Performance of various testing methodologies for detection of heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA) in bloodstream isolates

**RUNNING TITLE:** Testing methodologies for hVISA detection

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ABSTRACT

The best screening method for detecting heteroresistant vancomycin intermediate \textit{Staphylococcus aureus} (hVISA) remains unclear. Using the population analysis profiling – area under curve (PAP-AUC) method as the gold standard, we screened 458 consecutive MRSA bloodstream isolates to determine the most accurate and cost-effective testing strategy to detect the presence of heteroresistance. All isolates were also tested using the macromethod Etest (MET) and glycopeptide resistance detection (GRD) Etest. Minimum inhibitory concentration (MIC) was determined by several methods including standard vancomycin Etest, vancomycin broth microdilution (BMD) and Vitek2\textregistered. 55 (12\%) hVISA and 4 (1\%) VISA isolates were detected by PAP-AUC. When compared to PAP-AUC, the sensitivity and specificity of MET, GRD Etest, BMD (using MIC cut-off $\geq 2$mg/L) and standard vancomycin Etest (using MIC cut-off $\geq 2$mg/L) was 89\% & 55\%; 71 \& 94\%; 82 \& 97\% and 71 \& 94\% respectively. Combination testing increased overall testing accuracy by reducing the number of false positives. Cost was predominately determined by the number of PAP-AUCs required following a screening assay. The most cost-effective strategy was BMD (using a MIC cut-off $\geq 2$µg/ml) as a stand-alone assay or in combination with PAP-AUC, provided that BMD testing was batched. GRD remained an alternative with 71\% of hVISAs detected. Prevalence influenced both cost and test accuracy with results remaining unchanged for a hVISA prevalence up to 25\%. Implementation of any testing strategy would therefore be dependent on balancing cost with accuracy in a given population and clinical context.
Heterogenous vancomycin-intermediate (heteroresistant) *Staphylococcus aureus* (hVISA) is characterized by the presence of a resistant subpopulation usually at a frequency of 1 in $10^6$ in an otherwise fully susceptible isolate (26). Increased cell wall thickness is a consistent feature of these isolates (11). *In vitro*, it has been demonstrated that these isolates emerge under the appropriate selection pressure from vancomycin-sensitive *Staphylococcus aureus* (VSSA) isolates (6).

Subsequent to the first description in Japan in 1997 (8), hVISA has been reported worldwide with the first Australian isolates reported in 2001 (24). The prevalence of hVISA varies worldwide with rates dependent on multiple factors. These include testing methodologies employed; patient populations tested and the source of the original *Staphylococcus aureus* isolates selected (e.g. infection versus colonization isolate) (20). In Australia, hVISA represents approximately 9 to 13% of all MRSA blood stream isolates (3, 9).

The clinical significance of these isolates remains uncertain. Vancomycin treatment failures have been associated with hVISA infections (2,3, 24). In addition these isolates have been associated with high bacterial load infections and persistent bacteremia (7, 13, 15). Despite these associations, no study has detected worse outcomes with hVISA infections compared to susceptible isolates.

The optimal laboratory detection of hVISA remains unclear (10). Many of the traditional susceptibility platforms prove unreliable because the inocula used are
below the required threshold to detect resistant subpopulations (10, 26). Modified population analysis profile (PAP) using the area under the curve (AUC) is considered the gold standard and most reliable method (25). However, PAP-AUC is very labor intensive and time consuming which limits its use. As a consequence several screening assays including the macromethod Etest (MET) and glycopeptide resistance detection Etest (GRD) have been developed (10, 22-23). Controversy remains however, regarding what testing algorithm to use for the accurate detection of hVISA. A recent review attempted to address this question and suggested either using a laboratory directed approach utilizing the following assays: a vancomycin broth microdilution (BMD) minimum inhibitory concentration (MIC), MET, GRD Etest and/or PAP or a clinically directed approach based on treatment failure defined as persistent bacteremia despite adequate vancomycin trough levels (10).

