

Technical Data

Selective Broth for MRSA

Intended Use:

Recommended for improved detection of Methicillin Resistant Staphylococcus aureus (MRSA).

Composition**

Ingredients	g / L
Tryptone	11.000
Peptone	3.000
Dextrose (Glucose)	2.000
Sodium chloride	26.000
Starch, soluble	1.000
Disodium hydrogen phosphate	2.000
Sodium acetate	1.000
Magnesium glycerophosphate	0.200
Calcium gluconate	0.100
Cobaltous sulphate	0.001
Cupric sulphate	0.001
Ferrous sulphate	0.001
Zinc sulphate	0.001
Manganous chloride	0.002
Menadione	0.001
Cyanocobalamin	0.001
L-Cysteine hydrochloride	0.020
L-Tryptophan	0.020
Pyridoxine hydrochloride	0.003
Calcium pantothenate	0.003
Nicotinamide	0.003
Biotin	0.0003
Thiamine hydrochloride	0.040
Adenine	0.010
Guanine	0.010
Xanthine	0.010
Uracil	0.010
Final pH (at 25°C)	7.4 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 46.4 grams in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add rehydrated contents of 1vial of CAC Selective Supplement (FD299). Mix well and dispense as desired.

Principle And Interpretation

Staphylococcus aureus is a common bacterium found on the skin of healthy people. It is responsible for infections ranging from superficial to systemic (1,2). *Staphylococcus aureus* resistant to the antibiotic methicillin are referred to as Methicillin Resistant *Staphylococcus aureus* (MRSA) (3). The proportions of both hospital acquired and community acquired infections caused by MRSA have steadily been increasing worldwide. Initially staphylococcal infections were treated using penicillin. But over the years, resistance to penicillin developed, so methicillin was the next drug of choice. Unfortunately certain strains (MRSA) have now developed resistance to methicillin also. Patients with breaks in their skin due to wounds, indwelling catheters or burns are those with certain risk of developing MRSA infection (4).

Symptoms in serious cases may include fever, lethargy and headache. MRSA can cause UTI, pneumonia, toxic shock syndrome and even death. Spread of MRSA infections can be controlled to a great extent by maintaining personal hygiene

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hygiene after interaction with an MRSA infected person (3).

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) were recognized in 1980's as a major clinical and epidemiological problem. MRSA strains were heterogeneous in their expression of resistance to β - lactam agents, in that large differences in the degree of resistance were seen among the individual cells in a population. The basis of methicillin-resistance is the production of an additional penicillin-binding protein mediated by the mec A gene, an additional gene found in methicillin-resistant Staphylococci.

Tryptone, Peptone, dextrose provides nitrogen, carbon compounds, long chain amino acids and other essential growth nutrients. Sodium chloride maintains the osmotic equilibrium of the medium as well as supports the growth of *Staphylococcus* species. CAC Selective Supplement (FD299) is used for the selective growth of MRSA. It contains cefoxitin which is principally aimed at inducing the expression of methicillin resistance (5) and inhibiting the growth of Methicillin Sensitive *Staphylococcus aureus* (MSSA). The supplement also contains aztreonam to inhibit most isolates of the family *Enterobacteriaeceae* and colistin which is active against *Pseudomonas* species.

Type of specimen

Clinical samples - Swab of nose, wound or skin lesion, etc.

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic Use only. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1.Additional biochemical or antibiotic susceptibility as per CLSI are to be carried out for complete identification of MRSA species.

2.Some border line Staphylococcus aureus may show poor growth.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow coloured clear solution in tubes

Reaction

Reaction of 4.64% w/v aqueous solution 25°C. pH : 7.4±0.2

pН

7.20-7.60

Cultural Response

Cultural characteristics observed with added CAC Selective Supplement (FD299) after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth
Escherichia coli ATCC 25922 (00013*)	>=10 ⁴	inhibited
Klebsiella pneumoniae ATCC 13881	>=10 ⁴	inhibited
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10 ⁴	inhibited

Staphylococcus aureus
subsp. aureus ATCC
6538 (00032*)>=104inhibitedStaphylococcus aureus,
MRSA ATCC 43300
(00211*)50-100good-luxuriantKey : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Doyle, Beuchat and Montville, (Eds.), 1997, Food Microbiology Fundamentals and Frontiers. American Society for Microbiology, Washington, D.C.

2. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

3. Methicillin Resistant *Staphylococcus aureus*, Copyright © 1997-2005, Canadian Centre for Occupational Health and Safety, Sept 19th, 2005.

4. Dr. Alan Johnson, Methicillin resistant *Staphylococcus aureus* (MRSA) infection, The support group for MRSA sufferers and Dependents, AUG 1st, 2005.

5. Okonogi, K., Y. Noji, M. Kondo, A. Imada, and T. Yokota. 1989. Emergence of methicillin-resistant clones from cephamycin-resistant *Staphylococcus aureus* J. Antimicrob. Chemother. 24:637-645.

6. Isenberg, H.D. Clinical Microbiology Procedures Handbook.2nd Edition.

7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, Richter, S.S and Warnock., D.W. (2015) Manual of M.L., Clinical Microbiology, 11th Edition. Vol. 1.

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