CAT
Critically Appraised Topic

Comparison of Eswab with dry swab and Amies swab in maintaining viability of microorganisms and comparison of Eswab with dry swab for Gram stain quality

Author: Veroniek Saegeman
Supervisor: Prof. dr. Jan Verhaegen
Search/methodology verified by: Prof. dr. Pieter Vermeersch
Date: 19-05-2010

CLINICAL BOTTOM LINE

One of the routine procedures in the screening for and diagnosis of bacteriological infections involves the collection and safe transportation of swab samples. Both the swab tip material and the transport medium must be made of material that is not toxic for microorganisms. Recently, a new swab technology, the Eswab (Copan Liquid Amies Elution Swab) collection and transport system has been introduced onto the market. The secret of this swab is a tip flocked with soft nylon fiber providing stronger capillary action and strong hydraulic uptake of liquids. This should result in better specimen collection on the one hand and in more efficient release of specimen material than occurs with cotton/rayon or Dacron fiber-tipped swabs. From the available literature, it is obvious that Eswab design indeed leads to a better bacterial recovery. Another advantage of the Eswab system is the use of a liquid Amies medium and its multiple uses: both culture, rapid antigen testing and PCR can be performed with one collected sample.
At UH Leuven, we will investigate this Eswab system (n = 500) in comparison with 1) the dry Copan swab for MRSA screening and 2) the blue swab in Amies gel transport medium for sampling wounds from burn wound and septic orthopaedic patients. Preliminary results confirmed the literature data: statistically significant higher bacterial recovery with Eswab, leading to a higher MRSA detection rate. Gram stains performed with Eswab liquid have a better quality i.e. more bacteria, more bacterial morphotypes and more human cells visualised than Gram stains performed with a dry Copan swab.
In conclusion, in case the financial implications of switching to the Eswab transport system would be comparable with the conventional swab systems, the use of Eswabs is advisable.

CLINICAL/DIAGNOSTIC SCENARIO

Selection and collection of an appropriate specimen and maintenance of microorganism viability during transport to the microbiology laboratory are critical for appropriate isolation and identification of potential pathogens from that clinical specimen [1]. Specimens are selected according to the affected body site and the collection process must utilise appropriate techniques to minimise exposure to commensal flora [1]. The sample collection device should be chosen in such a way that the most appropriate specimen sample may be collected [1]. Specimens that may be collected include tissue by biopsy, needle aspiration of exudates/fluid/drainage, and swab for certain anatomic sites (mucosal surfaces, wound secretions and perineal skin) [1, 2]. The swab collection method, although not ideal, is frequently used to collect material that could be collected more optimal with biopsy or aspiration [1]. The swab transport system has gained importance recently in regards to transport delays [1], patient discomfort, time saving compared with taking a biopsy.
For the assessment of swab transport systems, it is important to check that i) the swab tip material and ii) the transport medium are made of materials sufficiently non-toxic or non-inhibitory to maintain microorganism viability throughout the collection and transport process [1]. i) There are several types of swab tip systems: cotton, Dacron, and rayon swabs only absorb bacteria onto their surface, thereby enmeshing them in their dense fiber matrix. Release of bacteria therefore gets compromised [3]. Moreover, there is often only scarce material present on the sampling surface [2]. There should be at least 10^6 bacteria present on the swab tip to be able to visualise them with microscopy [2]. The bacteria usually stay on the swab until they are cultured by physically rubbing the swab on the culture plate [4]. This design is inherently flawed as many bacteria stay trapped inside the swab fiber matrix[4-6]. In optimal

