BD BBL™ CHROMagar™ Staph aureus

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 100503

INTENDED USE

BBL CHROMagar Staph aureus is a selective medium for the isolation, enumeration and identification of *Staphylococcus aureus* from clinical and food sources. Confirmatory testing of typical isolates from clinical sources is not required.

BBL CHROMagar Staph aureus (prepared plated medium) has been validated by the AOAC™ Research Institute under the Performance Tested Methods[SM] Program for the analysis of shell eggs, smoked salmon and cooked roast beef when using AOAC and ISO methods.[1,2] Confirmatory testing of mauve-colored colonies obtained from the food matrices mentioned above is required.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

S. aureus is a well documented pathogen. It is responsible for infections ranging from superficial to systemic.[3,4] Due to the prevalence of this organism and its clinical implications, detection is of utmost importance.

Staphylococcal food poisoning caused by *S. aureus* is one of the most common types of foodborne illness worldwide. Its detection and enumeration help provide information about the potential health hazard of food, as well as being an indicator of poor hygiene.[5] It is also recommended that this organism be used as an indicator of water quality.[6]

BBL CHROMagar Staph aureus is intended for the isolation, enumeration and identification of *S. aureus* based on the formation of mauve-colored colonies. The addition of chromogenic substrates to the medium facilitates the differentiation of *S. aureus* from other organisms.

An advantage **BBL CHROMagar Staph aureus** has over some traditional media, such as Baird-Parker Agar, is the ability to identify *S. aureus* in 24 h as opposed to 48 h.

BBL CHROMagar Staph aureus was originally developed by A. Rambach, CHROMagar, Paris, France. BD, under a licensing agreement, has optimized this formulation utilizing proprietary intellectual property used in the manufacturing of the **BBL CHROMagar Staph aureus** prepared plated medium. Specially selected **Difco™** peptones supply nutrients. The addition of selective agents inhibits the growth of gram-negative organisms, yeast and some grampositive cocci. The chromogen mix consists of artificial substrates (chromogens), which release an insoluble colored compound when hydrolyzed by specific enzymes. This facilitates the detection and differentiation of *S. aureus* from other organisms. *S. aureus* utilizes one of the chromogenic substrates, producing mauve-colored colonies. The growth of mauve-colored colonies at 24 h is considered positive for *S. aureus* on **BBL CHROMagar Staph aureus**. Bacteria other than *S. aureus* may utilize other chromogenic substrates resulting in blue, blue-green, or if no chromogenic substrates are utilized, natural colored colonies.

*PRODUCER-SUPPLIED SAMPLES OF THIS TEST KIT MODEL WERE INDEPENDENTLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AND WERE FOUND TO PERFORM TO THE PRODUCER'S SPECIFICATIONS AS STATED IN THE TEST KIT'S DESCRIPTIVE INSERT. THE PRODUCER CERTIFIES THIS KIT CONFORMS IN ALL RESPECTS TO THE SPECIFICATIONS ORIGINALLY EVALUATED BY THE AOAC RESEARCH INSTITUTE AS DETAILED IN *Performance Tested Methods*[sm] CERTIFICATE NUMBER 100503.

REAGENTS

BBL CHROMagar Staph aureus

Approximate Formula* Per Liter of Purified Water

Chromopeptone	40.0 g
Sodium Chloride	25.0 g
Chromogenic Mix	0.5 g
Inhibitory Agents	0.07 g
Agar	14.0 g

pH: 6,8 +/- 0,2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Protect from light during drying. See **STORAGE AND SHELF LIFE**.

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. Standard precautions[7-10] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

USER QUALITY CONTROL

Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions (for details, see **GENERAL INSTRUCTIONS FOR USE** document). The test strains mentioned in the Table below are recommended. Incubate aerobically for 18 to 24 hours at 35 ± 2° C in the dark.

Strains	**Growth Results**
Staphylococcus aureus ATCC 25923	Growth; mauve colonies
Staphylococcus saprophyticus ATCC 15305	Growth; green to blue-green colonies
Proteus mirabilis ATCC 12453	Inhibition (partial to complete)
Uninoculated	Colorless to light amber, clear to trace hazy

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the clinical user refer to pertinent Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines for appropriate Quality Control practices.

PROCEDURE

Materials Provided

BBL CHROMagar Staph aureus, provided in 90 mm **Stacker** dishes

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and other laboratory equipment as required.

Specimen Types
Refer to appropriate texts or standards for details in specimen/sample collection and handling procedures. This medium is used for the isolation of *Staohylococcus aureus* from all types of clinical specimens. See also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**.
For agrifood testing, follow appropriate standard methods for details on sample preparation and processing according to sample type and geographic location.

Test Procedure
Observe aseptic techniques. The agar surface should be smooth and moist, but without excessive moisture. For clinical specimens, as soon as possible after receipt in the laboratory, inoculate onto a **BBL CHROMagar Staph aureus** plate and streak for isolation. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate plates aerobically at 35 ± 2°C for 20-24 h in an inverted position (agar-side up).

