OPEN ACCESS Freely available online

Journal of Food: Microbiology, Safety & Hygiene

Research Article

An Evaluation on the Recovery of *Staphylococcus aureus* in CHROMagar *versus* Conventional Selective Agar in Food Sample Analysis

Lei Benitez*

Department of Food Microbiology, University of Hamburg, Hamburg, Germany

ABSTRACT

In this study, we tried to evaluated the performance criteria's of CHROMagar™ (CHROMagar – Paris FRANCE) in comparison with the conventional standard Baird Parker Agar as per the verification protocols.

The objective of the study is to adopt the CHROMagar in the enumeration of *Staphylococcus aureus* in food samples which requires a lesser incubation time in comparison with standards methods which in turn reduces the turnaround time of the test. Performance criteria's such as Precision, Accuracy, Specificity sensitivity and selectivity were assessed. CHROMagar is highly effective for the isolation and presumptive identification of *S. aureus* from food samples and that it compares favourably with conventional media. CHROMagar for *Staphylococcus aureus* was highly specific (97.94%), The specificity increased from 95.24% to 100% when the result was evaluated in conjunction with Mast latex Kit. In the present study, one of the biggest advantage observed was the shortest Incubation period of 24hrs with CHROMagar yields a highest recovery rate of 95% over the conventional media which required 48hrs of Incubation as per the standard method for food analysis.

Keywords: Rapid; *Staphylococcus aureus*; Accuracy; Food sample analysis; Recovery; Conventional method

INTRODUCTION

The development and implementation of novel diagnostic techniques has had a profound effect on microbiology laboratory services in recent decades. Current follow-up culture-based isolation of the food microbes can be labor-intensive and time-consuming due to the long incubation period. In some cases, such isolation is even unsuccessful due to lack of sufficiently selective isolation media and tedious biochemical tests. Recently, media allowing the detection of specific bacteria genus by the incorporation of chromogenic substrates have been introduced. Compared to conventional selective media chromogenic media had higher specificities, with more false-negative results.

Therefore, any new medium or method with the ability to streamline culture processing in a meaningful way, such as reducing technologist workload, improving result Turn Around Times (TATs), or reducing laboratory costs, would be welcomed and has the potential to have considerable laboratory impact. The conventional methods for detecting the foodborne bacterial pathogens present in food are based on culturing the microorganisms on agar plates followed by standard biochemical identifications [1]. Conventional methods are usually inexpensive and simple but these methods can be time consuming as they depend on the ability of the microorganisms to grow in different culture media such as pre-enrichment media, selective enrichment media and selective plating media. Problems associated with the direct culture of microbial species include difficulties in identification of pathogens, the time and expense involved in identifying and typing of pathogens isolates and the effect of selective media and/or selective isolation methods [2].

In recent years, the use of chromogenic media has become a key method for the rapid identification of microorganisms. These media detect key microbial enzymes as diagnostic markers for

Correspondence to: Lei Benitez, Department of Food Microbiology, AUniversity of Hamburg, Hamburg, Germany, E-mail: benit@lei.gr

Received: 18-Jun-2024, Manuscript No. JFMSH-24-32034; **Editor assigned:** 21-Jun-2024, PreQC No. JFMSH-24-32034 (PQ); **Reviewed:** 05-Jul-2024, QC No. JFMSH-24-32034; **Revised:** 12-Jul-2024, Manuscript No. JFMSH-24-32034 (R); **Published:** 19-Jul-2024, DOI: 10.35841/2476-2059.24.9.298.

Citation: Benitez L (2024) An Evaluation on the Recovery of *Staphylococcus aureus* in CHROMagar *versus* Conventional Selective Agar in Food sample analysis. J Food Microbial Saf Hyg. 9:298.

pathogens through the use of "chromogenic" substrates incorporated into a solid-agar-based matrix [3]. Recently in the United States of America and United Kingdom, and more recently in Australia, chromogenic substrate technologies have been accepted as standard methods [4-7]. Over the last two decades, there has been a rapid expansion in the development and commercial availability of chromogenic agar media for the detection of pathogenic bacteria and yeasts [3,8].