conditions, maximal 10% of the bacteria present on the swab will be cultured [2]. Dacron or rayon tipped swabs are considered optimum while cotton has been shown to be inhibitory to some microorganisms [1].
ii) Bacteria will soon die on dry swabs, partly by oxidation and drying. Transport media therefore offer a better protection of bacterial viability. In traditional transport swab systems such as Stuart's medium, the fiber swab is kept moist by surrounding or submerging it in a buffered half-solid salt medium without nutritional substances but with thioglycolate as reducing substance for maintenance of anaerobic microorganisms [2,4]. The Amies transport medium additionally contains charcoal which inactivates toxic substances [2].
A new type of swab system has recently been introduced onto the market and has been introduced in a growing number of laboratories. This Copan Eswab™ consists of a screw-cap tube filled with 1 ml of Liquid Amies medium and a small peel pouch containing a specimen collection swab which has a tip flocked with soft nylon fiber [7]. This design provides a stronger capillary action and strong hydraulic uptake of liquids, which should result in better specimen collection [8, 9]. This technology would also release specimen material more efficiently and, therefore, entrap specimen less than occurs with typical rayon or Dacron fiber-tipped swabs [8, 9]. According to the product insert, the Eswab may be stored at room temperature (RT) (20-25°C) or in the refrigerator (4-8°C) and processing may be delayed up to maximally 48 hours [7].

The aim of this study was to evaluate this new Eswab technology for two conditions regularly leading to the use of a swab: i) MRSA screening with dry swabs taken from anterior nares and perineum, the predilection area of *Staphylococcus aureus* and ii) patients with burn or orthopaedic wounds which are sampled with a swab because too little material is available for needle aspiration/drainage.
Methicillin resistant *Staphylococcus aureus* (MRSA) is a major health problem in hospitals because these bacteria are resistant to most routine antistaphylococcal antibiotics. A substantial part of the hospital population (1/1000 patient days in 2006 [10]) is MRSA carrier although without being ill. Invasive MRSA infections count for 1/1000 hospital admissions [11]. In order to prevent hospital infections with MRSA, it is important to detect and decontaminate patients who are carrier of this microorganism. In UH Leuven, 4000 to 5000 MRSA screening tests are performed monthly in the microbiology laboratory. On average 3% of these tests are positive for MRSA.
Burn wounds are regularly swabbed with the aim of having a background knowledge of the colonising wound flora. In case the patient would become septic, one can rely on these microorganisms to initiate antibacterial therapy. Patients with infected open/fistulated bone wounds are frequently swabbed with the ultimate aim of finding the causative microorganism. Antibiotic therapy will depend on the susceptibility pattern of this microorganism.
All this makes it easy to understand that a swab system with a maximal absorption and a high efficiency in releasing microorganisms would be very helpful in search for microorganisms in these specific conditions.

In particular, this study compared the Eswab™ (Eswab for wounds and Eswab MRSA collection kit for MRSA screening) with the conventional blue swabs in Amies transport medium (International Medical Products, Brussels, Belgium) and with the red dry Copan swabs (wooden applicator, cotton tip)(150C, Copan, USA) respectively.

The following hospital units of UH Leuven took part in gathering the samples: Department of Septic Orthopaedics (E231, UH Pellenberg) and Burn Care Unit (E519) for wound Eswabs, Department of Geriatric Medicine (E640, E641, E455; E230 (UH Pellenberg), Department of General Internal Medicine (E454), Burn Care Unit (E519) for MRSA screening with Eswabs.

The recommendations of the CLSI guideline M40-A: Quality Control of Microbiological Transport Systems were used for this evaluation [12].
Bacterial recovery (CFU/mL) of the paired swabs 'MRSA Eswab – Copan dry swab' and 'wound Eswab – swab in Amies transport medium' was compared and the difference in the spectrum of species recovered with both systems was assessed. Factors possibly influencing the outcome of the culture are 1) time interval between sample collection and processing and 2) which type of swab system was used first to sample the body site. Both factors were registered and their influence evaluated. Remark that all swabs were stored at room temperature. In practice, swabs from clinical samples are transported to the laboratory at room temperature immediately after retrieval and they are processed during the day. Swabs taken in the evening/night are stored at room temperature too until the next morning. Accordingly, influence of storage temperature was not evaluated specifically.

