



**HAL**  
open science

## **Clinical evaluation of the Copan ESwab for methicillin-resistant detection and culture of wounds**

V. Saegeman, J. Flamaing, J. Muller, W. E. Peetermans, J. Stuyck, J. Verhaegen

### ► **To cite this version:**

V. Saegeman, J. Flamaing, J. Muller, W. E. Peetermans, J. Stuyck, et al.. Clinical evaluation of the Copan ESwab for methicillin-resistant detection and culture of wounds. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, 30 (8), pp.943-949. <10.1007/s10096-011-1178-1>. <hal-00666672>

**HAL Id: hal-00666672**

**<https://hal.science/hal-00666672v1>**

Submitted on 6 Feb 2012

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



## 10 Introduction

11 Critical points for appropriate isolation and identification of potential pathogens from clinical  
12 specimens are the selection and collection of an appropriate specimen and maintenance of  
13 microorganism viability during transport [1]. The sample collection device should be chosen in such a  
14 way that the most appropriate specimen sample is collected. The collection process must utilise  
15 appropriate techniques to minimise exposure to commensal flora. Possible specimens to collect  
16 include tissue by biopsy, needle aspiration of exudates/fluid/drainage, and swab for certain anatomic  
17 sites (mucosal surfaces, wound secretions and perineal skin). Remark that the swab collection method  
18 is frequently used to collect material from for instance burn wounds that would be better collected by  
19 means of biopsy or aspiration. Swabs have the advantages of omitting biopsy, patient discomfort,  
20 transport delay and costs.

21 It is important that the swab tip material and the transport medium are made of materials sufficiently  
22 non-toxic or non-inhibitory to maintain microorganism viability. Cotton, Dacron, and rayon swabs  
23 absorb bacteria onto their surface, thereby enmeshing bacteria in their dense fiber matrix and  
24 compromising their release [2]. A new type of swab system has recently been introduced in a growing  
25 number of laboratories. This Copan ESwab (Brescia, Italy) consists of a screw-cap tube filled with 1  
26 ml of Liquid Amies medium and a small peel pouch containing a specimen collection swab with a tip  
27 flocked with soft nylon fiber [3]. This design provides a stronger capillary action and strong hydraulic  
28 uptake of liquids, which should result in better specimen collection [1]. This technology would also  
29 release the specimen material more efficiently than typical rayon or Dacron fiber-tipped swabs [1,4,5].  
30 The aim of this study was to evaluate this new ESwab technology for two common applications: i)  
31 methicillin resistant *Staphylococcus aureus* (MRSA) screening from anterior nares and perineum, the  
32 predilection area of *Staphylococcus aureus* colonisation and ii) burn or septic orthopaedic wounds  
33 which are swabbed because too little material is available for needle aspiration/drainage.

34 In particular, we compared the ESwab MRSA collection kit (MRSA ESwab) with the dry cotton  
35 Copan swabs (dry swab, wooden applicator, cotton tip, 150C, Sterilin - Copan, Brescia, Italy) and the  
36 ESwab for wounds (wound ESwab) with Amies agar gel swab (CE0344, Copan, Brescia, Italy).

37 Gram stain smears prepared from dry cotton swabs and ESwabs were compared semiquantitatively in  
38 terms of number of bacterial morphotypes and human eukaryotic cells.

39 ESwab and conventional swab systems were evaluated in terms of bacterial recovery (semiquantitative  
40 and quantitative) and spectrum of isolated species.

41 Additionally, two factors possibly influencing the culture results were evaluated: 1) time interval  
42 between sample collection and processing and 2) effect of the sequence of use of the different swab  
43 systems in the recovery of bacteria.

44

## 45 **Materials and methods**

46

### 47 *Samples (Table 1)*

#### 48 *MRSA screening*

49 Hospital wards from a large tertiary care hospital (University Hospitals Leuven) where MRSA  
50 screenings are routinely performed were included in the study: 3 wards of Geriatric Medicine, and 1  
51 ward of General Internal Medicine. Among 164 patients with a positive MRSA screening test, a  
52 MRSA ESwab and two conventional dry swabs were taken. At the Burn Care Unit, 36 consecutive  
53 patients were sampled both with the MRSA ESwab and with the conventional dry swabs without  
54 previously knowledge of the patient's MRSA colonisation status. The MRSA ESwab collection kit  
55 was used according to the manufacturer's instructions [3] sampling both nares and perineum but  
56 omitting sampling of the throat. Two dry swabs were taken, one from nares and one from perineum.  
57 MRSA ESwab and dry swabs were streaked onto identical agar plates.

#### 58 *Wounds*

59 Among 25 patients of the Septic Orthopaedics Department with positive wound culture results, swabs  
60 were taken of the wounds with both swab systems: the wound ESwab and the combination dry swab  
61 for Gram staining and Amies agar gel swab for culture. At the Burn Care Unit, 178 consecutive swabs  
62 were taken of burn wounds with both swab systems without previous knowledge of the  
63 colonisation/infection status of the wound. The ESwab for wound sampling was used according to the  
64 manufacturer's instructions [3].