For food samples, consult appropriate references and follow applicable standard methods. Inoculate the homogenized food samples onto **BBL CHROMagar Staph aureus** using the spread plate technique. Incubate plates aerobically at 35-37°C for 20-28 h in an inverted position (agar-side up).

Results
After proper incubation, read plates against a white background. *S. aureus* will produce mauve to orange/mauve colored colonies on the **BBL CHROMagar** medium. Most gram-positive organisms, if not inhibited, will produce blue, blue-green or natural color (colorless, white or cream) colonies. Gram-negative organisms and yeasts are partially to completely inhibited.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE
Performance Results[12]
Clinical Testing

1. In a field trial conducted at a large US metropolitan hospital, 201 throat and sputum specimens from cystic fibrosis patients and 459 nasal specimens from other hospital patients were evaluated on **BBL CHROMagar Staph aureus**. **BBL CHROMagar Staph aureus** was compared to blood agar or Mannitol Salt Agar, with isolate confirmation by slide coagulase. *S. aureus* was recovered from 190 combined specimens. **BBL CHROMagar Staph aureus** detected 9 additional *S. aureus* positive cultures which were not recovered on conventional media. Four potential false positives were also observed on the **BBL CHROMagar Staph aureus** medium following 24 h incubation: two corynebacteria and two coagulase-negative staphylococci. **BBL CHROMagar Staph aureus** produced an overall sensitivity of 99.5% and a specificity of 99.2%.[11]

2. In a European study, one hundred sixty five (165) specimens from a routine lab, consisting of 100 specimens shown to contain *S. aureus* by standard methods (= known positive specimens) and 65 known negative specimens, were streaked on **CHROMagar Staph aureus**, Mannitol Salt Agar and Columbia Agar with 5% Sheep Blood. The specimen types are shown in Table 1. Plates were incubated for 20 to 24 hours at 35 to 37°C and were read for colonies suspicious of *S. aureus*. Tube coagulase tests were set up from all suspicious colonies on all three media. Also, the amount of *S.aureus* growth (scored semi-quantitatively), was determined.

Table 1: Specimen Types

Specimen Types	S. aureus known positive specimens	S. aureus known negative specimens
Abscesses	14	6
Ascites fluid	1	0
Bone	1	0
Bursa	1	0

Catheter	4	0
Drainage	1	0
Fistula	0	1
Surgery specimens	12	15
Miscellaneous swabs	12	19
Phlegmona	1	0
Tracheal secretions	1	0
Wounds	52	24
Total	100	65

Results: Of the 165 specimens, 100 yielded growth of *S. aureus* on any medium, as confirmed by coagulase testing from all media.
On **CHROMagar Staph aureus**, 100 specimens yielded growth of S. aureus; on Mannitol Salt Agar, 91 yielded *S. aureus*; on Columbia Agar together with coagulase testing, 98 specimens were positive for *S. aureus*. There was one false positive on **CHROMagar Staph aureus** that turned out to be *Streptococcus agalactiae*. Upon restreaking the strain on **CHROMagar Staph aureus**, the colonies were violet rather than rose to mauve.
Among the known negative specimens, there were 5 cultures with violet or lilac colonies which were similar to *S. aureus* in color. However, they could be easily differentiated from *S. aureus* colonies (=rose to mauve) by the reader who was not too familiar with the colony colors of the different species growing on the medium (only a summary of colors and organisms occurring on the medium had been given to her before the study was begun).
The sensitivities of **CHROMagar Staph aureus** (based on rose to mauve colony color), Mannitol Salt Agar (based on colonies surrounded by yellow medium), and Columbia Agar (growth of typical *S. aureus* colonies together with coagulase testing) were 100%, 91%, and 98%. The specificity of **CHROMagar Staph aureus** was 98.5%.
On **CHROMagar Staph aureus**, the growth intensity was significantly more frequently higher than on MSA (p= 0.05); see Table 2.
CHROMagar Staph aureus can be used for a wide variety of specimens.

Table 2: Growth intensity of S. aureus isolates on CSA* as compared to MSA*

	Growth intensity					
	CSA = Col	CSA> Col	Col>CSA	CSA=MSA	CSA>MSA	MSA>CSA
Number of specimens	74	12	14	74	18**	8

* CSA, **CHROMagar Staph aureus**; MSA, Mannitol Salt Agar; Col, Columbia Agar with 5% Sheep Blood
** difference statistically significant (p= 0.05)

