**Certificate of Analysis**

**PREPARED MICROBIOLOGICAL MEDIA**

**METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AGAR**

Catalogue Number CMS178-10-10

Product Description (Methicillin-Resistant Staphylococcus aureus Agar, 10cm Petri Dish, Package of 10

Lot Number 1252

Date of Manufacture 17 November 2022

Expiry Date 16 January 2023

**INTENDED USE**

Use: For the isolation and cultivation of methicillin-resistant *Staphylococcus* *aureus* (MRSA).

**PRINCIPLE**

*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 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. Selective Supplement for MRSA (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.

**REAGENTS (FORMULA)**

Composition per liter:

Agar .............................................................. 20.0g

Casein enzymic hydrolysate ......................... 10.0g

Glycine.......................................................... 10.0g

Mannitol........................................................ 10.0g

NaCl ............................................................. 10.0g

Sodium pyruvate .......................................... 10.0g

LiCl .............................................................. 5.0g

Beef extract .................................................. 5.0g

Indicator mix................................................ 0.13g

MRSA selective supplement........................10.0mL

Deionized Water……….………………….1000ml

**Source:** This medium, without MRSA selective supplement, is available as a premixed powder from Sigma.

MRSA Selective Supplement: Composition per 10.0mL:

Methicillin...................................................4.0mg

Preparation of MRSA Selective Supplement: Add methicillin to distilled/deionized water and bring volume to 10.0mL. Mix thoroughly. Filter sterilizes. Preparation of Medium: Add components, except MRSA selective supplement, to distilled/deionized water and bring volume to 990.0mL. Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks. Autoclave for 15 min at 15 psi pressure–121°C. Cool to 50°C. Aseptically add 10.0mL MRSA selective supplement. Pour into sterile Petri dishes or leave in tubes

**EXPECTED RESULTS**

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 growt

**QUALITY CONTROL**

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

|  |  |  |
| --- | --- | --- |
| **QA Testing:**  | **Result:** | **Expected:** |
| **Characteristics** | **Pass** | **Pass** |
| **Sterility** | **Pass** | **Pass** |
| **Performance** | **Pass** | **Pass** |

pH 7.4±0.2 @ 25°C

Appearance Light yellow, Clear

Storage Condition Refrigerate, 2-8°C

Sterility Method Autoclave

Sterility Test Pass

(Absence of growth following 72 hours at 35-37°C)

|  |  |  |  |
| --- | --- | --- | --- |
| **Organisms** | **(CFU)** | **Incubation** | **Results** |
| *Escherichia coli ATCC 25922 (00013\*)* | >=104 | 35-37°C for 18-48 hours | Inhibited |
| *Klebsiella pneumoniae ATCC\*\* 13881* | >=104 | 35-37°C for 18-48 hours | Inhibited |
| *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034\*) | >=104 | 35-37°C for 18-48 hours | Inhibited |
| *Staphylococcus aureus subsp. aureus ATCC 6538 (00032\*)* | >=104 | 35-37°C for 18-48 hours | Inhibited |
| *Staphylococcus aureus* MRSA ATCC 43300, (00211\*) | 50-100 | 35-37°C for 18-48 hours | Good-luxuriant |

(\*) Corresponding WDCM numbers

ATCC\*\* is a registered trademark of American Type Culture Collection.

**BIBLIOGRAPHY**

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5. Okonogi, K., Y. Noji, M. Kondo, A. Imada, and T. Yokota. 1989. Emergence of methicillin-resistant clones

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All our prepared media products are manufactured at our site in RCFFN, University of Manitoba and tested both at our site and by department of Microbiology, University of Manitoba.

The generation of this certificate confirms all sterilization and performance criteria have been achieved.

NOTE: Expiry Date only valid if packs stored unopened at Ambient Room Temperature not exceeding 25°C.

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