A Gram stain has its relevance in the rapid presumptive diagnosis of infectious agents and serves to assess the quality of clinical specimens [13]. To evaluate Eswab compliance with the Gram stain, Gram stain smears prepared from the dry Copan swab and the Eswab system were compared in terms of number of bacteria, number of bacterial morphotypes, and presence of human cells.
Next to the diagnostic performance of the Eswab system, the clinical and organisational impact and relative costs of Eswab were compared with those of the conventional swab systems.

QUESTION(S)

1) Are there previous studies performed with Eswabs?
1.1) Evaluation of bacterial recovery
1.2) Gram stain
2) UH Leuven study protocol:
2.1) Is the minimal detection limit for microorganisms better with Eswab than with the conventional Copan dry swab and the conventional swab on Amies transport medium?
2.2) With which swab system, bacterial recovery is highest?
2.3) Are there bacterial species which are missed by a particular swab system?
2.4) Is there a difference in the quality of the Gram stain of the Eswab and the Copan dry Swab?

SEARCH TERMS

1) *MeSH Database (PubMed): MeSH term: "[bacteriological techniques] and[specimen handling] and [comparison] and swab (no MESH term)"*
2) *PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters: (bacteriological techniques and specimen transport) AND (Diagnosis/Narrow[filter]); bacteriological techniques and specimen transport) AND (Diagnosis/Broad[filter]);*
3) *Pubmed (Medline; from 1966), SUMSearch (http://sumsearch.uthscsa.edu/: search term: "microbiology and transport system" "Copan and transport system", National Guideline Clearinghouse (http://www.ngc.org/), Institute for Clinical Systems Improvement (http://www.icsi.org), The National Institute for Clinical Excellence (http://www.nice.org.uk/), Cochrane (http://www.update-software.com/cochrane, Health Technology Assessment Database (http://www.york.ac.uk/inst/crd/htahp.htm)*
4) *National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/), Clinical Laboratory Improvement Amendments (CLIA; http://www.cms.hhs.gov/clia/)*
5) *Copan website: http://www.copaninnovation.com/studies/index.php?topic=4&year=2009*
6) *UpToDate Online version 17.3 (2009)*

1) Are there previous studies performed with Eswabs?

1.1) Evaluation of bacterial recovery

From all studies described in Table 1 (Attachment 1), it is clear that Eswab has an equal or even better recovery of most microorganisms compared with other swab systems. More details are given in the table.

Characteristics of Eswab

- With electron microscopy, Human and Jones showed that bacteria adhere to the fibers of the flocked swab while this was more difficult to demonstrate in conventional swabs; presumably because bacteria were absorbed onto the surface and enmeshed/trapped within the dense fiber matrix [14]. The brush-like flocked nylon fiber traps bacteria by capillary action.
- Human and Jones showed by means of radioactive tracers that flocked swabs release much more particles (92% of the initial inoculum) than conventional cotton wool/rayon swabs (30% of the initial inoculum) [14]. Numerous studies confirmed this better release of bacteria because i) the zero-time counts of Eswab were higher than of the other swab systems tested [1, 9, 15-21] and ii) CT values of RT-PCRs were similar to that of the initial inoculum when using Eswab [19].
- Since the Eswab system provides increased organism release, this would theoretically mean that a greater number of all organisms including pathogens and commensal microorganisms would be recovered. Infections with a low bacterial load might be detected more efficiently if collected with such a swab with enhanced releasing capability [20].
- Another advantage of the flocked swab in liquid medium is that every inoculated plate receives the same inoculum. Conventional swabs, when used to plate a number of agar plates, result in less inoculum on the last plate [14].
- A previous study of Moore et al showed that dry flocked swabs could keep viral RNA stable up to 15 days storage at room temperature [22]. Moreover, the amount of nucleic acid released from the swab is highly consistent over the time [22]. This study indicates that flocked swabs can reliably be used for molecular testing.

