery Diluent or other suitable diluent. Take two sterile petri dishes and by means of a sterile pipette, transfer 1ml of the test sample, if the product is liquid, or 1ml of the initial suspension in the case of other products. Take two other sterile petri dishes and transfer, by means of an other sterile pipette to each dish 1ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products. If necessary repeat the procedure with the further dilutions using a fresh sterile pipette for each decimal dilution. Pour about 15ml of the Violet Red Bile Agar cooled to 45 °C into each petri dishes. Carefully mix the inoculum with the medium by rotating the plates and allow to solidify the petri dishes on a cool horizontal surface. Pour about 4ml of the Violet Red Bile Agar cooled to 45°C on the surface of inoculated medium and allow to solidify. Invert the prepared petri dishes and place them in the incubator at 30°C or 37°C for 24 +/- 2 hours. After incubation for 24 hours coliforms grow on Violet Red Bile Agar with purplish red colonies, 0.5mm or more in diameter.

Quality assurance (37°C-24 hrs)

Productivity control
E.coli ATCC 25922: growth, purplish red colonies

Selectivity control
E.faecalis ATCC 19433: inhibited

Specificity control
P.aeruginosa ATCC 27853: growth, pale green colonies

Storage
Dehydrated medium: 15-30°C
User prepared flasks or plates: 5 days at 2-8°C

References
APHA (1978), Standard Methods for the Examination of Dairy Products, 14th edition
APHA (1978), Compendium of Methods for the Microbiological Examination of Foods
ISO 4833:1991 Microbiology-General guidance for the enumeration of coliforms–colony count technique.

VIOLET RED BILE AGAR + MUG

A fluorogenic medium for the isolation and enumeration of coliforms and *E.coli* in foodstuff

Code: KM5123

Typical formula	**(g/l)**
Peptone	7.00
Yeast Extract	3.00
Bile Salts No.3	1.50
Lactose	10.00
Sodium Chloride	5.00
Neutral Red	0.030
Crystal Violet	0.002
Agar	15.00
MUG	0.10

pH 7.4 +/- 0.2

Directions
Suspend 41.6g in 1000ml of cold distilled water. Heat to boiling and boil for about 2 minutes, cooling in a water bath to approximately 45°C and transfer to inoculated petri dishes. After solidification, add a covering layer of medium to prevent surface microbial growth. Sterilisation is not necessary. However, if the plates have to be stored in the refrigerator it is advisable to autoclave them at 121°C for 15 minutes. This will not appreciably diminish the fertility of the medium or productivity.

Description
Violet Red Bile Agar + MUG is used for the detection of *E.coli* with direct plating method in water and foods. Incubate the inoculated plates for 18-24 hours and observe periodically for the development of fluorescence under a Wood's lamp. Refer to various compendia for the test being performed and to technical sheet of Violet Red Bile Agar for the details of the procedure for the detection and confirmation of coliforms.

Quality assurance (37°C-24 hrs)

Productivity Control
E.coli ATCC 25922: growth, red purple colonies, fluorescent under a Wood's lamp

Specificity Control
E.aerogens ATCC 13048: growth, red purple colonies, no fluorescent under a Wood's lamp

Selectivity control
S.aureus ATCC 25923: inhibited

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