65

1  
2 66 ESwab (MRSA ESwab or wound ESwab) or the two Copan swabs (dry swabs or dry swab and Amies  
3  
4 67 agar gel swab) were alternately used first to sample the infected/colonised body region.

5  
6 68 The day and time of sampling and the order of swab use were registered.  
7  
8

9 69

10  
11 70 *Gram staining*

12  
13 71 At the microbiology laboratory, a Gram stain was performed of both ESwab (MRSA ESwab and  
14  
15 72 wound ESwab) and dry swab. Gram stains of the ESwab were performed by depositing 1 to 2 drops of  
16  
17 73 Liquid Amies medium recovered from the vortex mixed ESwab tube on a glass slide [3]. For the  
18  
19 74 preparation of a Gram stain of the dry swab, the swab was shortly suspended in 1 mL of sterile saline  
20  
21 75 and streaked onto the surface of a glass slide. The specimens were air dried on the slide, fixed with  
22  
23 76 heat and stained with a Mirastainer® colouring instrument (Merck KGaA).

24  
25  
26 77 Gram stains were evaluated by one experienced person. Gram stain smears prepared from the ESwab  
27  
28 78 and the dry swab were compared semiquantitatively in terms of number of bacterial morphotypes  
29  
30 79 (gramnegative rods, streptococci, grampositive cocci, grampositive rods, yeast cells) per high power  
31  
32 80 field (N = 403 paired samples), and number of polymorphonuclear cells, lymphocytes, red blood cells,  
33  
34 81 and epithelial cells per high power field (N = 350 paired samples).  
35  
36

37 82

38  
39  
40 83 *Quantitative swab elution method*

41  
42 84 The quantitative 'swab elution method' was performed on 200 ESwabs (100 MRSA ESwabs and 100  
43  
44 85 wound ESwabs) and 200 corresponding conventional swabs (100 dry swabs and 100 Amies agar gel  
45  
46 86 Copan swabs). Because we used clinical samples without previous knowledge of the bacterial load, a  
47  
48 87 modified CLSI M40-A protocol was used [3].  
49  
50

51 88 The ESwab (MRSA ESwab and wound ESwab) was suspended in 1mL of Liquid Amies transport  
52  
53 89 medium and was vortex mixed for 10-15 seconds.  
54

55 90 The Amies agar gel swab from wounds and the two dry swabs taken from nose and perineum (MRSA  
56  
57 91 screen) were suspended in 1 mL of sterile saline during 10-15 seconds by means of vortex mixing.  
58  
59  
60  
61  
62  
63  
64  
65

92 From all suspensions, three tenfold serial dilutions ( $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ ) were performed in sterile  
93 saline. From each of these dilutions, 50  $\mu$ L was inoculated on a Mannitol Salt agar (MSA) and a  
94 MRSA Chromagar (dilution  $10^{-1}$ ) for the MRSA screening tests and for the wound swabs 50  $\mu$ L was  
95 inoculated on a blood agar plate (all dilutions) and the dilution  $10^{-1}$  on the following 4 plates: MSA,  
96 MacConkey agar, chocolate agar and blood agar for anaerobic incubation. Plates were inoculated with  
97 the spiral plater (Spiral Biotech, autoplate 4000) and incubated at 36°C during 48 hours, in 5% CO<sub>2</sub>  
98 when indicated.

99 Counts (CFU/mL) were read with the IUL Counterstat Flash 4.2 automatic reader system.

100 For the determination of MRSA positivity, dark green colonies were investigated further by means of  
101 tube test, DNase and/or Maldi Biotyper<sup>®</sup> Flex series (Bruker, Germany) for confirmation.

102 Microorganisms isolated from wound cultures were identified by means of Vitek 2 system  
103 (Biomérieux, France), Maldi Biotyper<sup>®</sup> Flex series or conventional biochemical tests.

104

#### 105 *Semiquantitative 'roll plate method'*

106 The semiquantitative 'roll plate method' was performed on 203 ESswabs (100 MRSA ESswabs and 103  
107 wound ESswabs) and 203 corresponding conventional swabs (100 dry swabs and 103 Amies agar gel  
108 swabs).

109 To avoid overgrowth when streaking a swab over the entire surface of an agar plate a modified CLSI  
110 M40-A protocol was used [3].

111 The ESswab tube (MRSA ESswab and wound ESswab) was vortex mixed and the swab tip of both  
112 ESswab and Amies agar gel swab were rolled onto one quadrant of the culture plate to provide the  
113 primary inoculum. Further streaks were made starting from the primary inoculum over the second,  
114 third and fourth quadrant of the plate. Because two or more culture media were inoculated (identical  
115 plates as in swab elution method), the ESswab applicator/Amies agar gel swab were returned into the  
116 transport medium tube to absorb and recharge the applicator tip with medium. The total CFU/mL was  
117 judged semiquantitatively (0-5) after 48 hours of incubation at 36°C, in 5% CO<sub>2</sub> when indicated.