We therefore undertook this study to determine the utility of various testing strategies; the accuracy of testing algorithms and most cost effective approach to detect hVISA in patients with MRSA bacteraemia using PAP-AUC as the gold standard.

(This work has been presented in part at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Boston, MA, 12-15 September, 2010) (21).
MATERIALS AND METHODS

Isolate selection. All stored (-80°C) consecutive non-duplicate MRSA blood stream infection (BSI) isolates from Liverpool Hospital, Sydney, Australia during the period 1997 to 2008 were included in the study. Retrieved isolates were sub-cultured twice for 48hrs on Horse Blood Agar (HBA) prior to testing. For each patient episode the initial isolate was examined. Multiple isolates from the same patient were included if these isolates represented 1) a new BSI episode; defined as occurring greater than 14 days from the initial isolate and/or 2) a persistent BSI episode; defined as a positive blood culture isolate occurring greater than 3 days and less than 14 days from the initial isolate.

All runs included the appropriate negative and positive controls: Mu3 (ATCC 700698; hVISA) Mu 50 (ATCC 700699; VISA) and a vancomycin sensitive Staphylococcus aureus (ATCC 29213). All testing results were read by 2 independent scientists. Discordant results between scientists were resolved by a third scientist with 2 similar readings accepted as the true result.

Laboratory methods.

Modified population analysis profile area under the concentration-time curve (PAP-AUC) ratio. PAP-AUC was performed on all isolates as previously described (23). Briefly, following an overnight culture in Brain Heart Infusion (BHI) broth (BBL, Becton Dickinson, MD) serial dilutions of the isolate were inoculated onto BHI agar (BBL, Becton Dickinson, MD) plates containing increasing vancomycin concentrations (0 to 8 µg/ml) and incubated for 48 hours (in air at 35°C). All subsequent colonies were counted and plotted against
vancomycin concentration. Isolates were classified as hVISA or VISA if the ratio
of the AUC of the test strain to Mu3 (ATCC 700698) was 0.9 to 1.3 or >1.3
respectively (23, 25). PAP-AUC results close to the cut-off (AUC of 0.8 to 1)
were repeated.

**Vancomycin broth microdilution (BMD).** Vancomycin BMD minimum
inhibitory concentrations (MICs) were determined according to CLSI criteria (4).
In brief, isolate suspensions prepared in Mueller-Hinton II broth (MH II; cation-
adjusted) (BBL, Becton Dickinson, MD) were serial diluted from a starting
concentration of $1 \times 10^6$ CFU/ml with increasing concentrations of vancomycin
(0.25-32 µg/ml). Following 24 hours incubation at 35°C in air the MIC was
recorded as the first well with complete inhibition of growth by the naked eye. As
BMD is not a routine test in our laboratory a subset of isolates (n=137, including
all hVISAs by PAP-AUC) with high MICs (>1ug/ml) were repeated to determine
the reproducibility of our results.

**Macromethod Etest (MET).** Modified Etests were performed according to the
product information (AB BIODISK, Solna, Sweden). Briefly, 200ul of a 2
McFarland standard (prepared in BHI broth) suspensions were inoculated onto a
90mm BHI agar plate and swabbed evenly. Vancomycin and teicoplanin Etest
strips were applied to the dry agar surface and read after an incubation of 48hrs
at 35°C in air. The test was considered positive if i) teicoplanin ≥ 12mg/L or (ii)
teicoplanin ≥ 8mg/L and vancomycin ≥ 8mg/L. Discordant Macromethod Etest
(MET) results compared to the gold standard were repeated.
Glycopeptide resistance detection (GRD) Etest. The GRD Etest (AB BIODISK, Solna, Sweden) was performed according to the product information. Briefly, a 0.5 McFarland saline suspension was inoculated onto MH+5% blood agar plates (BBL, Becton Dickinson, MD) and incubated for 48 hrs at 35 °C in air. Isolates were considered GRD positive if the MIC to vancomycin or teicoplanin was ≥8 µg/ml. Discordant Glycopeptide resistance detection (GRD) Etest compared to the gold standard were repeated.

