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Running Title: Comparison of Staphylococcus aureus detection by automated and manual processing.

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The sensitivity of automated culture of *S. aureus* from flocked swabs versus manual culture of fiber swabs was prospectively compared using nasal swabs from 867 patients. Automated culture from flocked swabs significantly increased the detection rate, by 13.1% for direct culture and 10.2% for enrichment culture.

MRSA screening is a key component of strategies to reduce methicillin-resistant *S. aureus* (MRSA) (2). In 2008 the UK Department of Health mandated screening all elective hospital admissions by March 2009 and hospital admissions from the emergency department by March 2011 (3). There is also increasing evidence that screening for methicillin-sensitive *S. aureus* (MSSA) reduces the rate of surgical site infections (SSIs) (1). PCR detection of MRSA remains significantly more expensive than culture methods, and consequently screening specimens are often cultured manually using fiber-based swabs. A deficiency of this method is that fiber swabs only release a small proportion of the organisms sampled onto solid media, which may reduce sensitivity (5).

In 2005, Copan Italia introduced the ESwab (Elution Swab), a flocked nylon swab transported in 1ml of liquid Amies transport medium. The increased efficiency of this system has been demonstrated *in-vitro* (5) and *in-vivo* on volunteers (7). Another advantage of the liquid medium is the potential for automation (6). In 2008, Copan Italia launched a new generation of specimen processor, the Walk-Away Specimen Processor (WASP). This system automatically de-caps and re-caps sample containers, and inoculates a variety of culture plates with minimal manual intervention.
A prospective trial was undertaken to compare the sensitivity of detection of *S. aureus*, including MRSA, from nose swabs using the automated WASP and the ESwab against the laboratory’s current manual method that uses a regular fiber swab. The study population attended the Medical Assessment Unit of Derriford Hospital, Plymouth between January and September 2009.

An ESwab (Copan Italia, 480CE) was used to sample one of the nostrils, the other nostril being swabbed with an M40 charcoal-based Transystem swab (Copan Italia 408CST). Each swab was pre-wetted with 50 µl sterile saline. 30 µl aliquots of the ESwab nose samples were quad-streaked by the WASP onto two plates, a Staphylococcus selective medium (Columbia Blood Agar with Aztreonam and Polymxyn (CAP) - Oxoid PB0122A) and an MRSA selective agar plate (ChromID MRSA Agar - Biomerieux 43451). M40 nose samples were inoculated and quad-streaked manually onto the same two media (4). All plates were incubated at 37°C for 48 h. The use of a 30 µl loop for streaking ESwab liquid Amies was based on previous experimentation that showed this volume to produce visually comparable cultures with in-house manual inoculations (6).

The remaining 800 µl of the ESwab sample and the swab tip plus 3 cm of the shaft of the M40 sample were inoculated into separate tubes of 7% Salt Broth (Oxoid EB1040E) for enrichment. Broths were incubated at 30°C for 24 h and 10 µl subcultured automatically or manually as appropriate. Resultant cultures were screened for the presence of *S. aureus* (MSSA and MRSA) using standard methods. Positive nasal *S. aureus* carriage was defined as a positive culture result obtained from one or more of the methods under investigation. The Sensitivity and negative
predictive value were calculated for each method. The matched pair Sign Test was used to analyze the data.

From the 867 patients enrolled 41% were found to be positive for S. aureus. 237 M40 swabs were culture positive for S. aureus compared to 268 positives for the ESwabs, an increase of 13.1% (P <0.001).

Enrichment culture was more sensitive than direct plating for both types of swab (Table 1); an increase of 24.5% was observed with the M40 samples and 21.6% for the ESwab samples. The most sensitive single method was enrichment culture of ESwabs (91.6%).

Previous studies used to validate flocked swabs and automated plating have been laboratory-based using bacterial cultures or human volunteers. These may not always reflect clinical practice where variables such as the presence of mucus and pus may influence swab performance. This study examines the performance of different swabs and plating methods in the hospital environment using routine clinical samples collected from patients.

The results demonstrate that use of ESwabs increases S. aureus recovery. The ability to automate processing enables laboratories to potentially improve efficiency. A comparison of timings showed that automated processing of a batch of 72 samples took 2.7 minutes hands-on time; this compared with 57 minutes for manual processing. This represents a reduction in “hands-on” time of 95%. It would be possible to improve this further by using alternative streaking protocols.

MRSA enrichment culture is used for screening Derriford Hospital’s elective pre-operative patients and is performed 10 to 14 days in advance of surgery. This study has shown that this method improves detection of S. aureus and justifies its use in this setting where an early result is less important. A short turn around time is more
important when screening emergency admissions, and in this setting direct culture using the most sensitive swab/transport system available is recommended. During the course of this trial the WASP produced 50,000 culture plates from ESwab samples. It consistently provided isolated colonies; there was also no evidence of cross contamination when 50 ESwab samples spiked with $10^7$ colony-forming-units of *S. aureus* were interspersed with 50 blank ESwab samples. The equipment was generally reliable and user friendly during the study with only a couple of plate carousel and label printer faults being encountered. These were resolved remotely by Copan Italia within one hour causing minimal delay to processing.

In conclusion, this study has shown ESwabs to be more sensitive than fiber swabs for the detection of *S. aureus* colonisation. It has also shown that sensitivity can be increased further by enrichment culture. Processing ESwabs using the WASP was much less labor intensive making the combination ideally suited for screening for MSSA and MRSA.

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**References:**


Table 1. Method sensitivity and negative predictive value for detecting +ve patients.

<table>
<thead>
<tr>
<th>No. S. aureus +ve patients</th>
<th>All methods</th>
<th>ESwab direct</th>
<th>ESwab enrich.</th>
<th>M40 direct</th>
<th>M40 enrich</th>
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<tr>
<td></td>
<td>355</td>
<td>268</td>
<td>325</td>
<td>237</td>
<td>295</td>
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<tr>
<td>Sensitivity of method</td>
<td>75.5%</td>
<td>91.6%</td>
<td>66.8%</td>
<td>83.1%</td>
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<tr>
<td>NPV</td>
<td>85.5%</td>
<td>94.5%</td>
<td>81.3%</td>
<td>89.5%</td>
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