chromID® MRSA agar / chromID® S. aureus agar (MRSA/SAID)

IVD

MULTIMEDIA

Chromogenic medium for the screening of methicillin-resistant *Staphylococcus aureus* (MRSA).
Chromogenic medium for the selective isolation of staphylococci and the direct identification of *S. aureus*.

SUMMARY AND EXPLANATION

This product consists of two culture media dispensed into one Petri dish containing separate compartments.
It is intended for use with clinical specimens.

CONTENT OF THE KIT

	Ready-to-use media:
REF **43466**	Pack of 20 plates (90 mm)
	MRSA/SAID *

* printed on each plate

- **MRSA** identifies the compartment on the plate containing chromID® MRSA agar.
- **SAID** identifies the compartment on the plate containing chromID® S. aureus agar.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- This kit contains products 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. Refer to "CLSI® M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Current Revision". For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH – Latest edition", or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.
- Do not expose the media to light.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates or plates that exude moisture.
- Interpretation of the test results should be made taking into consideration the patient's history, the source of the specimen, colonial and microscopic morphology and, if necessary, the results of any other tests performed.
- Use of the medium may be difficult for people who have problems recognizing colors.
- The performance data were obtained using the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- **Store the plates in their box at 2–8°C until the expiry date.**
- If not in the box, plates can be stored in the cellophane sachet for 2 weeks at 2–8°C **in the dark**.

WASTE DISPOSAL

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

CHROMID® MRSA AGAR (MRSA)

SUMMARY AND EXPLANATION

chromID® MRSA agar is a chromogenic medium for the screening of methicillin-resistant *S. aureus* (MRSA) in chronic carriers or patients who are at risk for MRSA (1, 7). This medium does not replace conventional antimicrobial susceptibility tests.
MRSA are multi-resistant bacteria which may cause nosocomial infections (3, 4, 9). The detection of MRSA carriers is particularly important for the epidemiological prevention and monitoring 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 nutrient base combining different peptones. It also contains a chromogenic substrate of α-glucosidase and a combination of several antibiotics including cefoxitin, which favour (2, 6):

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

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

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 l

pH 7.3

MATERIAL REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.
- Brain-Heart Infusion Broth (ref. 42081)
- Todd Hewitt Broth + Antibiotics (ref. 42116)

SPECIMENS

Different types of specimens may be used (nose, throat, perineum, etc.) and should be collected using swabs. A recent study has shown that the use of nylon flocked swabs and transport medium (Eswab) improves the detection of MRSA on chromID® MRSA agar (see PERFORMANCE section, 4th evaluation).

For nose and throat specimens only, the presence of MRSA can be detected after enrichment of the sample.

Good laboratory practices for collection and transport should be respected and adapted to each type of specimen.

A. Direct inoculation

1. **Allow the plates to come to room temperature**.
2. Inoculate the specimens directly onto the chromID® MRSA agar.
3. Incubate the plates inverted at 37°C in aerobic conditions. The cultures are generally examined after 18-24 hours of incubation.
   A positive result may be obtained after 18 hours of incubation, however if a negative result is obtained (no growth or coloration) incubation must be extended to 24 hours.
   If a negative result is obtained at 24 hours, the medium can be incubated for an additional 24 hours to increase the sensitivity of detection.

   If a nylon flocked swab with transport medium (Eswab) is used, this additional incubation time is not necessary.

B. Inoculation after enrichment

1. Inoculate the sample in the enrichment broth (Brain-Heart Infusion Broth or Todd Hewitt Broth + Antibiotics).
2. Incubate the broth at 37°C for 18-24 hours.
3. Allow the plates to come to room temperature.
4. Inoculate the chromID® MRSA agar using the enrichment broth.
5. Incubate the plates inverted at 37°C in aerobic conditions. The cultures are generally examined after 18-24 hours of incubation.

The performance data were obtained using the procedure and a 37°C incubation temperature. The user is responsible if any other incubation temperature is used, and should check that performance is maintained.