118 Identification of microorganisms was performed in the same way as in the swab elution method.

119

120 *Statistical analysis (GraphPad Prism 4 Software and Excel software)*

121 Results of the Gram stains were compared in a semi-quantitative manner (Wilcoxon signed rank test).

122 Culture results with both methods were compared both in a qualitative (MRSA positive/negative,

123 isolates species; McNemar Chi square test), semiquantitative (Wilcoxon signed rank test) and

124 quantitative (paired t-tests of log-transformed data) way. A p-value < 0.05, with Bonferroni

125 correction when indicated, was considered significant.

126 Chi square was used to evaluate the influence on culture results of the time interval from sampling up

127 to culture processing (Chi square test of independence between time delay before culture shorter or

128 longer than 0.21 days and MRSA culture result), and the effect of the sequence of use of ESwab or dry

129 swab in the recovery of MRSA.

130

## 131 **Results**

### 132 *Gram stain quality/quantity*

133 Details of polymorphonuclear cells, lymphocytes, red blood cells and epithelial cells appeared to be

134 better preserved in the Gram stains prepared from ESwab. No obvious difference between the two

135 swab systems was noticed in staining quality and shape of microorganisms.

136 There were significantly more gramnegative rods, streptococci, grampositive cocci, grampositive rods,

137 polymorphonuclear cells, lymphocytes, and red blood cells on Gram stains made from ESwab

138 compared with Gram stains made from dry swab (Table 2; p < 0.006). Although there was a tendency

139 towards more epithelial cells visualized on Gram smears from dry swab compared with those from

140 ESwab, this difference was not statistically significant.

### 141 *Bacterial recovery*

142 With the swab elution quantitative study, we determined the difference in bacterial recovery between

143 ESwab and the dry swab (100 paired samples, MRSA screen) and Amies agar gel swab (100 paired

144 samples, wound specimen). Total colony counts with ESwab (mean  $6.30 \times 10^6$  CFU/mL for MRSA

145 and mean  $1.86 \times 10^6$  CFU/mL for wounds) were on average 9 times higher than with the dry swab

146 (mean  $7.06 \times 10^5$  CFU/mL)(Figure 1) and 6 times higher than with the Amies agar gel swab (mean

147 3.06 x 10<sup>5</sup> CFU/mL)(Figure 2). This difference was statistically significant both for the dry swab and  
148 the Amies agar gel swab (p < 0.05).

149 Results of the semiquantitative roll-plate method confirmed this; total bacterial colony counts with  
150 ESwab (mean 2.3 quadrants growth for MRSA and mean 1.2 quadrants growth for wounds) were 1.3  
151 times higher than with the dry swab (mean 1.7 quadrants growth) (100 paired samples, MRSA screen)  
152 and the Amies agar gel swab (mean 0.9 quadrants growth) (103 paired samples, wound  
153 specimen)(Supplementary Figures 3 and 4). This difference was statistically significant both for the  
154 dry swab and for the Amies agar gel swab (p < 0.05).  
155 ESwab resulted in a slightly higher recovery of MRSA than the dry swab, although not statistically  
156 significant (Table 3, p = 0.50).

157 Considering the wound swabs cultured with the quantitative swab elution method, significantly more  
158 coagulase-negative *Staphylococcus* spp. and more *Enterococcus* spp. were isolated from ESwab than  
159 from Amies agar gel swab (p < 0.006, Table 4). Wound swabs cultured with the semiquantitative roll-  
160 plate method showed a higher recovery of coagulase-negative *Staphylococcus* spp. and  
161 *Corynebacterium* spp. with Eswab versus Amies agar gel swab although not statistically significant (p  
162 > 0.006).

#### 163 *Influence of culture delay time and order of swab use on MRSA screening result*

164 There was no statistical significant relation between i) time interval between sampling and inoculation  
165 of the culture (p > 0.05 both with ESwab and with dry swab, data for 198 samples, Table 5) or ii) order  
166 of swab system used first (p > 0.05 both with ESwab and with dry swab, data for 199 samples, Table  
167 6) and the MRSA culture result.

168 There is no effect of time delay until culturing for neither the ESwab nor the conventional dry swab.  
169 This implicates that survival of MRSA on both swab systems is not significantly different.

## 171 **Discussion**

172 The goal of microbiological evaluation is to provide accurate, clinically pertinent results in a timely  
173 manner. Several techniques can be applied to collect appropriate specimens. The swab collection