In contrast to conventional culture media, chromogenic media allow direct colony color-based identification of the pathogen from the primary culture. This reduces the need for subculture for further biochemical testing and hence the time until a result is obtained [9]. Chromogenic media are intended to correctly identify more-frequently occurring bacteria and yeasts or organism groups on primary culture with no further testing or a minimum number of confirmatory tests [10]. Target enzymes hydrolyze chromogenic substrates generating colored products which allow for easy identification of specific organisms [3,11]. This facilitates the differentiation of species within polymicrobial cultures and the targeting of pathogens with high specificity. When specific pathogens are targeted, selective agents such as antibiotics are employed to limit the number of species able to grow. Most media rely upon the inclusion of indoxylic substrates in order to generate colonies with contrasting colors. For example, the release of green and red chromogens from two distinct substrates can result in the formation of green, red or purple colonies depending on whether one or both enzyme activities are present [12]. Pathogens may therefore be differentiated from commensal bacteria by their possession of either one or both enzymes [12,13].

The purpose of this study is to evaluate the accuracy, specificity, sensitivity and selectivity of the CHROMagar media for *Staphylococcus aureus* when compared with conventional selective agar as specified in international standards in different types of food. In addition, the limit of detection for the chromogenic media was investigated in this study.

MATERIALS AND METHODS

Strains

A total 10 bacterial strains included in the study which includes lab isolates from natural samples, isolates from proficiency samples and quality control strains from National Collection of Type Cultures (NCTC) and American Type Culture Collection (ATCC). All strains were identified by VITEK 2 Compact system or respective biochemical tests (Table1).

Culture media

Chromogenic media was purchased as powdered media from CHROMagar™ through Biomed Dubai. The plates were prepared according to the manufacturers' instructions. Briefly, weight as specified by grams of dehydrated media were reconstituted in 1 liter of distilled water. They were brought to boil by repeated heating's and then cooled in a water bath at 45°C by swirling and stirring. Then 20 ml of the media were dispensed into petri dishes. They were kept in the dark at 4°C and used for further studies.

Baird Parker Agar (Biolife Italiana Srl °491116) for *Staphylococcus aureus* was used as conventional agar as per the standard ISO method. All medias were prepared as per manufacturer's instructions and stored at 4°C and used for further studies.

Identification system

All isolates were identified using VITEK 2 compact system using

Table 1: Parameters of inclusivity strains and exclusivity strains.

Parameters	*Staphylococcus aureus*
Inclusivity strains	*Staphylococcus aureus* NCTC 6538
	Staphylococcus aureus ATCC 25923
	Staphylococcus aureus -Lab Isolate Ref# PT PHE-562
	Staphylococcus aureus ATCC 259213
	Staphylococcus aureus NCTC 6571
	Staph aureus- # LAB Isolate 1 from natural sample
Exclusivity strains	*Pseudomonas aeruginosa ATCC 25668*
	Enterococcus faecalis ATCC 19433
	Staphylococcus xylosus ATCC 29971
	Staphylococcus epidermis ATCC 12228

respective cards such as GN for Salmonella, Cronobacter sakazakii. GP cards used for *Staphylococcus aureus* and Listeria monocytogenes identification. Bacillus Identification Card (BCL) VITEK cards used to identify Bacillus species.

Matrixes

Different matrixes were used in the study based on the strains of choice and the regulatory requirements. Details as mentioned on below Table 2.

Inoculum preparation

Purified isolates of all strains were prepared by sub culturing the reference strains and lab isolates onto plate count agar and incubation at 37°C for 24 hrs. A standard turbid preparation of 0.5 for each microbes prepared with McFarland standard using Densi Check plus (Biomeriux). Further serial dilutions conducted to achieve the desired concentration of inoculum. Three different inoculum levels prepared for each strains (Low (5-10 cfu), Medium (50-100) and High (1000 and above)). After appropriate dilutions, each desired dilution of each of the isolates was plated onto the five chromogenic media and onto standard agar, which served as a control.

Food sample preparation

A wide variety of food samples selected based on the UAE.S GSO 1016/2017, microbiological criteria for food stuffs. Details of the matrixes selected as shown in Table 2.