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

READING AND INTERPRETATION

The presence of at least one typical green colony gives the sample a positive MRSA status. The green color is more vivid if the colonies are observed through the agar.

A. Direct inoculation

- After 18-24 hours of incubation, for nose specimens, a green color is characteristic of MRSA. For the other types of specimens the typical colonies must be identified using biochemical or immunological tests (*S. aureus*). If identification of S. aureus is confirmed, check the resistance of the strain to methicillin.
- After 48 hours of incubation and whatever the type of specimen, the typical colonies should be identified following the same procedure.

B. Inoculation after enrichment

- After 18-24 hours of incubation, identify the typical colonies.

QUALITY CONTROL

Protocol:

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

- *Staphylococcus aureus* ATCC® 43300™
- *Staphylococcus aureus* ATCC® 29213™

Range of expected results:

Strain	Results at 33-37°C	
Staphylococcus aureus ATCC® 43300™	Growth within 24 hours	Green colonies
Staphylococcus aureus ATCC® 29213™	No growth within 48 hours	

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, etc.).

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 typical colonies on this type of medium after 24 or 48 hours of incubation.
- Certain coagulase-negative staphylococci may develop a pale green color. After 48 hours of incubation, this result should be considered negative.
- Certain organisms other than S. aureus produce green colonies which have a different phenotypic appearance, enabling them to be differentiated from MRSA (Bacillus, Gram-negative bacilli, enterococci, ESBL strains).
- If a susceptibility test is performed using colonies from chromID® MRSA agar, the results obtained for the glycopeptides will not be interpretable. A tendency towards too resistant results has been observed for these antibiotics.

PERFORMANCE

Performance of chromID® MRSA agar was evaluated at 4 sites using human clinical specimens, within the context of MRSA carrier screening.

The first evaluation (France) (8) was performed using 278 nasal swabs (including 28 frozen and presumed positive specimens). chromID® MRSA agar was compared to 2 other media:

- A commercially available screening medium whose typical colonies have to be confirmed by a coagulase test.
- A Columbia CNA agar + 5% sheep blood in combination with tests for confirmation of suspect colonies (coagulase + *mecA* gene detection by PCR).

The agar were inoculated directly with the specimens. Readings were performed after 18-24 h and 48 h of incubation at 33-37°C in aerobic conditions.

45 specimens (including 23 frozen ones) were found to be positive for at least one of the 3 media.

	Recovery rate of MRSA		
	chromID® MRSA	Other medium	CAN + coagulase test + *mec*A detection
18-24 h	42/45 (S = 93.3%) (Sp = 98.7%)	38/45 (S = 84.4%) (Sp = 88.8%)	42/45
48 h	43/45 (S = 95.6%)	43/45 (S = 95.6%)	43/45

S: Sensitivity of detection
Sp: Specificity

The second evaluation (Belgium) (5) was performed using 491 specimens corresponding to nasal (363), throat (47) and perineum (46) swabs or other types of specimens (35). chromID® MRSA agar was compared to a Columbia agar + 5% sheep blood (COS) in combination with confirmation tests (coagulase + *mecA* gene detection by PCR).

The specimens were inoculated directly onto the agars. Reading of the agars was performed after 18-24 hours and 48 hours of incubation at 33-37°C in aerobic conditions.

Fifty-five specimens were found to be positive on at least one of the 2 media, whatever the method.

	Direct inoculation	
	chromID® MRSA	COS+ coagulase test + *mecA detection*
18-24 h	35/55 (S = 63.6%) (Sp = 99.8%)	30/55
48 h	54/55 (S = 98.1%)	38/55

S: Sensitivity of detection
Sp: Specificity

The third evaluation (Belgium) was performed using 770 specimens corresponding to nasal (119) and throat (355) swabs or other types of specimens (296).

