A Comparative Evaluation of ChromID MRSA Agar and Brilliance MRSA 2 Agar for detection of MRSA in clinical samples

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Application Notes

Key Words
MRSA, screening plate, infection control, 24 hour result, healthcare associated infection

Goal
Evaluate the performance of Oxoid™ *Brilliance*™ MRSA 2 Agar (Thermo Fisher Scientific™) alongside ChromID MRSA Agar (bioMérieux).

Abstract
A study was conducted to evaluate the performance of Oxoid *Brilliance* MRSA 2 Agar (Thermo Fisher Scientific) alongside ChromID MRSA Agar (bioMérieux). Four hundred and eighty-four routine MRSA screening samples were processed using both plates. Presumptive MRSA colonies were confirmed by our routine methods.

Brilliance MRSA 2 Agar equalled or improved on the performance of ChromID MRSA Agar with regard to sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and showed a statistically significant improvement in terms of sensitivity. The reduced incubation time required for *Brilliance* MRSA 2 Agar (18-24 hrs, as recommended by the manufacturer) allows infection control procedures to be adopted earlier.

In the light of recent concerns regarding reliability of expensive rapid MRSA PCR investigation, *Brilliance* MRSA 2 Agar may also improve the cost effectiveness of MRSA infection control measures.

Introduction
MRSA is a major cause of nosocomial and community-acquired infection worldwide. Screening of high risk populations and isolation of carriers are cost effective measures in prevention of transmission in hospital when screening results are reliable and readily available.[1]

Methods of detecting MRSA in clinical samples ideally should be cost effective, have a high sensitivity and specificity, combined with a short time to reporting of results (2). Various chromogenic media have been used in Altnagelvin Area Hospital Microbiology Laboratory to screen for MRSA in recent years. These include ORSAB (2001), MRSA ID (2005) and currently ChromID MRSA Agar (bioMérieux). The choice of screening media used by Altnagelvin Area Hospital has previously changed when a new product has demonstrated improved performance.

Brilliance MRSA 2 Agar is a chromogenic medium which has been enhanced over that of its original formulation in two ways. New inhibitory components in the medium inhibit the growth of non-target organisms. In addition, those organisms that do grow are more easily distinguished as distinctive blue MRSA colonies through inclusion of a novel pink counter-stain, further improving ease of interpretation.

The purpose of this study was to evaluate the performance of *Brilliance* MRSA 2 Agar compared to ChromID MRSA Agar.

The Study
Four hundred and eighty-four routine MRSA screening swabs were processed over a period of approximately two weeks. Most were nasal (n=249), axilla and groin swabs (n=171), but others such as catheter, wound and other body site swabs were also included. Swabs were received from routine admissions as well as Intensive Care, High

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Dependency and Special Care Baby Units and on admission from previously identified MRSA carriers.

Swabs were broken into 1ml of sterile distilled water and thoroughly emulsified using a vortex mixer. One hundred µl of this inoculum was immediately transferred to the primary bed of a *Brilliance* MRSA 2 Agar and ChromID MRSA Agar. Using a 10ul loop, a portion of the inoculum was streaked onto each plate using the diminishing streak technique.

All plates were incubated aerobically at 36±1°C. *Brilliance* MRSA 2 Agar was inspected for blue colonies after 20±1 hrs. incubation and ChromID MRSA Agar inspected for green colonies after 24 hrs. incubation. As per manufacturer's instructions, ChromID MRSA Agar that showed no growth after 24 hrs. was incubated for a further 24 hours at 36±1°C then re-examined. *Brilliance* MRSA 2 Agar does not require the re-incubation step.

Quantity of growth was noted (+ = growth in primary bed only, ++ = growth in primary bed and 1st streak, +++ = growth in primary bed, 1st and 2nd streak and ++++ = growth in primary bed, 1st, 2nd and 3rd streak). Any variation in colony size was also noted.

Typical MRSA colonies were picked from *Brilliance* MRSA 2 Agar and ChromID MRSA Agar plates, streaked onto Columbia Blood Agar (CBA) (Thermo Fisher Scientific) plates and incubated overnight aerobically at 36±1°C. Colonies were confirmed as MRSA using Prolex™ Staph Xtra Latex Kit (Pro-Lab Diagnostics), Oxoid Penicillin Binding Protein (PBP2') latex agglutination test (Thermo Fisher Scientific), ID and AST panels on Phoenix Automated Microbiology System (Becton Dickinson) and Oxoid 30 µg cefoxitin antimicrobial susceptibility testing discs (Thermo Fisher Scientific). MRSA classification was determined by resistance to cefoxitin on the Phoenix Automated Microbiology System and when using antimicrobial susceptibility testing discs according to European Committee on Antimicrobial Sensitivity Testing (EUCAST) guidelines.

Results

A total of 41 samples from 35 patients were confirmed positive for MRSA according to one or both chromogenic MRSA screening media, representing the expected prevalence rate of approx 8%.

In samples where MRSA colonies were found on both *Brilliance* MRSA 2 Agar and chromID MRSA Agar, no appreciable difference in colony numbers or size was observed. Much more consistent coloration of MRSA colonies was achieved on *Brilliance* MRSA 2 Agar compared to ChromID MRSA Agar, due to the distinctive intense blue colony appearance on *Brilliance* MRSA 2 Agar. The blue colour was confined only to the colonies on *Brilliance* MRSA 2 Agar and did not affect the surrounding agar. However green colouration was frequently found surrounding both MRSA and non-MRSA colonies on ChromID MRSA Agar, even when individual colonies were not green. Non-MRSA colonies on *Brilliance* MRSA 2 Agar were either white or the same colour as the medium, smaller than on ChromID MRSA Agar and easily distinguished compared to non-MRSA colonies on ChromID MRSA Agar.

