

MRSA Select /Agar (Methicillin-Resistant *Staphylococcus aureus*)

356-3747

DEFINITION

MRSA Select is a selective chromogenic medium for the isolation and direct identification of Methicillin-resistant *Staphylococcus aureus* (MRSA).

PRINCIPLE

The selectivity of this medium is based on the presence of an optimised salt concentration and an antibiotic-antifungal mixture that inhibits the majority of microbes, with the exception of Methicillin-resistant staphylococci.

Identification is based on the demonstration of a specific enzymatic activity of *Staphylococcus aureus*: cleavage of a chromogenic substrate, leading to a strong pink coloration of the *Staphylococcus aureus* colonies.

- Methicillin resistant *Staphylococcus aureus* (MRSA): little pink colonies
- Methicillin-resistant coagulase negative staphylococci (MRCNS) : little white colonies (possibly tinged with pink)
- Methicillin sensitive staphylococci (MSS): no growth.

PRESENTATION

• Ready-to-use

90 mm x 20 dishes

code 356-3747

STORAGE

- Ready-to-use: + 2° to 8°C, **protected from light**
- Expiration date and batch number are shown on the package.

COMPOSITION

MRSA Select is a selective medium for the isolation of MRSA, composed of :

- An Optimised base for the growth of staphylococci
- An antibiotic-antifungal mixture and a high salt concentration, which inhibits the growth of yeasts, the majority of Gram – and Gram + bacteria, and thus methicillin-resistant staphylococci.
- A chromogenic substrate enabling the direct identification of *Staphylococcus aureus*.

PROTOCOL

Veterinary samples for detection of carriage, colonization or infection in animals:

Main types of sample:

1. Swabs: nasal, throat, skin, rectal samples
2. Foremilk: detection of mastitis

Detection Methods:

Swabs or samples taken from normally non-sterile sites expected to be colonized or infected, are streaked directly onto MRSA Select which prevents the growth of contaminating microorganisms and allows the easy direct identification of Methicillin Resistant *S. aureus*.

In case of very low levels of MRSA and/or MRSA in the presence of a high background flora, the specimen can be pre-enriched in selective broth containing salt and cefoxitin/oxacillin before subculture onto MRSA Select (Bocher, S., Smyth, R. et al., 2008).

When the sample originates from normally sterile sites (e.g. blood) from an animal with clinical symptoms of disease, use a general isolation method which can identify a range of potential pathogens

Environmental samples

• Main types of sample:

Moistened swabs, dust

• Detection Methods:

Swabs or samples (see EN ISO 6887-6) are streaked directly onto MRSA Select.

In case of low levels of MRSA and/or MRSA in the presence of a high background flora, the specimen can be pre-enriched in selective broth containing salt and cefoxitin/oxacillin before subculture onto MRSA Select (Bocher, S., Smyth, R. et al., 2008).

Samples of Food from animal origin (meat and dairy products) and **ready to eat foods** which have been significantly handled:

- **Main types of sample** : See EN ISO 6887 parts 1-5 for sample preparations

• Enumeration and detection methods:

1. Enumeration of coagulase+ staphylococci followed by MRSA screening

Coagulase + *Staphylococci* are first enumerated in food samples according to the specifications of the EN ISO 6888-1 standard. Representative presumptive or confirmed *S. aureus* colonies are subcultured onto MRSA Select for direct confirmation of their MRSA phenotype.

2. Enumeration of MRSA

Direct enumeration of MRSA can be achieved by inoculation of MRSA Select plates with a defined volume of the initial suspension (or subsequent dilutions) of the food.

3. Detection of MRSA

In order to detect low levels of MRSA, one or more enrichment steps can be introduced prior to MRSA Select inoculation. (van Loo, I., van Dijk, S. et al., 2007; Bocher, S., Smyth, R. et al., 2008). Direct streaking of the initial food suspension onto MRSA Select can provide earlier results.

INCUBATION

Incubate the plate for 18 to 24 hours at 37°C.

READING AND INTERPRETATION

- Little pink colonies: MRSA
- White colonies (possibly tinged with pink): lack of MRSA (strongly suggestive of coagulase negative Methicillin-resistant *staphylococci*).

PRECAUTIONS

- Very rare slow-growth MRSA strains require over 24 hrs to develop pink colonies. However, since the medium's performances are optimized for reading at 24 hours, it is recommended to confirm the identification of colonies showing late coloring (if incubation is extended after 28h)
- If it is necessary to defer the interpretation of a 24-hour culture, store the plate at +2-8°C, protected from light.
- It is possible that rare strains of *Staphylococcus epidermidis* will develop a faint pink coloration. However, the intensity of their coloration enables them to be differentiated from MRSA.
- It is possible that certain strains of *Acinobacter* will develop a dark pink coloration. However, their domed and mucoid appearance enables them to be differentiated from MRSA.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

Culture performance is controlled using specific strains. Please refer to the control certificate for each product and batch.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

BIBLIOGRAPHY

Bocher, S., Smyth, R., Kahlmeter, G., Kerremans, J., Vos, M.C. and Skov, R., 2008. Evaluation of four selective agars and two enrichment broths in screening for methicillin resistant *Staphylococcus aureus*. J Clin Microbiol 46 (9), 3136-3138.

Graveland, H., et al., Evaluation of isolation procedures and chromogenic agar media for the detection of MRSA in nasal swabs from pigs and veal calves. Vet. Microbiol. (2009), doi : 10.1016/j.vetmic.2009.05.019

EN ISO 6887 - Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination

EN ISO 6887-6 - Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination
Part 6 - Specific rules for the preparation of samples taken at the primary production stage.