The specimens were inoculated directly onto the agar or after enrichment in Brain-Heart Infusion Broth and Todd Hewitt Broth + Antibiotics.

Reading of the agars was performed after 22-24 hours and 48 hours of incubation at 33-37°C in aerobic conditions in the case of direct inoculation and after 18-24 hours in the case of enrichment.

	Direct inoculation on chromID™ MRSA		Enrichment in Brain-Heart Infusion Broth		Enrichment in Todd Hewitt Broth + antibiotics	
	Nose	Throat	Nose	Throat	Nose	Throat
22-24 h	17/22 S=77.3 % Sp=100 %	15/22 S=68.2 % Sp=97.9 %*	21/22 S=95.5 % Sp=96.9 %*	17/22 S=77.3 % Sp=90.7 %*	20/22 S=90.9% Sp=95.9%*	19/22 S=86.4 % Sp=92.2 %*
48 h	19/22 S=86.4 %	17/22 S=77.3 %	N/A	N/A	N/A	N/A

S: Sensitivity of detection

Sp: Specificity

* Specificity without confirmation

The fourth evaluation (France) was performed using 167 specimens corresponding to nasal (144) or wound (23) swabs.

In this study, the Eswab (flocked nylon tip and its transport medium) was compared to a dry swab used routinely in the laboratory.
Each swab containing the same specimen was inoculated directly onto a chromID™ MRSA agar plate.

Reading of the agars was performed after 18, 24 and 48 hours of incubation at 33-37°C in aerobic conditions.

59 specimens were found to be positive with at least one of the 2 types of swabs during the whole study.

	Inoculation using Eswab	Inoculation using dry swab (polyurethane)
18 h	51/59 S=86.4% Sp=97.2%	45/59 S=76.2% Sp=98.2%
24 h	55/59 S=93.2% Sp=97.2%	48/59 S=81.4% Sp=98.2%
48 h	55/59 S=93.2% Sp=96.3%	51/59 S=86.4% Sp=97.2%

S: Sensitivity of detection

Sp: Specificity

Use of the Eswab significantly increases the detection of MRSA in 18 and 24 hours on chromID® MRSA agar. Additional incubation and reading after 48 hours prove to be unnecessary.

INTERFERENCE STUDY

An internal study has shown that the use of the nylon flocked dry swab (Ref. 280101) is compatible with chromID® MRSA agar.

LITERATURE REFERENCES

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10. C. Nonhoff, O. Denis, A. Brenner, P. Buidin, C. Thiroux, M. Struelens. (Brussels, BE) - Comparison of three chromogenic media for rapid detection of methicillin-resistant Staphylococcus aureus from screening swabs in hospitalised patients - ECCMID MUNICH 31.03.2007 au 03.04.2007 poster n°1605.

CHROMID® S. AUREUS AGAR (SAID)

SUMMARY AND EXPLANATION

chromID® S. aureus agar is a chromogenic medium for the selective isolation of staphylococci and the identification of *S. aureus* in human specimens.

PRINCIPLE

chromID® S. aureus agar is composed of a rich nutritive base which combines different peptones and two chromogenic substrates to enable:

- the growth of all staphylococci.
- the detection of activities of specific enzymes (patent pending) and therefore the differentiation of mixed cultures (1, 2).

Direct identification of *S. aureus* is based on the following principle: spontaneous green coloration of colonies producing α-glucosidase.

The presence of a second substrate enables the differentiation from other species of staphylococci which demonstrate specific enzyme activity (pink or mauve colonies). The species of staphylococci which do not utilize any substrates produce white colonies.

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

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	0.53 g
Selective mixture	4.1 g
Agar	14 g
Purified water	1 l

pH 7.3

MATERIAL REQUIRED BUT NOT PROVIDED

Bacteriology incubator.

POSSIBLE ADDITIONAL REAGENTS

- ATCC® quality control strain.
- Slidex® Staph-Kit (Ref. 73112).

SPECIMENS

All types of specimens may be used and should be inoculated directly onto the agar.