174 method is one of them and although often inferior to biopsy or aspiration it has the advantage of being  
1 non-invasive. The ESwab system was introduced in 2008 and data on its clinical use are limited.  
2  
3  
4 176 The aim of this study was to evaluate the ESwab system by using clinical samples in comparison with  
5  
6 177 dry cotton Copan swab for MRSA screening and with Amies agar gel Copan swab for sampling of  
7  
8 178 wounds. Gram stain and bacterial recovery were the examined parameters.  
9  
10  
11 179 The high number of gramnegative rods, streptococci, grampositive cocci, grampositive rods,  
12  
13 180 polymorphonuclear cells, lymphocytes, and red blood cells on Gram stains made from the ESwab  
14  
15 181 could be attributed to the flocced swab system that enhances bacterial absorption and release in  
16  
17 182 comparison with the traditional cotton, Dacron or rayon swabs that entrap bacteria in the fiber matrix.  
18  
19 183 Our findings were in accordance with those of previous authors. Fontana et al compared the quality of  
20  
21 184 80 pairs of Gram stains performed with ESwab in liquid Amies medium (Copan) versus rayon swab in  
22  
23 185 Amies gel transystem (Copan) from vaginal, cervical, urethral, and wound origin. ESwab slides  
24  
25 186 demonstrated more human cells and more bacterial species than the traditional Amies gel system [6].  
26  
27 187 Daley et al reported a statistically better yield in respiratory epithelial cells by direct fluorescent  
28  
29 188 antibody staining and accordingly also more infected epithelial cells with the use of a flocced swab  
30  
31 189 system compared with rayon swabs [7,8].  
32  
33  
34  
35 190 Details of polymorphonuclear cells, lymphocytes, red blood cells and epithelial cells appeared to be  
36  
37 191 better preserved in Gram stains prepared from ESwab. This most probably is the result from the  
38  
39 192 ESwab that was not streaked onto the slide while preparing the Gram stain. As such mechanical stress  
40  
41 193 to the eukaryotic cells is avoided.  
42  
43  
44 194 We used a modified version of the CLSI M40-A standard for ‘Quality Control of Microbiological  
45  
46 195 Transport Systems’ because we did not use standard initial inoculums for the evaluation of the swab  
47  
48 196 systems [9]. The swab elution method allows a quantitative measurement of the ability of a transport  
49  
50 197 system to maintain viable organisms [9]. The roll plate technique takes into consideration some  
51  
52 198 mechanical variables of the direct swabbing action that exist in the clinical laboratory that can  
53  
54 199 influence the release of the sample onto culture plates [9]. Previous authors have already examined the  
55  
56 200 ESwab system according to the CLSI M40-A standard [1,4,5]. We therefore focused on the  
57  
58 201 performance of ESwab when using clinical samples and therefore samples with a potential low  
59  
60  
61  
62  
63  
64  
65

202 bacterial load and with possible sample transport delay. In this way, pre-analytical variables are  
1 included in the study.  
2

3  
4 204 We showed that the bacterial recovery with ESwab was significantly higher (quantitatively and  
5  
6 205 semiquantitatively) compared with the dry swab and compared with the Amies agar gel swab. In  
7  
8 206 theory, the higher recovery with the ESwab system would also increase the chance to detect MRSA or  
9  
10 207 potential pathogenic microorganisms. In this study, there was indeed a tendency for a higher rate of  
11  
12 208 MRSA detection while using ESwab compared with the dry swab. Comparing the recovery of  
13  
14 209 different bacterial species between ESwab and Amies agar gel swab, bacterial recovery was higher  
15  
16 210 with ESwab both quantitatively and semiquantitatively for the subgroup coagulase-negative  
17  
18 211 *Staphylococcus* spp.  
19

20  
21 212 With the swab elution method, the 6- to 9-fold higher bacterial recovery with ESwab compared with  
22  
23 213 the Amies agar gel swab and dry swab respectively was comparable with the reported 9-times higher  
24  
25 214 recovery with ESwab compared with the Venturi Amies agar gel swab from the study of Smismans et  
26  
27 215 al [10]. This means that ESwab has a lower detection limit than the dry swab and the Amies agar gel  
28  
29 216 swab. Therefore, MRSA colonising skin in low numbers and microorganisms in wounds present in  
30  
31 217 low numbers might be more efficiently detected with such a swab system with a better bacterial uptake  
32  
33 218 and release capability. The reason for the significant higher recovery of coagulase-negative  
34  
35 219 *Staphylococcus* spp. with wound ESwab (quantitative method) could be related to the frequency of  
36  
37 220 staphylococcal (burn or orthopaedic) wound infection in combination with the lower detection limit of  
38  
39 221 the ESwab.  
40  
41

42  
43 222 This study had a few limitations. According to routine practice in our laboratory, all swabs were stored  
44  
45 223 at room temperature. The influence of storage temperature was not specifically evaluated. Overgrowth  
46  
47 224 of microorganisms, particularly gram-negative bacilli, in swab systems transported or held at 20-25°C  
48  
49 225 can be a significant problem [9]. At present, none of the commercially available swab transport  
50  
51 226 devices are able to inhibit microbial overgrowth at room temperature [9].  
52

53  
54 227 Because this was a real practice study, we did not include zero-time controls by processing swabs  
55  
56 228 immediately after sampling [9].  
57  
58  
59  
60  
61  
62  
63  
64  
65