Sensitivity of *Brilliance* MRSA 2 Agar was statistically significantly higher (P<0.0001; McNemar test) than ChromID MRSA Agar after 24 hr. incubation (45.2% versus 97.6%); see table 1. Of the 40 samples which were positive on both media, only 19 (48%) were positive at 24 hours on ChromID MRSA Agar. Hence the remaining 52% of positive MRSA results were available 24 hours earlier when using on *Brilliance* MRSA 2 Agar compared to ChromID MRSA Agar.

One MRSA strain grew on ChromID MRSA Agar at 48 hours but not on *Brilliance* MRSA 2 Agar after 24 hours.

Table 1. Performance summary of *Brilliance* MRSA 2 Agar versus ChromID MRSA Agar.

Performance (%)	*Brilliance* MRSA 2 Agar	ChromID MRSA Agar	
		18-24 hr incubation	48 hr incubation
Sensitivity	97.6% (95% CI = 96.2-99.0%)	45.2% (95% CI = 40.8-49.6%)	97.6% (95% CI = 96.2-99.0%)
Specificity	99.8% (95% CI = 99.4-100%)	100% (95% CI = 73.0-100%)	99.5% (95% CI = 98.9-100%)
PPV	97.6% (95% CI = 96.2-99%)	100% (95% CI = 73.0-100%)	95.3% (95% CI = 93.4-97.2%)
NPV	99.8% (95% CI = 99.4-100%)	95.0% (95% CI = 93.1-96.9%)	99.8% (95% CI = 99.4-100%)

This was determined as resistant to cefoxitin by Phoenix, but was classified as sensitive to oxacillin by M.I.C.Evaluator™ strip (Thermo Fisher Scientific). It was also found to be PBP2' positive. One MRSA strain grew on *Brilliance* MRSA 2 Agar but not on ChromID MRSA Agar. This was also determined as resistant to cefoxitin but sensitive to oxacillin. This was also found to be PBP2' positive.

One MSSA strain produced small but distinctly blue-coloured colonies on *Brilliance* MRSA 2 Agar but did not grow on ChromID MRSA Agar. This isolate was negative for PBP2'. Two false positive results showing typical green colonies on ChromID MRSA Agar tested negative by Staph Xtra latex kit. No further testing was done on these isolates as they were recorded as coagulase negative staphylococci (CNS) and discarded as per our normal protocol.

Discussion

In previous studies involving *Brilliance* MRSA 2 Agar and ChromID MRSA Agar, the specificity of both media has been reported as consistently high (3,4,5,6,7,8). In agreement with all of these articles, we found that the sensitivity, specificity, NPV and PPV of *Brilliance* MRSA 2 Agar are similar to ChromID MRSA Agar, but the specificity of *Brilliance* MRSA 2 Agar at 20±1 hrs. is much superior to ChromID MRSA Agar.

The sensitivity of ChromID MRSA Agar has previously been reported lower than seen in this study, at 75.0% after 42 hours (12) and 73.0% at 48 hours (8). Sensitivity of ChromID MRSA Agar at 18 hours of 45.6% reported in (12) compared with 45.2% at 20 hours in this study.

Our results demonstrate an increase in true positive and true negatives obtained, in addition to a decrease in false positive and false negative results when using *Brilliance* MRSA 2 Agar compared to our existing method.

Recently developed rapid nucleic acid amplification methods for MRSA such as the GeneXpert System (Cepheid), which is in use in our laboratory, offer an alternative to conventional culture methods and can substantially reduce turnaround time, typically to 2-4 hours. These time reductions have been associated with reduced transmission of MRSA (10 ,11). However a recent report has shown that the GeneXpert System can produce false positive MRSA results in *Staphylococcus aureus* lacking the *mecA* gene (13). A similar ongoing study in this laboratory reflects these findings (data not published). The infection control implications of wrongly identified MRSA positive patients are likely to include unnecessary decolonisation of patients and overuse of antibiotics. In a study involving the use of the rapid GeneOhm MRSA assay (Becton Dickinson) it was found that rapid results led to more efficient use of isolation resources, although no evidence was found of significant reduction in MRSA transmission (14). The report concluded that when compared with existing screening methods for MRSA, the increased cost of rapid tests are unlikely to be justified.

Conclusion

In this study *Brilliance* MRSA 2 Agar performed as least as well as ChromID MRSA Agar with regard to specificity, PPV and NPV. The sensitivity of *Brilliance* 2 MRSA Agar (97.6%) was markedly better than that of ChromID MRSA Agar (45.2%) at 20±1 hrs. Fifty two percent of all positive MRSA results and all negative results were available 24 hours earlier using *Brilliance* MRSA 2 Agar.

Distinctive blue colonies on *Brilliance* MRSA 2 Agar plates make interpretation straightforward, facilitating isolation of colonies for confirmatory testing. A reduction in false positive results seen on *Brilliance* MRSA 2 Agar (compared to ChromID MRSA Agar) reduces the requirement for confirmatory testing. Examination of *Brilliance* MRSA 2 Agar is only necessary at 20±1 hours, eliminating the need to re-incubate and re-examine plates at 48 hours as in our current method.

Our results suggest that in addition to contributing to improving the efficiency of infection control procedures, *Brilliance* MRSA 2 Agar can act as a cost-effective routine screening method for MRSA, particularly when doubts exist regarding the reliability of expensive rapid alternative methods. On completion of this study the decision was taken to adopt *Brilliance* MRSA 2 Agar as our routine MRSA screening method.

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