Good laboratory practices for collection and transport should be respected and adapted to the type of specimen.

INSTRUCTIONS FOR USE

This medium must not be exposed to light other than during the inoculation and reading steps.

1. Allow plates to come to room temperature in the dark.
2. Inoculate the specimen.
3. Immediately incubate the plates, inverted, in the dark at 37°C in aerobic conditions. The user is responsible for choosing the appropriate temperature for the intended use, in accordance with current standards.

   The cultures are generally examined after 24 hours of incubation.

   Note: If negative results are obtained after 24 hours, the medium can be incubated for an additional 24 hours in order to increase the sensitivity of detection.
   In this case, any green colonies must be identified using supplementary tests, for example Slidex® Staph-Kit.

READING AND INTERPRETATION

- After incubation, observe the bacterial growth and the appearance of the colonies: colonies of *S. aureus* are green (very pale green to dark green).
  To facilitate reading, it is recommended to observe the colonies through the agar (with the plate upside down).
- The identification of colonies other than green ones (white, pink or mauve) must be followed by biochemical and/or immunological tests.

QUALITY CONTROL

Protocole:

The nutrient capacity of the medium can be tested using the following strain-:

- *Staphylococcus aureus* ATCC® 25923™.

Range of expected results:

Strain	Results at 33-37°C	
Staphylococcus aureus ATCC® 25923™	Growth within 24 hours*	Green colonies

* In case of a negative result, prolong incubation for an additional 24 hours.

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, etc.).

LIMITATIONS OF THE METHOD

- Certain coagulase-positive *Staphylococcus* strains, other than *S. aureus*, also produce green colonies.
- Certain strains other than *S. aureus* may produce green colonies e.g. *S. saprophyticus*, *S. haemolyticus*, *S. warneri*, *Micrococcus*) (3, 4).
- Some species may produce green colonies which differ morphologically from those of *S. aureus* (small colonies e.g. *Enterococcus*, *Streptococus*, *Stomatococcus* and *Candida*).
- With certain strains of *S. aureus*, the color of colonies may turn from green to violet/grey after 48 hours of incubation.

- Growth depends on the requirements of each individual microorganism. It is therefore possible that certain strains of staphylococci, which have specific requirements, may not grow or may not develop the green color.
- If the instructions for use are not complied with (exposure to light), a lack of coloration for *S. aureus* colonies, or even inhibited growth of certain strains may be observed.
- Dispose of non-inoculated plates if they have been exposed to light.
- Depending on the specimens analyzed, it is recommended to use chromID® S. aureus agar in conjunction with non-inhibitory media (e.g. Columbia agar + 5% sheep blood).
- Some *S. aureus* strains that show little or no alpha glucosidase activity, may not produce the expected characteristic color.

PERFORMANCE

chromID® S. aureus agar was compared with another chromogenic medium by testing 514 human specimens of various origins (blood cultures, urine, feces, nasal swabs, suppurations, ENT and genital specimens etc.).

Performance was evaluated after 24 hours of incubation at 35–37°C.

With chromID® S. aureus, 365 specimens produced *Staphylococcus*-positive cultures.

Nutrient capacity and sensitivity of *S. aureus* detection

Among the 514 specimens studied, 129 produced *S. aureus*-positive cultures on at least one of the media (identification confirmed).

	SAID	Other medium
Nutrient capacity of *S. aureus*	128/129 (99%)	115/129 (89%)
Sensitivity of *S. aureus* detection	127/129 (98%)	114/129 (88%)

Coloration specificity

Among the characteristic colonies observed on each of the media (136 green colonies on SAID and 118 pink colonies on the other medium), the proportion of colonies identified as *S. aureus* is the following:

	SAID	Other medium
Coloration specificity	127/136 (93%)	114/118 (97%)

LITERATURE REFERENCES

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INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Cotnains sufficient for <n> tests
	Protect from light

WARRANTY

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