229 By using clinical samples, we did not know the initial inoculum density. Therefore, the recovery  
1  
2 230 percentage of the evaluated swab systems could not be determined.  
3  
4 231 Fastidious anaerobic microorganisms might be missed after longer storage (> 6 hrs) at room  
5  
6 232 temperature [1]. For example, the difficulty in recovering *Prevotella melaninogenica* from swab  
7  
8 233 transport systems stored at room temperature is a known problem [1,11]. However, Van Horn et al  
9  
10 234 have previously shown that several anaerobic microorganisms can survive 48 h at room temperature in  
11  
12 235 ESwab [1]. In this study, *Bacteroides* spp. were the only anaerobic microorganisms isolated and this  
13  
14 236 after a swab storage time at room temperature of maximum 3h47 min (data not shown).  
15  
16 237 We found a higher recovery capacity of the ESwab system compared with the dry swab and Amies  
17  
18 238 agar gel swab system. This may increase the likelihood of detecting MRSA and wound pathogens with  
19  
20 239 ESwab. The extra costs of the ESwab should be evaluated against the hospital savings of detecting  
21  
22 240 more MRSA carriers. These savings originate mainly in the avoidance of extended length of hospital  
23  
24 241 stay and more expensive antibiotic treatment due to MRSA infection. Also, in case potential  
25  
26 242 pathogenic microorganisms are recovered from wounds with a lower limit of detection with ESwab  
27  
28 243 than with the conventional Amies agar gel swab, one can initiate proper antibacterial therapy sooner.  
29  
30 244 This possibly can reduce length of hospital stay and patient morbidity/mortality. Finally, the liquid  
31  
32 245 medium makes it possible to perform several tests with one ESwab (f.eg. performing MRSA PCR and  
33  
34 246 culture with the same swab).  
35  
36  
37  
38  
39

#### 40 247 *Conclusions*

41  
42 248 The advantage of the ESwab system is the possibility of performing different laboratory tests (culture,  
43  
44 249 PCR, rapid antigen testing) from a single sample [12]. Avoiding multiple samples reduces sampling  
45  
46 250 bias. Moreover, ESwab is the ideal swab specimen to use in automated plating instruments [13].  
47  
48 251 Gram stains performed with ESwab were superior to the dry swab in terms of number of bacterial  
49  
50 252 morphotypes and human eukaryotic cells visualized. Moreover, the significantly higher bacterial  
51  
52 253 recovery with ESwab compared with dry swab and Amies agar gel swab make it a valuable  
53  
54 254 alternative for MRSA screening and wound sampling.  
55  
56  
57  
58  
59

60 255  
61  
62  
63  
64  
65

256 **Acknowledgements**

1  
2 257 We sincerely thank Rudi Deleus (Intensive Care Unit Burn Wounds), Godelieve Nicaise, Raf Beneens  
3  
4 258 and Bart Vander Elst (Geriatric Medicine), Marleen Van Eygen (General Internal Medicine), and Jan  
5  
6 259 Bosque (Septic Orthopaedics) for their readiness to procure all the clinical samples and Jos Vandeven  
7  
8 260 (Laboratory Medicine) for the help in identification of microorganisms.

10  
11 261 Both the ESwab MRSA collection kits and the conventional ESwabs were kindly provided by Copan.  
12  
13 262

15 263 **References**

- 17 264
- 20 265 1. Van Horn K, Audette C, Sebeck D, Tucker K (2008) Comparison of the Copan ESwab  
21  
22 266 system with two Amies agar swab transport systems for maintenance of microorganism  
23  
24 267 viability. J Clin Microbiol 46(5):1655-1658
  - 27 268 2. Osterblad M, Järvinen H, lönnqvist K, Huikko S, Laippala P, Viljanto J, Arvilomni H,  
28  
29 269 Huovinen P (2003) Evaluation of a new cellulose sponge-tipped swab for microbiological  
30  
31 270 sampling: a laboratory and clinical investigation. J Clin Microbiol 41:1894-1900  
32  
33  
34  
35 271 3. Copan Innovation (2008) ESwab collection and transport system, Product insert and How to  
36  
37 272 Use Guide. Brescia, Italy  
38  
39  
40  
41 273 4. Nys S, Vijgen S, Magerman K, Cartuyvels R (2010) Comparison of the Copan eswab  
42  
43 274 system with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*,  
44  
45 275 *Streptococcus agalactiae* and *Candida albicans*. Eur J Clin Microbiol Infect Dis 29:453-456  
46  
47  
48  
49 276 5. Van Horn K, Audette C, Tucker K, Sebeck D (2008) Comparison of 3 swab transport  
50  
51 277 systems for direct release and recovery of aerobic and anaerobic bacteria. Diagn Microbiol  
52  
53 278 Infect Dis (62):471-473  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