Vancomycin Etest. Vancomycin Etest MIC was obtained according to the product information (AB BIODISK, Solna, Sweden). Briefly, a 0.5 McFarland saline suspension was inoculated onto MHA (BBL, Becton Dickinson, MD) and incubated for 24hrs at 35°C in air. The MIC was determined as the point at which the growth inhibition ellipse intersected the Etest strip.

Vitek2®. (Gram positive susceptibility card; AST-P579; bioMerieux, Inc, Durham, USA): MIC by Vitek2® was performed according to the manufacturer’s instructions on all isolates.

Statistical analysis, multiple testing strategy, cost benefit and impact of disease prevalence. The chi-squared test was used for categorical data using SAS version 9.2 for Windows (SAS Institute, Cary, NC). All testing was compared to PAP-AUC as the gold standard. Testing accuracy was determined by the equation [(true negative + true positive)/all tested]. Costs analysis and multiple testing strategies were compared using the algorithm adapted from Crobach et al (Figure 1) (5) with test accuracy derived by the equation 

\[
\frac{[\text{Sensitivity Test 1} \times \text{Sensitivity Test 2} \times \text{prevalence}] + [1 - \text{prevalence}] \times \text{Cost Benefit}}{\text{All Compared Tests}}
\]
(Specificity Test 1 + [1-Specificity Test 1] x [1-Specificity Test 2]) x100} for a given prevalence. The impact of disease prevalence on single testing accuracy was calculated using the following equation {sensitivity x prevalence + [1-
prevalence] x specificity} x 100.
RESULTS

417 MRSA BSI cases were identified over the 12 year period (Table 1). 152 cases had a subsequent blood culture taken of which 41 (27%) were positive for MRSA (>3 and <14 days from the initial blood culture bottle). Population analysis profiling of all 458 isolates identified 55 (12%) hVISA and 4 (1%) VISA isolates. The 4 VISA isolates were excluded from the remaining analysis. The rates of hVISA varied widely throughout the 12 years with a biphasic pattern (Table 1). hVISAs were detected on the initial isolate in 93% (51/55) with the remaining 4 hVISA isolates confirmed on the subsequent isolate only. hVISA phenotype was stable with no reversion to VSSA detected in hVISA episodes with repeat blood cultures.

Persistent bacteremia occurred in 41% (7/17) of hVISA episodes compared to 25% (34/143) of VSSA episodes (p=ns). Although limiting hVISA testing to these isolates would increase the prevalence to 17% (7/34), the overall effect would be small and underestimate the total hVISA burden.

Vancomycin minimum inhibitory concentrations by broth microdilution, standard Etest and Vitek2 were determined for all isolates. A correlation between heteroresistance and MIC existed irrespective of method used. The likelihood of detecting heteroresistance increased with higher MICs (Figure 2) (p<0.001).

The sensitivity, specificity, negative and positive predictive values and testing accuracy compared to PAP-AUC as the gold standard was determined for the various methods including using various MIC cut-offs (Table 2). Vancomycin broth microdilution using an MIC cut-off of ≥2 ug/ml resulted in the greatest
specificity (97%) and was the most accurate tests at a hVISA prevalence of 12%. Decreasing the MIC cut-off increased testing sensitivity with a corresponding decline in specificity and test accuracy. GRD performed similar to a standard Etest using a MIC cut-off ≥2 ug/ml with an accuracy of 91.2%. Although the macromethod Etest was one of the more sensitive tests (89%) it had poor specificity and was thus the least accurate assay in determining the presence of heteroresistance. The low sensitivity and accuracy of the Vitek method precluded using this method for hVISA detection with no further cost or performance analysis done.

Cost estimates and labor time required for each of the different testing methods are shown in Table 3. For PAP-AUC and BMD, costs of consumables declined with increased numbers tested, with an optimal run size of 7. As the cost and labor would be prohibitive for single PAP-AUC and BMD the remaining calculations are for batched testing using optimal run sizes (i.e. 7).

