REF 04924

IVD

Chromogenic medium for the detection and identification of methicillin-resistant Staphylococcus aureus (MRSA).

SUMMARY AND EXPLANATION

chromID MRSA Agar is a chromogenic medium for the detection and identification of methicillin-resistant *S. aureus* (MRSA) in clinical specimens (1, 2)

MRSA are mutli-resistant bacteria which may cause nosocomial infections (3, 4, 5). The detection of MRSA carriers is particularly important for the epidemiological prevention and follow-up of these infections. In this context, the use of chromID MRSA Agar contributes towards the active surveillance of MRSA.

PRINCIPLE

chromID MRSA Agar consists of a rich nutritive base combining different peptones. It also contains a chromogenic substrate of α -glucosidase and an antibiotic (cefoxitin) which favours (6, 7):

- the growth of methicillin-resistant *staphylococci* (MRSA) including hetero-resistant strains.
- the direct detection of MRSA strains by revealing αglucosidase activity (patent registered): green colonies.

The selective mixture inhibits most bacteria not belonging to the genus *Staphylococcus*, as well as yeasts.

PRESENTATION

Ready-to-use medium REF 04924 Pack of 1x10 plates (90 mm) *MRSA

* printed on each plate.

COMPOSITION

Theoretical formula

This medium can be adjusted and/or supplemented according to the performance criteria required:

| Plant and animal peptones (porcine or bovine) | 20.1 g |
|---|--------|
| Tris | 0.65 g |
| Chromogenic mixture | 0.4 g |
| Selective mixture | 4.1 g |
| Agar | 13 g |
| Purified water | 1 Ľ |
| pH 7 3 + 0 3 | |

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

Material:

• Bacteriology incubator.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional use only.
- This product contains materials of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual

precautions for handling the bacterial group studied should be observed throughout this procedure.

- Culture media should not be used as manufacturing material or components.
- Do not use past the expiration date.
- Do not use if the packaging is damaged.
- Do not use contaminated plates, or plates that exude moisture.
- The use of this medium may be difficult for people who have problems recognising colours.

STORAGE CONDITIONS

Protect from light.

Store product in original packaging at 2-8°C until the expiry date.

SPECIMENS

All types of specimens may be used (mainly nose, throat, perineum, groin etc). They should be inoculated etiher directly onto the agar or after enrichment in a broth.

Good laboratory practices for collection and transport should be respected.

INSTRUCTIONS FOR USE

- 1. Allow plates to come to room temperature.
- Inoculate the specimens directly onto the chromID MRSA Agar.

The medium can also be inoculated using an enrichment broth.

3. Incubate with the agar inverted at 35±2°C. The cultures are generally examined after 18-24 hours incubation.

If a negative result is obtained (no growth or colouration) the medium should be incubated for an additional 24 hours.

The user is responsible for choosing the appropriate incubation temperature depending on intended use and in accordance with current standards.

Note: Test conditions (use of an enrichment phase, incubation time) whould be adapted to the local epidemiology.

READING AND INTERPRETATION

- After 18-24 hours incubation, observe the bacterial growth and the appearance of the colonies. A green colour is characteristic of MRSA colonies (no additional tests).
- After 48 hours incubation, identificaton of characteristic colonies should be confirmed using biochemical or immunological tests (*S. aureus*).
- The presence of at least one green colony indicates a positive result: presence of MRSA. The colour is more vivid if the colonies are observed through the agar (plate inverted).

Note: To determine the resistance profile of an MRSA strain, a complete susceptibility test should be performed.

QUALITY CONTROL

Protocol:

The capacity of the medium can be tested using the following strains:

- Staphylococcus aureus BMR ATCC 43300
- Staphylococcus aureus ATCC 29213
- Escherichia coli ATCC 8739
- Candida albicans ATCC 10231
- Enterococcus faecalis ATCC 29212
- Staphylococcus aureus 05-04-605
- Staphylococcus aureus 05-06-614

Range of expected results:

| Strain | Results at 35±2°C after 18-48 hours |
|---|--|
| Staphylococcus aureus BMR ATCC 43300 | Growth, green colonies |
| Staphylococcus aureus ATCC 29213 | Partial to complete inhibition |
| Escherichia coli ATCC 8739 | Partial to complete inhibition |
| Candida albicans ATCC 10231 | Partial to complete inhibition |
| Enterococcus faecalis ATCC 29212 | Partial to complete inhibition |
| Staphylococcus aureus 05-04-605 | Partial to complete inhibition |
| <i>Staphylococcus aureus</i> 05-06-614 | Partial to complete inhibition |

Note:

It is the responsibility of the user to perform Quality Control taking into consideration the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature...).