279 6. Body B, Arbique J, Bourbeau P et al (2003) Quality Control of Microbiological Transport  
1 Systems; Clinical Laboratory and Standards Institute CLSI Approved Standard M40-A.  
2 280  
3  
4 281 Pennsylvania, USA Dec. Report No.: M40-A, volume 23, Number 34  
5  
6  
7 282 7. Fontana C, Favaro M, Limongi D, Pivonkova J, Favalli C (2009) Comparison of the eSwab  
8  
9 283 collection and transportation system to an amies gel transystem for Gram stain of clinical  
10  
11 284 specimens. BMC Research Notes 2:244  
12  
13  
14  
15 285 8. Daley P, Castriciano S, Chernesky M, Smieja M (2006) Comparison of flocced and rayon  
16  
17 286 swabs for collection of respiratory epithelial cells from uninfected volunteers and  
18  
19 287 symptomatic patients. J Clin Microbiol 44:2265-2267  
20  
21  
22  
23 288 9. Loens K, Van Heirstraeten L, Malhotra-Kumar S, Goossens H, Ieven M (2009) Optimal  
24  
25 289 sampling sites and methods for detection of pathogens possibly causing community-  
26  
27 290 acquired lower respiratory tract infections. J Clin Microbiol 47(1):21-31  
28  
29  
30  
31 291 10. Smismans A, Verhaegen J, Shuermans A, Frans J (2009) Evaluation of the Copan ESwab  
32  
33 292 transport system for the detection of methicillin-resistant Staphylococcus aureus: a  
34  
35 293 laboratory and clinical study. Diagn Microbiol Infect Dis 65(2):108-111  
36  
37  
38  
39 294 11. Human R, Jones G (2004) Evaluation of swab transport systems against a published  
40  
41 295 standard. J Clin Pathol 57:762-763  
42  
43  
44 296 12. Human R, Jones G (2006) A new concept for transporting clinical material on flocced swabs  
45  
46 297 in liquid Amies medium. ASM, 10<sup>th</sup> General Convention, Florida, USA May 22-24  
47  
48  
49  
50 298 13. Bourbeau P, Swartz B (2009) First evaluation of the WASP, a new automated microbiology  
51  
52 299 plating instrument. J Clin Microbiol 47(4):1101-1106  
53  
54  
55 300

58 301 **Tables and Figures**

302 Table 1. Number of patients and samples  
1  
2 303 Table 2. Comparison of Gram stain with ESwab and dry swab for MRSA/wounds  
3  
4 304 Table 3. MRSA screening results with ESwab and dry swab, 137 paired samples  
5  
6 305 Table 4. Comparison of microorganisms cultured from ESwab and Amies agar gel swab, 203 paired  
7  
8 306 wound culture samples  
9  
10 307 Table 5. Relation between time interval sampling – culture inoculation and MRSA culture results  
11  
12 308 related to the median time interval (0.21 days)  
13  
14 309 Table 6. Relation between swab used first and MRSA culture results  
15  
16 310 Figure 1. Total bacterial counts on Eswab versus dry swab for MRSA screen (expressed in log  
17  
18 311 CFU/mL)  
19  
20 312 Figure 2. Total bacterial counts on Eswab versus Amies agar gel swab for wound cultures (expressed  
21  
22 313 in log CFU/mL)  
23  
24 314 **Supplementary Figures**  
25  
26 315 Figure 3. Bacterial colonies counted semiquantitatively with roll-plate method for MRSA  
27  
28 316 Eswab and dry swab (100 MRSA screening samples)  
29  
30 317 Figure 4. Bacterial colonies counted semiquantitative with the roll-plate method for Eswab  
31  
32 318 and Amies agar gel swab (103 wound samples)  
33  
34 319  
35  
36 320  
37  
38 321  
39  
40 322  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Table 1. Number of patients and samples

	MRSA screening		Wound specimen	
Number of patients				
General wards	164			
Burn Care Unit	36		178	
Septic Orthopaedics			25	
Number of samples	Eswab	2 dry swabs	Eswab	dry swab / Amies agar gel swab <sup>a</sup>
Gram stain	200	200	203	203
Swab elution method	100	100	100	100
Roll-plate method	100	100	103	103

<sup>a</sup>The dry swab was used for Gram staining, the Amies agar gel swab was used for bacterial recovery tests

Table 2

Table 2. Semiquantitative evaluation of Gram stains with ESwab and dry swab for MRSA/wounds. The data presented are the number of Gram stains with a specific categoric quantity (+/-, +, ++) of bacterial morphotypes and eukaryotic cells per high power field.

Number of Gram stains	Bacterial morphotypes (N = 403 paired samples)					Eukaryotic cells (N = 350 paired samples)			
	Gram negative rods*	Strepto-Cocci*	Gram positive cocci*	Gram positive rods*	Yeast cells	Polymor-pho-nuclear cells*	Lympho-cytes*	Red blood cells*	Epithelial cells
<b>ESwab</b>									
+/-	17	2	37	21	7	10	2	1	43
+	17	10	59	53	10	21	7	6	49
++	27	4	25	19	4	15	5	7	5
<b>Dry swab</b>									
+/-	20	4	48	19	6	15	2	2	65
+	18	3	32	46	4	14	2	3	49
++	9	1	8	10	5	7	1	2	7

\*Significant more morphotypes/eukaryotic cells on ESwab than on dry swab Gram stains (p<0.006)

Table 3. MRSA screening results with Eswab and dry swab,  
137 paired samples

	Culture results	
	MRSA positive	MRSA negative
Eswab	128	9
Dry swab	124	13

Table 4. Comparison of microorganisms cultured from Eswab and Amies agar gel swab with the quantitative swab elution method and the semiquantitative roll-plate method