To increase over-all accuracy and determine the most cost effective testing strategy multiple testing algorithms were evaluated (Figure 1). Only testing algorithms that resulted in an overall accuracy of ≥90% and detected more than 70% of hVISA isolates are presented in Table 4. Regardless of the number of tests included, overall sensitivity (i.e. the % of hVISA detected) was predominantly determined by the sensitivity of the initial test. In contrast, overall testing accuracy was predominantly determined by the specificity of the final assay. As PAP-AUC is the most expensive test, the addition of PAP-AUC to any other test increased costs and labor time required. However, this corresponded
with an increase in overall accuracy by effectively differentiating true positives from false positives. Of all the testing strategies analyzed, BMD (cut-off of ≥2 µg/ml) was the most cost effective single test (11% of PAP-AUC) with 82% of hVISAs detected. The addition of PAP-AUC following a positive BMD (cut-off of ≥2 µg/ml) was the most effective combination. For laboratories that do not have access to BMD or PAP-AUC, testing using the GRD or standard Etest (cut-off of ≥2 µg/ml) had the best predictive value for a reasonable cost (Table 4).

Both accuracy and cost were co-dependent with hVISA prevalence. Thus, we undertook a sensitivity analysis of testing accuracy to determine whether our findings changed with differing prevalence rates (Figure 3). Tests with higher sensitivities were more accurate with increasing prevalence while tests with higher specificity became less accurate. Although thresholds existed at which certain tests would be more accurate than others, our finding remained unchanged for hVISA prevalences between 5 and 25%.

137 (30%) isolates had their BMD repeated with 99% (135/137) reproducibility. Similarly, 6% (29/454) of all PAP-AUCs were repeated and represented PAP-AUC results between 0.8 and 1.1. Although the AUC varied by a mean of ± 0.1, 90% (26/29) of the original PAP-AUC classifications were reproducible. Discordant GRD and MET results were repeated with overall agreement with the original discordant result in 99% of cases for both methods.
DISCUSSION

Screening for heteroresistance remains difficult as no optimal laboratory approach has been determined. Our study, containing the largest number of consecutive MRSA blood stream isolates categorised by the gold standard PAP-AUC, provides useful insights into the utility of the various proposed testing algorithms (10). Furthermore, the overall prevalence of hVISA (12%) detected, was sufficiently high to make assessment of the various screening assays possible.

A clinically directed approach, limiting testing to high risk patients, would underestimate the overall hVISAs burden. An alternate strategy is therefore required if documentation of the total burden is necessary. For patient management this may not be essential as hVISA in less severe infections are not associated with significant worse clinical outcomes (9, 20).

A laboratory directed approach, in contrast, significantly improves the ability to determine the hVISA burden. However, no single test was accurate enough when compared to PAP-AUC. The GRD sensitivity (74%) was lower than other published reports (89-93%) (12, 27). Similarly, the specificity of MET (54%) in our study was significantly less than previous reports (88-96%) (12, 22, 27). Reasons for these differences are unclear and may reflect specific MRSA subtype characteristics. However, recent MET and GRD assessments, performed on consecutive clinical isolates showed significantly lower sensitivities (44%-69%) and specificities (48%-98%) (1, 17, 18) than previous laboratory characterized
evaluations. This suggests that some of these differences may reflect hVISA phenotype instability (22).

The proportion of hVISA detected, similar to other studies, increased with higher MICs irrespective of method (14, 16, 19) and thus MICs cut-offs by method were evaluated as appropriate screening assays. An Etest (using an MIC cut-off of ≥1.5 µg/ml) had the highest sensitivity (91%) but was associated with a test accuracy of 69%. In contrast, vancomycin broth microdilution (using an MIC cut-off of 2µg/ml) was the most accurate test (95%) but with a corresponding decline in sensitivity. When including cost as part of the analysis, BMD was the most cost effective strategy. However, this requires batch testing which may be infrequent in low MRSA prevalent settings and prompt GRD to be a suitable alternative test.