Test method

For *Staphylococcus aureus*, quantitative techniques embraced to identify the sensitivity, specificity and accuracy of test methods. Sample preparation was performed based on ISO-6887-2 (Table 3).

For quantitative analysis, 20 gm of sample weighed in a sterile stomacher bag for each food matrix aseptically in quadruplicates. An initial 1:10 dilution was prepared for sample by adding 180 ml of maximum recovery diluent (Biolife EG7204) in each. Among the four parts weighed 3 were spiked with low, high and medium level of inoculum of each organism under study and one sample kept as control. Homogenize the sample using a stomacher (Smasher, AES #44497426) for 30 seconds, Later on 0.1 ml of Inoculum were added to the CHROMagar plates and corresponding standard control plates as mentioned in (Table 2), and incubated for 24 hrs ± 2 h at 37 ± 1°C for *Staphylococcus aureus*. 25 gm of each sample weighed in a sterile stomacher bag for each food matrix aseptically in quadruplicates. An initial 1:10 dilution was prepared for sample by adding 225 ml of maximum recovery diluent in each. Among the four parts weighed 3 were spiked with low, high and for quantitative analysis, colonies with typical morphology for each plates were counted and recorded separately. 5 presumptive colonies of each plates were purified in plate count agar for further confirmation. Confirmation was done by conventional techniques based on International Standard Organization (ISO) standards and on VITEK 2 Compact-Biomeriux-VK2C5407 according to the manufacturer's instructions (Table 4).

Table 2: Microbiological criteria for food stuffs and details of the matrixes selected for each parameter.

Type of matrixes selected for the study	No. of samples analysed
Fermented milk products-dairy	7
Dairy	8
Environment swabs	4
Frozen to be reheated before consumption	7
Candy	8
Beverages	5
Bakery	10
Ready to eat meals	10
Fresh fruits and vegetables	10
Sauce	5

Table 3: Comparison between CHROMagar and standard method media.

Parameter	CHROMagar	Standard method media
Staphylococcus aureus	CHROMagar™ *S. aureus* #TA672 #P000927	Baird Parker agar-biolife EM7507 (with egg yolk tellurite-3EH062)

Table 4: Confirmation techniques and kits used for identifying *Staphylococcus aureus*.

Parameter	Confirmation techniques	Kit
Staphylococcus aureus	Latex coagulase test	Mast-360815/371435
	Automated biochemical test	GP card-Lot-2420689403-VITEK 2 compact

Statistical analysis

Results were analysed statistically to identify accuracy, specificity, sensitivity and selectivity of alternative methods. Precision was calculated trough the comparison of test results with the results of reference method as well as with proficiency sample. Assessment of the sensitivity, specificity and selectivity of CHROMagar was achieved through the identification of typical colonies isolates from CHROMagar.

RESULTS AND DISUSSION

In total 97 samples were analyzed, On CHROMagar *S. aureus*, all typical colonies appeared as mauve colonies after 24 h of incubation compared with grey to black colonies with and without zone on Baird Parker agar after incubation for 48 h. Most of the competitive flora are inhibited and some appeared as blue color colonies in the CHROMagar. False positive result was observed with *Staphylococcus warneri* which appeared as mauve coloured and indistinguishable from the *Staphylococcus aureus* on CHROMagar. But the strain was identified later as coagulase negative in Latex Agglutination Kit for *S. aureus* (MASTSTAPH).

Precision

Precision (accuracy/trueness) of the test method using CHROMagar was determined by estimating the mean percentage recovery of test results in comparison with Baird Parker agar. A mean recovery of 95 % shows the high accuracy of test results, and therefore there is no indication of significant bias. At all levels of inoculums from low to high the recovery was more or less similar (Table 5).

Accuracy, sensitivity, specificity and selectivity

The outcomes of the analyses of the data show that for *S. aureus*, the CHROMagar™ method is sensitive and specific. 6 strains of *S. aureus* tested were detected by the alternative method. Of the 5 non-target strains tested, cross-reactivity observed only with *Staphylococcus warneri* in the alternative method. All *S. aureus* had a characteristic profile on CHROMagar™. The confirmation was done by coagulase test Mast Latex Agglutination Kit (MLAK). Colonies showing typical morphology and negative coagulase test were subjected for VITEK 2 confirmation and identified as *Staphylococcus warneri* (Table 6-9).

