WL Nutrient Medium

M115

Intended Use:

Recommended for cultivation and isolation of microorganisms encountered in brewing and industrial fermentation processes.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	4.000
Dextrose (Glucose)	50.000
Potassium dihydrogen phosphate	0.550
Potassium chloride	0.425
Calcium chloride anhydrous	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromo cresol green	0.022
Agar	20.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.25 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, to obtain a pH of 6.5, add 1% solution of sodium bicarbonate before sterilization. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

WL (Wallerstein Laboratory) media are formulated as described by Green and Gray for the examination of materials encountered in brewing and for industrial fermentations containing mixed flora of yeast and bacteria (1, 2). Bakers yeast counts can be carried out in this medium at a pH 5.5. By adjusting the pH to 6.5, the medium can be used for obtaining counts of Baker and distillers yeast (3).

WL Nutrient and WL Differential Media are used in combination. One plate of WL Nutrient Agar and two plates of WL Differential Agar are prepared (3). The WL Nutrient Agar plate is incubated aerobically to give a total yeast count while one WL Differential Agar plate gives the count of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacterial count when incubated aerobically. The other WL Differential Agar Plate is incubated anaerobically for the growth of lactic acid bacteria and *Pediococcus*. While determining microbial counts using these media, temperature and time of incubation will vary depending on the nature of material under test. Temperatures of 25°C are employed for brewing materials while 30°C are employed for baker's yeast and alcohol fermentation mash analyses.

Yeast extract which serves as a source of trace elements, vitamins and amino acids. Tryptone is used as a source of nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Buffering of the medium is done by potassium dihydrogen phosphate. Potassium chloride, calcium chloride and ferric chloride are essential ions that help to maintain the osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromo cresol green is a pH indicator.

Type of specimen

Brewing sample

Specimen Collection and Handling

For Brewing samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. While determining microbial counts using these media, temperature and time of incubation will vary depending on the nature of material under test.
2. Temperatures of 25°C are employed for brewing materials while 30°C are employed for baker's yeast and alcohol fermentation mash analyses.

d en l mbd mc u kt shnm

d en l mbd ne sgd ldchtl hr dwodbsdc vgdm trdc r od sgd ch dbshnm nm sgd k adk vhsgdnwsgd od hnc vgdm rsn dc s dbnlldmcdc sdlod st d

Quality Control

Appearance

Light yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Bluish green coloured very slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 8.02% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH

5.30-5.70

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 25922	50-100	fair-good	40-50%
Lactobacillus fermentum ATCC 9338	50-100	fair-good	40-50%
Proteus mirabilis ATCC 25933	50-100	fair-good	40-50%
Saccharomyces cerevisiae ATCC 9763	50-100	good-luxuriant	>=70%
Saccharomyces uvarum ATCC 28098	50-100	good-luxuriant	>=70%

Reference

1. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 12:43
2. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 13:357
3. MacFaddin J. F., 1985, Media for Isolation- Cultivation- Identification- Maintenance of Medical Bacteria, Vol.1,Williams & Wilkins, Baltimore, Md.

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