Acceptance criteria for Swabs

The performance of swab systems can be evaluated by means of the CLSI document M40-A: 'Quality Control of Microbiological Transport Systems; Approved Standard' [12]. When using the qualitative roll-plate method, a viability study with a swab system is considered acceptable when there are ≥ 5 CFU following the storage time from the plate with the same dilution as that with a zero-time count of +/- 300 CFU [12]. When using the quantitative swab elution method, a viability study with a swab system is considered acceptable if there is no more than a 3 $\log_{10}$ ($1 * 10^3$ +/- 10%) decline in CFU between the zero-time CFU count and the CFU of the swabs that were stored [12].
In the Copan Eswab product insert, all CLSI acceptance criteria (qualitative and quantitative) were met for *Haemophilus (H.) influenzae, Streptococcus (S.) pyogenes, S. pneumoniae, Pseudomonas (P.) aeruginosa, Neisseria (N.) gonorrhoeae* (24 hrs storage), *Peptostreptococcus (P.) anaerobius, Bacteroides (B.) fragilis, Fusobacterium (F.) nucleatum, F. necrophorum, Propionibacterium (P.) acnes, Prevotella (P.) melaninogenica, Enterococcus (E.) faecalis, Staphylococcus (S.) aureus, S. agalactiae, Clostridium (C.) sporogenes, C. perfringens, Peptococcus magnus* after storage for 48 hrs at 4-8°C and 20-25°C [23].

As is generally known, temperature has a significant effect on preservation of microorganisms in transport devices [12]. Previous studies have shown that cold temperatures are superior to room temperature. Room temperature is not the optimal holding temperature for maximum preservation of microbiological samples [12]. In UH Leuven though, most samples are taken and cultured within a couple of hours thanks to the pneumatic transport system. Therefore, microbiological samples are kept at room temperature until their inoculation. Only for external samples, i.e. UH Pellenberg, prolonged room temperature storage can be a problem. **This aspect will be considered in our study.**

There is no CLSI standard for the evaluation of overgrowth in swab transport systems at room temperature [9, 12]. At present, none of the commercial available transport devices are able to adequately inhibit microbial overgrowth at room temperature [12]. Van Horn et al observed heavy overgrowth of *P.*

aeruginosa after 24 and 48 hrs storage at room temperature with both the Eswab, BD CultureSwab MaxV and Remel BactiSwab system [9].

Anaerobic microorganisms

Caution is warranted in using swab systems such as Eswab not specifically designed for transport of specimens for anaerobic culture. Fastidious organisms might be missed after longer storage (> 6 hrs) at room temperature [9].

For example, the difficulty in recovering *P. melaninogenica* from swab transport systems stored at room temperature is a known problem [24]. Van Horn et al found that *P. melaninogenica* did not survive storage for more than 6 hrs at room temperature with neither of the swab systems tested: Eswab, BD CultureSwab MaxV, nor Remel BactiSwab [9]. However, Biggs[25, 26] and Sarina et al[25, 26] could recover *P. melaninogenica* after 48 hrs of storage in Eswab at room temperature.

Concerning *Peptostreptococcus anaerobius, Bacteroides fragilis, Fusobacterium nucleatum*, and *Propionibacterium acnes* literature is also contradictory (cfr table 1). Additional studies are warranted concerning Eswab and survival of anaerobic organisms.

MRSA

According to the study of Smismans et al, the recovery of MRSA with the Copan Eswab exceeded that of the Venturi Copan swab system by a factor of at least 3.6 [6]. Moreover Eswab had a 1 to 2 log higher recovery of microorganisms on MRSA chromogenic agar plate than Stuart liquid transystem (Copan)[27, 28]. This means that the theoretic chance of detecting MRSA increases too.

The MRSA Eswab collection kit (Copan) of nares, throat and axilla was fully concordant with the traditional Amies agar gel swab (Copan) of nares in terms of positive MRSA samples with culture and pre-enrichment culture on CHROMagar MRSA and PCR [29]. This pooling of specimens from multiple sites limits the test costs and broadens the screening capabilities [29]. Martens et al [30] validated the pooled MRSA Eswab from nose, throat and perineum for use instead of the nose, throat and perineum samples taken by a double Copan swab (cepheid collection device). Eswab proved to be a valid alternative as input for the Xpert MRSA® assay run on the GeneXpert® system (Cepheid) [30].

