Comparative evaluation of chromID MRSA agar and *Brilliance* 2 MRSA agar for detection of MRSA in clinical samples

P. McELHINNEY*, C. MILLAR* and E. SCOPES†

*Microbiology Department, Altnagelvin Area Hospital, Londonderry, Northern Ireland; and †Thermo Fisher Scientific, Basingstoke, UK

Methicillin-resistant *Staphylococcus aureus* (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 the microbiology laboratory at Altnagelvin Area Hospital 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 has previously changed when a new product has demonstrated improved performance.

Brilliance MRSA 2 agar is a chromogenic medium that has been enhanced over 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 pink counterstain, further improving ease of interpretation. The purpose of this study is to evaluate the performance of *Brilliance* MRSA 2 agar compared to chromID MRSA agar.

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 from intensive care, high dependency and special care baby units, and on admission from previously identified MRSA carriers.

Swabs were broken into 1 mL sterile distilled water and thoroughly emulsified using a vortex mixer. A 100 µL sample of this inoculum was immediately transferred to the primary bed of *Brilliance* MRSA 2 agar and chromID MRSA agar plates. Using a 10 µL 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 h incubation and chromID MRSA agar inspected for green colonies after 24-h incubation, following the manufacturers' instructions. chromID MRSA agar showed no growth after 24 h and was incubated for a further 24 h 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 first streak, +++: growth in primary bed, first and second streak, and ++++: growth in primary bed, first, second and third 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 on Columbia blood agar (CBA; Thermo Fisher Scientific) plates and incubated overnight aerobically at 36±1°C. Colonies were confirmed as MRSA using the Prolex Staph Xtra latex kit (Pro-Lab Diagnostics), Oxoid Penicillin Binding Protein (PBP2′) latex agglutination test (Thermo Fisher Scientific), ID and AST panels on the Phoenix automated microbiology system (Becton Dickinson) and Oxoid 30 µg cefoxitin antimicrobial susceptibility testing discs (Thermo Fisher Scientific). Classification of MRSA was determined by resistance to cefoxitin on the Phoenix system and when using antimicrobial susceptibility testing discs according to the European Committee on Antimicrobial Sensitivity Testing (EUCAST) guidelines.

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 both on *Brilliance* MRSA 2 agar and chromID MRSA agar, no appreciable differences in colony numbers or size were observed. Much more consistent colouration of MRSA colonies was achieved on *Brilliance* MRSA 2 agar compared to chromID MRSA agar, due to the distinctively 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 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-h incubation (45.2% versus 97.6%; Table 1). Of the 40 samples that were positive on both media, only 19 (48%) were positive at 24 h on chromID MRSA agar; hence, the remaining 52% of positive MRSA results were available 24 h earlier when using *Brilliance* MRSA 2 agar compared to chromID MRSA agar.

One MRSA strain grew on chromID MRSA agar at 48 h but not on *Brilliance* MRSA 2 agar after 24 h. This was determined as resistant to cefoxitin by the Phoenix system, but was classified as sensitive to oxacillin using an 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, and was also found to be PBP2′-positive.

One methicillin-sensitive *S. aureus* (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 the Staph Xtra latex kit. No further testing was performed on these isolates as they were

Correspondence to: Peter McElhinney
Altnagelvin Area Hospital, Western Health and Social Care Trust, Glenshane Road, Londonderry, Northern Ireland, UK
Email: peter.mcelhinney@westerntrust.hscni.net

Table 1. Performance summary of *Brilliance* MRSA 2 agar versus chromID MRSA agar.

	Brilliance MRSA 2 agar	chromID MRSA agar	
		18-24 h incubation	48 h incubation
Sensitivity	97.6%	45.2%	97.6%
	(95% CI: 96.2–99.0%)	(95% CI: 40.8–49.6%)	(95% CI: 96.2–99.0%)
Specificity	99.8%	100%	99.5%
	(95% CI: 99.4–100%)	(95% CI: 73.0–100%)	(95% CI: 98.9–100%)
PPV	97.6%	100%	95.3%
	(95% CI: 96.2–99%)	(95% CI: 73.0–100%)	(95% CI: 93.4–97.2%)
NPV	99.8%	95.0%	99.8%
	(95% CI: 99.4–100%)	(95% CI: 93.1–96.9%)	(95% CI: 99.4–100%)

recorded as coagulase-negative staphylococci (CNS) and discarded following the laboratory's normal protocol.

In previous studies involving *Brilliance* MRSA 2 agar and chromID MRSA agar, the specificity of both media has been reported as consistently high.[3–7] Similarly, the present study found that the sensitivity, specificity, negative predictive vale (NPV) and positive predictive vale (PPV) of *Brilliance* MRSA 2 agar were similar to chromID MRSA agar, but the specificity of *Brilliance* MRSA 2 agar at 20±1 h proved superior to chromID MRSA agar.

The sensitivity of chromID MRSA agar has been reported to be lower than that seen in this study, at 75% after 42 h[11] and 73% at 48 h.[7] Sensitivity of chromID MRSA agar at 18 h has been reported as 45.6%,[11] compared with 45.2% at 20 hours in this study.

The present results demonstrate an increase in true positives and true negatives obtained, in addition to a decrease in false-positive and false-negative results when using *Brilliance* MRSA 2 agar compared to the laboratory's existing method.

Recently developed rapid nucleic acid amplification methods for MRSA (e.g., GeneXpert, Cepheid) in use in the authors' laboratory offer an alternative to conventional culture methods and can substantially reduce turnaround time, typically to 2–4 h. These time reductions have been associated with reduced transmission of MRSA.[9,10] However, a recent report has shown that the GeneXpert system can produce false-positive MRSA results in *S. aureus* that lack the *mecA* gene.[12] A similar ongoing study in the authors' laboratory reflects these findings (data not shown).