An alternative strategy is employing a 2 or 3-step combination testing algorithm. Although these combinations are able to increase the overall accuracy of testing by reducing the number of false positives, the percentage of hVISA detected does not improve and is determined by the initial test. When assessing cost, the greatest cost and labor is associated with PAP-AUC thus decreasing or eliminating the need for PAP-AUC significantly impacts on the overall cost and labor required. Of the testing strategies examined a MET screening assay followed by PAP-AUC was similar in cost to PAP-AUC (92% of PAP-AUC) and thus would negate MET as a viable screening assay.

Both accuracy and cost are dependent on the prevalence of hVISA with our findings remaining unchanged for hVISA prevalences up to 25%. Application of
our results should only be instituted with knowledge of local hVISA epidemiology.

The optimal choice of testing strategy for a given institution is thus dependent on balancing the prevalence and required rate of hVISA detection against the cost and labor required. In our opinion, the combination of BMD (MIC cut-off ≥2 µg/ml) followed by PAP-AUC was the most cost and time effective strategy.

Alternatively, if detection of hVISA is critical, despite double the cost, BMD (MIC cut-off ≥1.5 µg/ml) followed by PAP-AUC is the testing strategy to use. However, batch testing is required for these cost savings and thus may not be a practical in instructing patient management.

Unfortunately, no ideal strategy was found that would reliably allow for hVISA testing to be introduced into routine laboratory testing and thus further research is needed in this area.

This study has several limitations. As no genetic marker for heteroresistance exists, PAP-AUC is assumed as the gold standard test for detecting hVISA phenotype (10). The variability and problems with reproducibility of laboratory testing with respect to heteroresistance is well documented (22). Repeat testing of all isolates may have altered our results. However, this was not detected in the subset of isolates retested. hVISA phenotypes are known to be unstable with the ability to revert to VSSA under various conditions including isolate passage on vancomycin free media (22). It is unclear whether storage and subcultures of the isolates impacted our results. However, all hVISA MICs were retested with no phenotype instability detected. VISA isolates were excluded from the analysis. However, our results did not change if these were considered as hVISA (data not
shown). Finally intra-observer variability was not corrected for with multiple operators over the 12 year study period.

In conclusion, we believe that this study contributes to the increasing understanding of detection of hVISA in the current literature. Previously accepted screening methods (MET, GRD) did not perform as well as expected in our study raising the possibility that they may not be as reliable as previously thought.

Based on our data, laboratory implementation of optimal hVISA testing strategies should take into account clinical settings with the aim of balancing maximum detection and cost. Ongoing research is required to determine better detection methods that are less costly and time intensive allowing for more widespread laboratory implementation.
REFERENCES


Table 1: Number of MRSA blood stream infection (BSI) episodes, isolates and hVISA frequency over a 12-year span.

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of MRSA BSI episodes*</td>
<td>19</td>
<td>52</td>
<td>80</td>
<td>79</td>
<td>83</td>
<td>104</td>
<td>417</td>
</tr>
<tr>
<td>Number of episodes with repeat blood cultures(^b)</td>
<td>5</td>
<td>17</td>
<td>26</td>
<td>20</td>
<td>39</td>
<td>45</td>
<td>152</td>
</tr>
<tr>
<td>Subsequent blood culture positive</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>Total number of isolates</td>
<td>19</td>
<td>54</td>
<td>82</td>
<td>81</td>
<td>100</td>
<td>121</td>
<td>458</td>
</tr>
<tr>
<td>hVISA</td>
<td>0</td>
<td>3</td>
<td>28</td>
<td>6</td>
<td>15</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>% hVISA</td>
<td>0</td>
<td>5.5</td>
<td>34.1</td>
<td>7.3</td>
<td>15</td>
<td>2.4</td>
<td>12</td>
</tr>
</tbody>
</table>

* Multiple isolates from the same patient were included if these isolates represented a new bacteremic episode: defined as occurring greater than 14 days from the initial isolate.