LIMITATIONS OF THE METHOD

- Certain strains of *S. aureus* which have the mec A gene but a low MIC in relation to cefoxitin (≤4 mg/L) may not develop on this type of medium,
- Certain strains of *S. aureus* which do not have the mec A gene may develop characteristic colonies on this type of medium due to the existence of other resistance mechanisms (hyperproduction of penicillinase).
- Certain coagulase negative *staphylococci* may develop a pale green colour.
- Certain organisms other than *S. aureus* produce green colonies which have a different appearance, enabling them to be differentiated from MRSA (*Bacillus*, Gram -ve bacilli, BLSE strains).
- If a susceptibility test is performed using colonies from chromID MRSA Agar, the results obtained for the glycopeptides will not be interpretable. A tendancy towards too many resistant results has been observed for these antibiotics.

PERFORMANCE

Performance was evaluated at 35±2°C using 20 bacterial strains (*Staphyloccus aureus* (MRSA), other

Staphylococcus, Enterococcus, Escherichia, Klebsiella and yeasts (Candida)).

Nutrient capacity:

All *Staphylococcus aureus* (MRSA) strains tested grew after 18 hours incubation.

All other *Staphylococcus aureus* (non-MRSA) strains tested grew after 18 hours incubation.

Selectivity:

All other Gram (+), Gram (-) and yeasts tested were inhibited after 48 hours incubation.

These results were obtained under controlled laboratory conditions using non-clinical strains. Interpretation of this performance data should include consideration for the limitations previously stated.

WASTE DISPOSAL

Unused product may be considered as non-hazardous waste and disposed of accordingly.

Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious materials.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazard and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

- Blanc, D. S., Francioli, P., Coustumier, A. et al, Reemergence of gentamicin-susceptible strains of methicillinresistance Staphylococcus aureus in France: a phylogenetic approach, J. Clin. Microbiol., Jun 2001, Vol. 39, No. 6, p2287-2290.
- Perry, J. D., Davies, A., Butterworth, L. A. et al, Development and evaluation of a chromogenic agar medium for methicillin-resistant Staphylococcus aureus, J. Clin. Microbiol., Oct 2004, Vol. 42, no. 10, p4519-4523.
- Lelievre, H., Lina, G., Jones, M. E. et al, Emergence and spread in French hospitals of methicillin-resistant Staphylococcus aureus with increasing susceptibility to gentamicin and other antibiotics, J. Clin. Microbiol., Nov 1999, Vol. 37, No. 11, p3452-3457.
- Muto, C. A., Jernigan, J. A., Ostrowsky, B. E. et al, Guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and Enterococcus, Infect. Control. Hosp. Epidermiol., 2003, Vol. 24, p362-386.
- Sevin, E., Larmaraud-Sevin, O., Legrand, P., Approache moléculaire de la résistance á la méticilline de Staphylococcus aureus, Revue française des laboratoires, 1999, Vol. 315, p25-31.
- Davies, A., Perry, J. D., Butterworth, L. A. et al, An evaluation of MRSA ID: a new chromogenic medium for the isolation and identification of methicillin-resistant Staphylococcus aureus, R2151, Pragues (République Tchéque) 2004, 14th, ECCMID.
- Perry, J. D., Rennison, C., Butterworth, L. A. et al, Evaluation of S. aureus ID, a new chromogenic agar medium for detection of Staphylococcus aureus, J. Clin. Microbiol., Dec 2003, Vol. 41, p5695-5698.

INDEX OF SYMBOLS

| Symbol | Meaning |
|------------|---------------------------------------|
| REF or REF | Catalogue number |
| IVD | In Vitro Diagnostic Medical Device |
| | Manufacturer |
| | Temperature limitation |
| Σ | Use by |
| LOT | Batch code |
| X | Protect from light |
| (H) | Consult Instructions for Use |
| Σ | Contains sufficient for <n> tests</n> |