Table 5: Comparison of bacterial recovery rates from various food samples using BPA and CHROMagar *S. aureus* media.

Sample Ref		Bisphenol A (BPA)		CHROMagar *S. aureus*		% of recovery
		cfu/G	Log_{10}	cfu/G	Log_{10}	
64998	Roasted coffee	30	1.48	28	1.45	97.97
64994	Akawi cheese	35	1.54	25	1.4	90.91
64990	Processed cheese	21	1.32	20	1.3	98.48
64989	Chilli sauce	3400	3.53	3000	3.48	98.58
64982	Fresh veg	2300	3.36	2600	3.41	101.49
64980	Fresh corn	2000	3.3	2200	3.34	101.21
64979	Fresh lemon	19,000	4.28	15000	4.18	97.66
64976	Orange juice	18500	4.27	16600	4.22	98.83
64950	Spices	17600	4.25	17000	4.23	99.53
64951	Spices	7	0.85	5	0.7	82.35
64952	Spices	8	0.9	7	0.85	94.44
64948	Fresh juice	6	0.78	4	0.6	76.92
64679	Chocolate milk	160	2.2	175	2.24	101.82
64678	Cream caramel	200	2.3	188	2.27	98.7
Average		-	2.45	-	2.41	95.64

Table 6: Confirmation of mauve colored colonies in various strains of *staphylococcus aureus* using LATEX.

Inclusivity			
Strains	**Expected results**	**Actual results**	**Confirmation by LATEX**
Staphylococcus aureus NCTC 6538	Mauve colored colonies	Mauve colored colonies	Positive
Staphylococcus aureus ATCC 25923	Mauve colored colonies	Mauve colored colonies	Positive
Staphylococcus aureus-Lab Isolate Ref# PT PHE-562	Mauve colored colonies	Mauve colored colonies	Positive
Staphylococcus aureus ATCC 259213	Mauve colored colonies	Mauve colored colonies	Positive
Staphylococcus aureus NCTC 6571	Mauve colored colonies	Mauve colored colonies	Positive
S. aureus #LAB Isolate 1 from natural sample	Mauve colored colonies	Mauve colored colonies	Positive

Table 7: Exclusivity testing of various bacterial strains on selective media, comparing expected colony morphology with actual results and confirmation by LATEX agglutination test.

Exclusivity			
Strains	**Expected results**	**Actual results**	**Confirmation by LATEX**
Pseudomonas aeruginosa ATCC 25668	Atypical colonies	No growth	Negative
Enterococcus faecalis ATCC 19433	Atypical colonies	Colorless colonies	Negative
Staphylococcus xylosus ATCC 29971	Atypical colonies	Blue colored colonies	Negative
Staphylococcus epidermis ATCC 12228	Atypical colonies	Blue colored colonies	Negative
S. warneri-# LAB isolate 2 from natural sample	Atypical colonies	Mauve colored colonies	Negative

Table 8: Results of inclusivity and exclusivity tests.

Staphylococcus aureus-CHROMagar				
a	b	c	b	Total
55	40	2	0	97

Note: (a): Total number of positive samples/typical colonies shown as positive in CHROMagar and confirmed as positive (true positives); (b): Total number of negative samples/atypical colonies confirmed as positive (false negatives); (c): Total number of typical colonies/positive samples and confirmed as negative (false positives); (d): Total number of atypical colonies/negative samples and confirmed as negatives (true negatives). Sensitivity: a/(a+b); Specificity: d/(c + d); Selectivity: Log10 ((a+c)/(a+b+c+d)); False positive rate: c/(a+c); Efficiency (E): Which gives the fraction of typical colonies correctly assigned, was calculated as E=(a+d)/(a+b+c+d).

Table 9: Performance metrics of the model.