\(^b\) Repeat cultures were taken greater than 3 days and less than 14 days from the initial isolate.
Table 2. Comparison of hVISA detection methods compared to a PAP-AUC gold standard at a hVISA prevalence of 12%.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>hVISA (TP=55)</td>
<td>(TN=399)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Etest ≥1.5ug/ml</td>
<td>50</td>
<td>263</td>
<td>91</td>
<td>66</td>
<td>98</td>
</tr>
<tr>
<td>BMD ≥1.5ug/ml</td>
<td>49</td>
<td>335</td>
<td>89</td>
<td>84</td>
<td>98</td>
</tr>
<tr>
<td>MET</td>
<td>49</td>
<td>218</td>
<td>89</td>
<td>55</td>
<td>97</td>
</tr>
<tr>
<td>BMD ≥2ug/ml</td>
<td>45</td>
<td>388</td>
<td>82</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Etest ≥2ug/ml</td>
<td>39</td>
<td>375</td>
<td>71</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>GRD</td>
<td>39</td>
<td>375</td>
<td>71</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Vitek2 ≥2ug/ml</td>
<td>14</td>
<td>382</td>
<td>25</td>
<td>96</td>
<td>90</td>
</tr>
</tbody>
</table>

Total of 454 isolates – 4 VISA isolates excluded

*VSSA: Vancomycin Susceptible Staphylococcus Aureus

*Accuracy= (TP + TN/ total tested) with 454 isolates tested.

TP = true positive; TN = true negative; NPV = negative predictive value; PPV = positive predictive value; BMD = vancomycin broth microdilution; GRD = Glycopeptide resistance detection Etest; MET = Macromethod Etest.
Table 3. Labor time and costs for hVISA detection by testing method.

<table>
<thead>
<tr>
<th>Test</th>
<th>No. isolates per run</th>
<th>Cost in AUS of reagents per run</th>
<th>Total labor time (hrs) per run</th>
<th>Total cost AUS per isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP-AUC</td>
<td>1-7</td>
<td>14 - 40</td>
<td>7-9</td>
<td>320-70</td>
</tr>
<tr>
<td>MET</td>
<td>1</td>
<td>9</td>
<td>0.5</td>
<td>29</td>
</tr>
<tr>
<td>GRD</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
<td>12</td>
</tr>
<tr>
<td>BMD</td>
<td>1-7</td>
<td>3 - 8</td>
<td>0.75</td>
<td>38-8</td>
</tr>
<tr>
<td>Vancomycin Etest</td>
<td>1</td>
<td>5</td>
<td>0.25</td>
<td>15</td>
</tr>
<tr>
<td>Vancomycin “MIC” (Vitek2®)</td>
<td>1</td>
<td>8</td>
<td>0.25</td>
<td>18</td>
</tr>
</tbody>
</table>

*Labor time includes set up, result reading and interpretation.

*Total per isolate cost = (reagent cost + Labor cost (at AU$40/hr Australian) x time)/number of isolates per run. The cost range represents between 1 and 7 isolates per run. Costs are quoted in Australian (AU) dollars.

PAP-AUC = Population analysis profiling; MET = Macromethod Etest; GRD = Glycopeptide resistance detection Etest; BMD = vancomycin broth microdilution.
Table 4. The performance, cost and labor time required of various testing strategies to test 454 MRSA isolates for the presence of \(12\)% hVISA with heteroresistance prevalence.