Since culture and PCR can be performed on the same sample, thanks to the 1 ml liquid Amies medium of the Eswab, sampling bias is reduced and the need for taking several clinical samples is eliminated [28, 30, 31].

1.2) Gram stain

Only one study compared the quality of Gram stains performed with different swab systems: Nylon flocked Eswab in liquid Amies medium (Copan) versus Rayon swab in Amies gel transystem (Copan). Eighty pairs of swabs were taken from vaginal, cervical, urethral, and wound specimen. Gram stains from Eswab (100 µl) had superior quality in terms of number of bacteria per slide, number of bacterial species visualised as morphological cell detail: more distinguishable morphology of bacteria (especially diplococci) and better details of human cells such as leucocytes and red blood cells [13, 32]. Microscopic examination of slides prepared from Eswab at 24 or 72 hrs after initial collection was equivalent to microscopy of slides prepared within 2 hrs of collection [32].

Daley et al reported a statistically better yield in respiratory epithelial cells by direct fluorescent antibody staining and accordingly also more infected epithelial cells with the use of a flocked swab system compared with rayon swabs [33, 34]. There was a two- to threefold increase in cell yield, which could result in an increased diagnostic sensitivity [33]. This finding indicates that epithelial cells and probably also polymorphonuclear cells could be taken up better with this new flocked Eswab system than with the conventional rayon-tipped swabs.

Although pernasal flocked swabs in universal transport medium recovered less epithelial cells than a nasopharyngeal aspirate in the study of Abu-Diab et al, pernasal flocked swabs collected and released enough cells to be detected by means of direct fluorescent antibody staining for respiratory viruses [22, 35].

2) UH Leuven study protocol

Previous studies [9, 20] have focused on the CLSI M40-A protocol using a high initial inoculum for testing the swab systems. In the UH Leuven study (cfr attachment 2), we wanted to test the Eswab system with clinical samples and therefore probably also with low numbers of microorganisms. In this way, the release capability of the Eswab system could be evaluated.

Limitations of the study

- By using clinical samples, we do know the initial inoculum density. Therefore, the recovery percentage of the Eswab/conventional swab systems could not be determined.
- We did not include swabs utilised as zero-time controls [12]. This was not possible for this real practice study, swabs could not be processed immediately after being taken.
- Overgrowth of microorganisms, particularly gram-negative bacilli, in swab systems transported or held at 20-25°C continues to be a significant problem [12]. At present, none of the commercially available swab transport devices are able to adequately inhibit microbial overgrowth at room temperature [12].

2.1) Is the minimal detection limit for microorganisms better with Eswab than with the conventional Copan dry swab and the conventional swab on Amies transport medium?

From our clinical study, we determined the difference in recovery between Eswab and the conventional dry swab (97 paired samples) and swab in gelatinous Amies transport medium (52 paired samples). The recovery with the Eswab on average was 9 times higher than with the red dry swab and 6 times higher than with the swab in Amies gel transport medium. It is to be expected that when the recovery is higher with the Eswab system, the chance to detect an MRSA strain or potential pathogenic microorganism is also higher. Indeed, in our study, Eswab had a higher recovery of MRSA (although not statistically significant) than the dry swab and a higher recovery of potential pathogens than the swab in gelatinous Amies transport medium (cfr 2.3).

Overall, there were 1 Eswab versus 4 of the paired dry swabs and 20 Eswabs versus 25 of the paired swabs in gelatinous Amies medium without growth. This indicates that the minimal detection limit of the Eswab is lower than for dry swab and for the swab in gelatinous Amies medium.

Smismans et al reported a 4-and 9-times higher recovery with Eswab compared with the Venturi swab in the roll-plate and the swab elution method respectively [6]. This means that the inoculum on the Venturi swab has to be 4- to 9-fold higher to reach a similar detectable growth as with the Eswab [6].

In the study of Giambra and Castriciano, the sensitivity limit was better with the Copan Eswab than with Stuart liquid transystem [28]. This means that MRSA could be detected up to higher dilutions with Eswab compared with Stuart liquid transystem for both culture and molecular techniques [28].