Quantitative swab elution method				
Microorganisms isolated	Eswab + , Amies agar gel swab +	Eswab + , Amies agar gel swab -	Eswab - , Amies agar gel swab +	p-value (*significant difference)
Coagulase-negative <i>Staphylococcus</i> spp.	26	18	3	0.001*
<i>Staphylococcus aureus</i>	4	2	2	1.000
<i>Enterococcus</i> spp.	7	8	0	0.005*
<i>Streptococcus</i> spp.	0	3	1	0.317
<i>Corynebacterium</i> spp.	9	1	1	1.000
<i>Enterobacteriaceae</i>	11	4	3	0.706
Non-fermenting bacteria	9	2	1	0.564
<i>Candida</i> spp.	5	0	0	/
Other	4	1	0	0.317
Semiquantitative roll-plate method				
Microorganisms isolated	Eswab + , Amies agar gel swab +	Eswab + , Amies agar gel swab -	Eswab - , Amies agar gel swab +	p-value (*significant difference)
Coagulase-negative <i>Staphylococcus</i> spp.	36	10	5	0.197
<i>Staphylococcus aureus</i>	5	0	0	/
<i>Enterococcus</i> spp.	17	2	2	1.000
<i>Streptococcus</i> spp.	1	1	1	1.000
<i>Corynebacterium</i> spp.	5	5	0	0.025
<i>Enterobacteriaceae</i>	18	2	1	0.564
Non-fermenting bacteria	19	0	2	0.157
<i>Candida</i> spp.	13	1	0	0.317
Other	2	4	0	0.046

Table 5. Relation between time interval sampling – culture inoculation and MRSA culture results related to the median time interval (0.21 days)

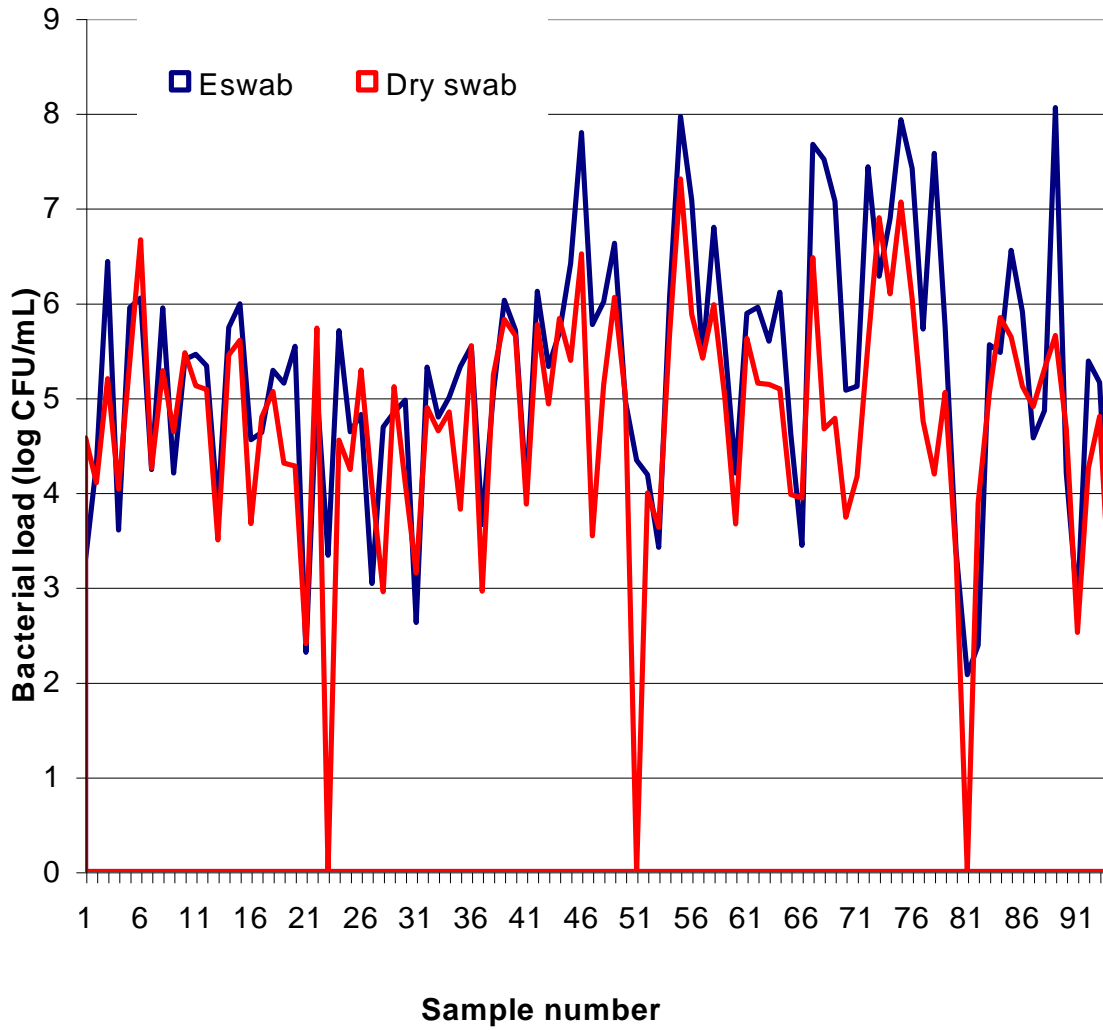
Number of swabs	> 0.21 days	< 0.21 days
Eswab +	63	65
Eswab -	36	34
Dry swab +	60	64
Dry swab -	39	35