Variables	Values
Relative accuracy/efficiency	97.94
Relative sensitivity	100
Relative specificity	95.24
Selectivity	-2
False positive rate	0.04%
False negative rate	0.00%
Efficiency	97.94%

CONCLUSION

Out of 97 total samples tested for exclusivity and inclusivity studies, 55 target colonies shown typical characteristics and confirmed as *Staphylococcus aureus* by mast staph latex agglutination kit. Most of the non-target strains suppressed and some shown atypical colonies on CHROMagar. However, the possibilities of coagulase negative *Staphylococcus* species shows typical colonies in CHROMagar still exists and can be rectified by adding the confirmation step of coagulase test. We conclude that CHROMagar is highly effective for the isolation and presumptive identification of *S. aureus* from food samples and that it compares favorably with conventional media. The preparation and use of CHROMagar *S. aureus* is much less labor intensive than conventional methods and requires fewer reagents for confirmation of suspect colonies of *S. aureus* as the cases of false positive and negative were less. CHROMagar for *Staphylococcus aureus* was also highly specific (97.94%), with few other species resembling *S. aureus*. The specificity increased from 95.24% to 100% when the result was evaluated in conjunction with mast latex kit. In the present study, one of the biggest advantage observed was the shortest Incubation period of 24 hrs with CHROMagar yields a highest recovery rate of 95% over the conventional media which required 48 hrs of Incubation as per the standard method for food analysis. The selectivity was -2 which was considerably better than the guidance value of -1 suggested by ISO/TR 13843 for colony count methods.

REFERENCES

1. Mandal PK, Biswas AK, Choi K, Pal UK. Methods for rapid detection of foodborne pathogens: An overview. Am J Food Technol. 2011;6(2):87-102.
2. Porter J, Edwards C, Pickup RW. Rapid assessment of physiological status in *Escherichia coli* using fluorescent probes. J Appl Bacteriol. 1995;79(4):399-408.
3. Perry JD, Freydiere AM. The application of chromogenic media in clinical microbiology. J Appl Microbiol. 2007;103(6):2046-2055.
4. Anon. AS/NZS 4276.21: Examination for coliforms and *Escherichia coli*-determination of Most Probable Number (MPN) using enzyme hydrolysable substrates. North Sydney, NSW, Australia: Standards Australia. 2005.
5. Brenner KP, Rankin CC, Roybal YR, Stelma Jr GN, Scarpino PV, Dufour AP. New medium for the simultaneous detection of total coliforms and *Escherichia coli* in water. Appl Environ Microbiol. 1993;59(11):3534-3544.
6. Oshiro R. Method 1604: Total coliforms and *Escherichia coli* in water by membrane filtration using a simultaneous detection technique (MI Medium). Washington, DC: US Environmental Protection Agency. 2002.
7. Hallas G, Giglio S, Capurso V, Monis PT, Grooby WL. Evaluation of chromogenic technologies for use in Australian potable water. J Appl Microbiol. 2008;105(4):1138-1149.
8. Aguilera-Arreola MG, Portillo-Muñoz MI, Rodríguez-Martínez C, Castro-Escarpulli G. Usefulness of Chromogenic CromoCen® AGN agar medium for the identification of the genus *Aeromonas*: Assessment of faecal samples. J. Microbiol Methods. 2012;90(2): 100-104.
9. Malhotra-Kumar S, Abrahantes JC, Sabiiti W, Lammens C, Vercauteren G, Ieven M, et al. Evaluation of chromogenic media for detection of methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol. 2010;48(4):1040-1046.
10. Rank EL. Chromogenic agar media in the clinical, food, and environmental testing arenas, part I. Clin Microbiol Newsl. 2012;34(6):43-47.
11. Manickam K, Karlowsky JA, Adam H, Lagacé-Wiens PR, Rendina A, Pang P, et al. CHROMagar Orientation medium reduces urine culture workload. J Clin Microbiol. 2013;51(4):1179-1183.
12. Al-Wasify RS. Sensitivity and specificity of chromogenic media for detection of some pathogens in water. Int J Environ Sustain. 2013;2(1):1-9.
13. Orenga S, James AL, Manafi M, Perry JD, Pincus DH. Enzymatic substrates in microbiology. J Microbiol Methods. 2009;79(2): 139-155.