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 there was no evidence of significant reduction in MRSA transmission.[13] The report concluded that, when compared to existing screening methods for MRSA, the increased cost of rapid tests is unlikely to be justified.

In the present study, *Brilliance* MRSA 2 agar performed at least as well as chromID MRSA agar in terms of 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 h. In addition, 52% of all positive MRSA results and all negative results were available 24 h 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 with *Brilliance* MRSA 2 agar (compared to chromID MRSA agar) reduces the need for confirmatory testing. Examination of *Brilliance* MRSA 2 agar is only necessary at 20±1 h, eliminating the need to re-incubate and re-examine plates at 48 h.

These 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 doubt exists regarding the reliability of expensive rapid alternative methods. On completion of this study, the decision was taken by the authors' laboratory to adopt *Brilliance* MRSA 2 agar for routine MRSA screening. □

Brilliance *MRSA 2 agar was provided by Thermo Fisher Scientific for inclusion in this study.*

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Observations on the number of saliva cotinine positives over a nine-year period

S. MORTLOCK, F. McLEAN, E. JONES and S. WILLIS
Department of Immunology and Molecular Biology, Quest Diagnostics, Heston, Middlesex, UK

In the past, many insurance companies would take customers at their word when completing application forms. With the rising cost of healthcare, these companies now increasingly insist on independent checks to identify fraudulent claims.[1,2] Companies are especially interested in using laboratory tests to identify controllable activities such as smoking, which in turn helps them to set insurance premiums correctly. Smokers may have to pay up to twice as much for some types of insurance, especially life assurance, because they face a higher risk of ill health and premature death. Passive smoking (usually seen in people who live with smokers) also has an impact on health.[3,4]

When nicotine from tobacco smoke is taken into the lungs and enters the bloodstream, it is metabolised in the liver and converted to cotinine by enzymes such as cytochrome P450 2A6, then eventually excreted in the urine as trans-3'-hydroxycotinine.[5,6] Cotinine diffuses easily from the blood into saliva, and salivary and blood levels have been shown to correlate.[7,8] Cotinine in saliva has a longer half-life than nicotine (greater than 10 hours), and is a specific and sensitive marker for determining exposure to tobacco and nicotine both in smokers and passive smokers.[9]

Correspondence to: Dr. Stephen Mortlock
Department of Molecular Biology, Quest Diagnostics, Cranford Lane, Heston, Middlesex TW5 9QA, UK
Email: stephen.x.mortlock@questdiagnostics.com

The levels of cotinine considered significant have changed over the years. Previous cut-off levels were 10 ng/mL: anything greater was considered as positive and consistent with smoking, and anything less was considered negative.[10] In more recent years, this cut-off has been changed to 13 ng/mL and now includes an equivocal range of 7–13 ng/mL, to try to discriminate between active smokers, passive smokers and non-smokers.[11–13]

Up to March 2011, Quest Diagnostics provided services for a number of insurance companies to test potential life insurance clients for smoking by measuring salivary cotinine levels as part of the application criteria. All samples were self-collected by insurance applicants using the Omin-SAL collection device. The collected samples were processed and assayed using a standard saliva cotinine assay (Cozart oral fluid microplate enzyme immunoassay [EIA]).

Over a nine-year period, 39,651 saliva samples were assayed for cotinine. Some of the samples either did not have the optimal amount of fluid in the specimen tube (2.1 mL) or lacked fluid completely. Of the samples received, 104 (0.3%) had to be discarded because of the absence of buffer in the collection tube.

Of the samples tested, the majority (93.6%) were reported as negative (<7 ng/mL); only 2433 (6.1%) samples were repeatedly positive (>13 ng/mL) (Table 1). There were also 80 samples that fell into the equivocal range (7–13 ng/mL).

From the 1970s onwards, smoking prevalence fell rapidly until the mid-1990s. Since then, the rate has continued to fall slowly, and in 2007 around a fifth (22%) of men (aged 16 and over) were reported as cigarette smokers. The rate remained stable between 2007 and 2009, but fell to 21% in 2010.[14,15] In 2007, smoke-free legislation was implemented in England, making virtually all enclosed public places and workplaces smoke-free.[16]

Over a nine-year period (2003–2011), the laboratory tested nearly 40,000 self-taken saliva samples for cotinine level. Some of these had to be discarded as they were unsuitable for testing due to lack of buffer; usually the container arrived with no buffer and only a dried, slightly blue collector strip. The assumption was that the buffer had leaked out during transport or the patient had discarded it, not realising its importance. Of the remaining samples, the majority were shown to be negative for cotinine, and only 6% were positive.

What was interesting was that, from 2003 to 2008, the positivity rate remained fairly constant (average: 6.3%), but in 2009 and 2010 this figure fell to 4.7%. Although the number of data points is small, a Fisher two-tailed test gave a P value of 0.0043, indicating a significant reduction in positivity rate. Further analysis of the number of positives for 2011 onwards is needed to see if this represented a true fall or simply a reflection of the reducing number of samples tested. The drop in the percentage of positive samples does seem to be in line with the drop in smoking among the general population since the introduction of smoke-free legislation in 2007. However, as the laboratory did not have access to the smoking habits of the people who provided the samples, it would be difficult to draw a solid conclusion.

What about the 80 samples (0.2%) that were in the 7–13 ng/mL range? Studies have shown that passive smokers, usually people who live with a smoker, will often