Table 6. Relation between swab used first and MRSA culture results

Number of swabs	Eswab first	Dry swab first
Eswab +	67	61
Eswab -	31	40
Dry swab +	64	60
Dry swab -	34	41

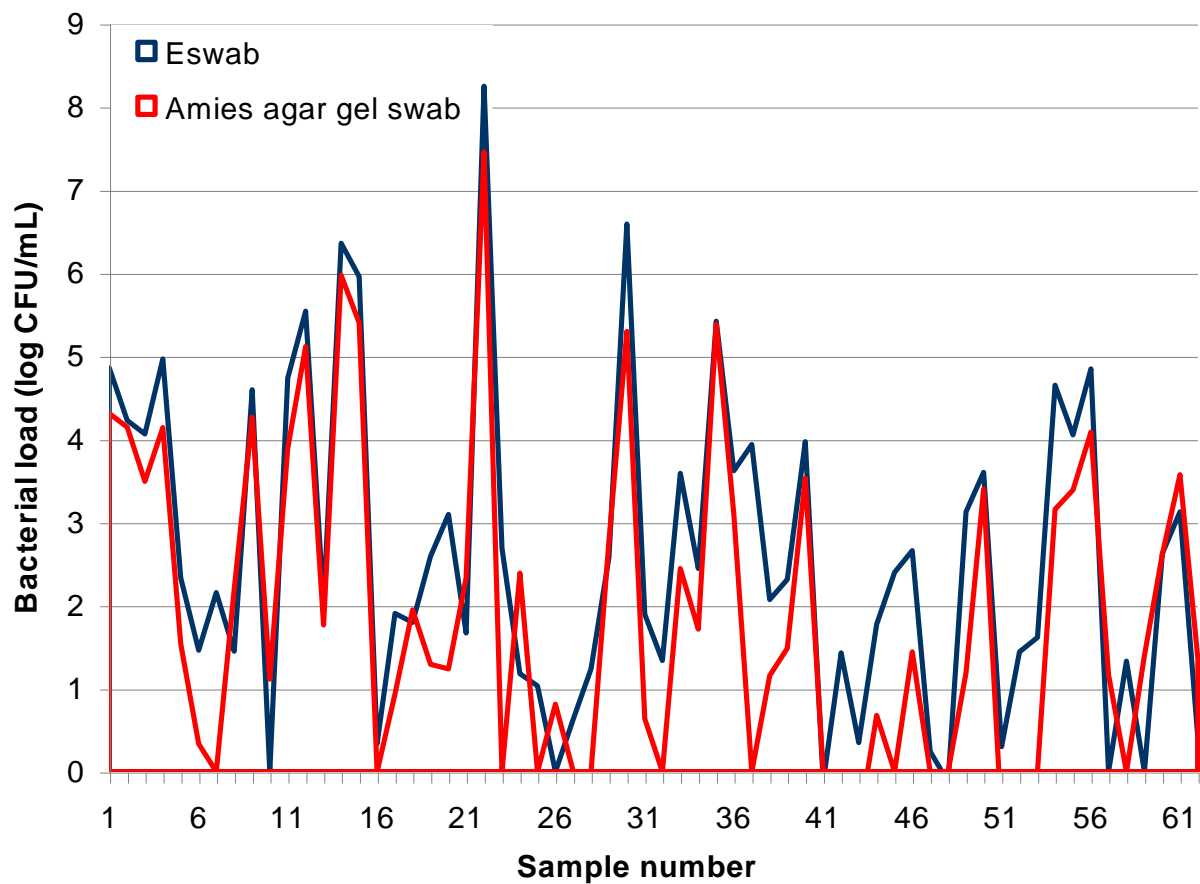
Figure 1

Figure 1. Total bacterial counts on Eswab versus dry swab for MRSA screen (expressed in log CFU/mL)



Remark that there were only 94 sample pairs with growth from at least one of the two swab systems. Swab pairs without growth on neither of the swab systems are not shown on this Figure (N=6).

Figure 2. Total bacterial counts on Eswab versus Amies agar gel swab for wound cultures (expressed in log CFU/mL)



Remark that there were only 62 sample pairs with growth for at least one of the two swab systems. Swab pairs without growth on neither of the swab systems are not shown on this Figure (N=38).

**Electronic Supplementary Material**

[Click here to download Electronic Supplementary Material: Figure 3.doc](#)

**Electronic Supplementary Material**

[Click here to download Electronic Supplementary Material: Figure 4.doc](#)