<table>
<thead>
<tr>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>% hVISA detected</th>
<th>Total Cost in AUS (% of PAP-AUC cost)</th>
<th>Total Labor time in hrs (% PAP-AUC time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP-AUC</td>
<td>PAP-AUC</td>
<td>100</td>
<td>100</td>
<td>32200 (100)</td>
<td>460 (100)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>Etest ≥1.5ug/ml</td>
<td>98.9</td>
<td>91</td>
<td>19789 (62)</td>
<td>352 (6)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>BMD ≥1.5ug/ml</td>
<td>98.7</td>
<td>89</td>
<td>11501 (36)</td>
<td>193 (33)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>MET</td>
<td>98.7</td>
<td>89</td>
<td>29145 (92)</td>
<td>520 (89)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>BMD ≥2ug/ml</td>
<td>97.8</td>
<td>82</td>
<td>7598 (24)</td>
<td>121 (21)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>Etest ≥1.5ug/ml</td>
<td>97.7</td>
<td>81</td>
<td>11360 (36)</td>
<td>188 (32)</td>
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<tr>
<td>PAP-AUC</td>
<td>BMD ≥1.5ug/ml</td>
<td>97.5</td>
<td>79</td>
<td>12374 (39)</td>
<td>206 (35)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>Etest ≥1.5ug/ml</td>
<td>96.9</td>
<td>75</td>
<td>8615 (27)</td>
<td>138 (24)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>BMD ≥2ug/ml</td>
<td>96.7</td>
<td>73</td>
<td>9329 (29)</td>
<td>151 (26)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>MET</td>
<td>96.5</td>
<td>71</td>
<td>11196 (35)</td>
<td>194 (33)</td>
</tr>
<tr>
<td>PAP-AUC</td>
<td>GRD</td>
<td>96.5</td>
<td>71</td>
<td>9834 (31)</td>
<td>194 (33)</td>
</tr>
<tr>
<td>Test</td>
<td>Accuracy</td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>True Positives</td>
<td>True Negatives</td>
</tr>
<tr>
<td>--------------</td>
<td>----------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------------</td>
<td>----------------</td>
</tr>
<tr>
<td>BMD ≥2ug/ml</td>
<td>95.4</td>
<td>82</td>
<td>3632 (11)</td>
<td>49 (8)</td>
<td></td>
</tr>
<tr>
<td>BMD ≥2ug/ml</td>
<td>MET</td>
<td>93.9</td>
<td>73</td>
<td>5623 (18)</td>
<td>83 (14)</td>
</tr>
<tr>
<td>BMD ≥2ug/ml</td>
<td>Etest ≥1.5ug/ml</td>
<td>93.5</td>
<td>75</td>
<td>4662 (15)</td>
<td>66 (11)</td>
</tr>
<tr>
<td>Etest ≥2ug/ml</td>
<td></td>
<td>91.2</td>
<td>71</td>
<td>6810 (21)</td>
<td>114 (20)</td>
</tr>
<tr>
<td>GRD</td>
<td></td>
<td>91.2</td>
<td>71</td>
<td>5448 (17)</td>
<td>114 (20)</td>
</tr>
</tbody>
</table>

Note: BMD and PAP-AUC costs are based on 7 samples per run. For multi-test strategies only positives from the prior test are tested (Figure 1).

*Accuracy = TP + TN/ total.

PAP-AUC = Population analysis profiling; MET = Macromethod Etest; GRD = Glycopeptide resistance detection Etest; BMD = vancomycin broth microdilution.
Figure 1: Application of a two-step algorithm, adapted from Crobach et al (5), testing 10000 isolates with a population hVISA prevalence of 10%. This would result in a testing accuracy of 97.8% (828+8955)/10000) based on the equation (total true positive + total true negative)/total tested).

Figure 2: Proportion of hVISA detected by method of vancomycin MIC determination

BMD = Broth microdilution; and Etest = standard Etest

Figure 3: Sensitivity analysis of testing accuracy by hVISA prevalence

BMD = Broth microdilution; and Etest = standard Etest. Glycopeptide Resistance Detection (GRD) Etest is equivalent in performance to a standard Etest using a MIC cut-off of ≥1.5ug/ml.
Prevalence of hVISA in MRSA Blood stream isolates 10%
Total number of isolates Tested = 10000

hVISA infection n=1000
VSSA infection n=9000

Sensitivity 0.92 hVISA screen - Test 1 Specificity 0.90

False negative 1 n=80
hvISA infection Negative test n=80
hvISA infection Positive test n=920
VSSA infection Positive test n=900
VSSA infection Negative test n=8100

True negative 1 n=8100

Sensitivity 0.90 hvISA confirmation - Test 2 Specificity 0.95

False negative 2 n=92
hvISA infection Negative test n=92
hvISA infection Positive test n=828
VSSA infection Positive test n=85
VSSA infection Negative test n=855

True positive n= 828 False positive n=45

True negative 2 n=855