Agrifood Testing

BBL CHROMagar Staph aureus was validated by the AOAC Research Institute under the Performance Tested Methods Program. The medium was evaluated by an external reference laboratory, as well as internally at BD, for the recovery and enumeration of S. aureus in cooked roast beef, smoked salmon and shell eggs. The recovery and enumeration of S. aureus on **BBL CHROMagar Staph aureus** was compared to the AOAC and ISO reference plated medium, Baird-Parker Agar, using the recommended diluents at low, medium and high inoculum levels of S. aureus. After 24 h incubation, enumeration was performed on **BBL CHROMagar Staph aureus** and after 48 h on Baird-Parker Agar. Based on statistical analysis, no significant difference was found between the reference methods and the **BBL CHROMagar Staph aureus** method for any food type or contamination level, with the exception of a low-level smoked salmon sample. The low contamination level of smoked salmon demonstrated a statistical difference in internal testing using the ISO method; i.e., the **BBL CHROMagar Staph aureus** method at 24 h recovered more colonies ($\log_{10}$ = 2.04) than the ISO reference at 48 h ($\log_{10}$ = 1.64). The repeatability precision estimates of the **BBL CHROMagar Staph aureus** method were satisfactory. The correlation coefficients ranged from 92.6% to 99.4%, demonstrating good correlation for all contamination levels in all food types. Data

is summarized in Tables 1 and 2. No false-positive colonies were recovered from the food matrices using **BBL CHROMagar Staph aureus**. All mauve colonies were confirmed as S. aureus with no discrepancies. Additionally, 30 strains of S. aureus, including known enterotoxin-producing strains, and 37 non-S. aureus isolates were evaluated producing both a sensitivity and specificity of 100% on **BBL CHROMagar Staph aureus**.[11]

Table 1. Summary of AOAC and ISO External Testing of Cooked Roast Beef and Shell Eggs [1-3]

		AOAC		
	Inoculum Level	**Paired t-test or One-way ANOVA[a]**	**Repeatability (standard deviation)[b]**	**Square of linear correlation coefficient**
Cooked Roast Beef	Low	Not significant	0.398	96.0%
	Medium	Not significant	0.04	
	High	Not significant	0.062	
Shell Eggs	Low	Not significant	0.302	95.5%
	Medium	Not significant	0.089	
	High	Not significant	0.143	
		ISO		
	Inoculum Level	**Paired t-test or One-way ANOVA[a]**	**Repeatability (standard deviation)[b]**	**Square of linear correlation coefficient[c]**
Cooked Roast Beef	Low	Not significant	0.315	94.6%
	Medium	Not significant	0.045	
	High	Not significant	0.117	
Shell Eggs	Low	Not significant	0.341	92.6%
	Medium	Not significant	0.223	
	High	Not significant	0.135	

Footnotes see Table 2

Table 2. Summary of AOAC and ISO External and Internal Testing of Smoked Salmon

	Inoculum Level	**Paired t-test or One-way ANOVA[a]**		**Repeatability (standard deviation)[b]**		**Square of linear correlation coefficient[c]**	
AOAC Smoked Salmon	Low	Not significant	Not significant	0.132	0.271	99.4%	93.2%
	Medium	Not significant	Not significant	0.055	0.095		
	High	Not significant	Not significant	0.064	0.161		
ISO Smoked salmon	Low	Not significant	Significant[d]	0.158	0.227	98.7%	97.4%
	Medium	Not significant	Not significant	0.135	0.165		
	High	Not significant	Not significant	0.116	0.033		

a Paired t-test and one-way ANOVA analysis used to evaluate comparable performance of **BBL CHROMagar Staph aureus** versus the reference medium by comparing the mean of the $\log_{10}$ of the colony counts.
b Repeatability demonstrates **BBL CHROMagar Staph aureus** produces comparable results between the tests run on the same material and method.
c Square of linear correlation coefficient is used to evaluate precision of quantitative methods over different *S. aureus* counts.
d **BBL CHROMagar Staph aureus** recovered more colonies than the ISO reference method.

Limitations of the Procedure

Occasionally some strains of staphylococci, other than *S. aureus*, such as: *S. cohnii*, *S. intermedius*, and *S. schleiferi*, as well as corynebacteria and yeasts, may produce mauve-colored colonies at 24 h.[11] Differentiation of *S. aureus* from non-*S. aureus* can be accomplished by coagulase, other biochemicals or Gram stain. Resistant gram-negative bacilli, which typically appear as small blue colonies, may also break through.

Incubation beyond 24 h (clinical) and 28 h (food) is not recommended due to an increase in potential false positives. If incubation time is exceeded, mauve-colored colonies should be confirmed prior to reporting as *S. aureus*.
Incubation less than the recommended 20 h may result in a lower percentage of correct results being obtained.
Due to the natural golden pigment of some *S. aureus* strains, colony color may appear orange-mauve.

REFERENCES

1. AOAC Official Method 9755.55. *Staphylococcus aureus* in foods. Surface plating method for isolation and enumeration. 1976.
2. International Organization for Standards (ISO). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive Staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker agar medium, 1st ed., ISO 6888-1:1999.
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11. Data on file, BD Diagnostic Systems.

PACKAGING/AVAILABILITY

REF 257074	Ready-to-use Plated Media, cpu 20
REF 257099	Ready-to-use Plated Media, cpu 120

FURTHER INFORMATION

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