2.2) With which swab system, bacterial recovery is highest?

There was a statistically significant higher bacterial recovery with Eswab compared with Copan dry swab (for MRSA screening) and swab in gelatinous Amies transport medium (for wounds) (paired t-test of log transformed data, *GraphPad Prism 4*, $p < 0.01$).
There was no statistical significant relation between i) time interval between sampling and culture (Chi square test of independence, Excel software, $p > 0.01$, Table 2) or ii) swab system used first and the MRSA culture result (Chi square test of independence, Excel software, $p > 0.01$, Table 3).
There is no effect of time delay until culturing for neither the Eswab nor the conventional dry swab. This implicates that storage time at room temperature is independent of the culture result: MRSA positive or negative.

Table 2: Culture results related to time interval to culturing (MRSA screen) – data for 95 samples

Eswab \ time	Mean storage time at RT > 9 h 24 min	Mean storage time at RT < 9 h 24 min	
MRSA +	15	43	58
MRSA -	15	22	37
	30	65	95

Chi square, p: 0.13

Dry swab \ time	Mean storage time at RT > 9 h 24 min	Mean storage time at RT < 9 h 24 min	
MRSA +	15	42	57
MRSA -	15	23	38
	30	65	95

Chi square, p: 0.61

Table 3: Culture results related to swab type used first (MRSA screen) – data for 96 samples

Eswab \ swab first used	Eswab	dry swab	
MRSA +	32	26	58
MRSA -	14	24	38
	46	50	96

Chi square, p: 0.08

Dry swab \ swab first used	Eswab	dry swab	
MRSA +	31	26	57
MRSA -	15	24	39
	46	50	96

Chi square, p: 0.13

2.3) Are there bacterial species which are missed by a particular swab system?

MRSA swabs

The dry swab missed 4 MRSA strains, while the Eswab missed 3 MRSA strains on 97 paired samples. This difference was not significantly different (McNemar Chi square test of paired data, Excel software, p=0.75).

Wound swabs

In 17 of the 52 paired samples, the results between both swab systems were discordant. *Staphylococcus* spp. were significantely missed by the swab in gelatinous Amies transport medium. Remark that in 5/52 samples, the swab system in gelatinous Amies transport medium also missed *Enterococcus* spp. (Table 4).

Table 4: Comparison of isolated species on Eswab versus swab in Amies transport medium

	Gram negatives	*Staphylococcus* spp., *Micrococcus* spp.	*Enterococcus* spp., *Streptococcus* spp.	*Corynebacterium* spp.
Eswab positive, gelatinous Amies medium negative	2	10	7	0
Eswab negative, gelatinous Amies medium positive	3	1	1	1
p-value (Mc Nemar Chi square)*	0.65	0.0067	0.034	0.32

* p-value < 0.008 is considered statistically significant

In general, Eswab missed less MRSA strains than dry swab and had a higher yield of bacterial species than swab in gelatinous Amies transport medium. Anaerobic microorganisms and fungi unfortunately were isolated by neither of the assessed swab systems.

2.4) Is there a difference in the quality of the Gram stain of the Eswab and the Copan dry Swab?

For both MRSA screening and wound sampling, the Eswab system has an equal or overall better performance than the Copan dry swab system in terms of visualisation of No. of bacterial morphotypes, number of bacteria per high power field (HPF) and a pick-up of eukaryotic cells like epithelial cells and leukocytes (cfr Table 5 and 6). When comparing the 95% confidence intervals of these respective proportions, the range was not overlapping for number of bacterial morphotypes and No.bacteria per HPF for MRSA screening. This means that there was a significant higher number of morphotypes and higher No. of bacteria visualised per HPF on the Eswab slide compared with the Copan dry swab slide for MRSA screening.

Table 5: Comparison of Gram stain with Eswab and Copan dry swab for MRSA

	No. of bacterial morphotypes (%)	No. bacteria/HPF (%)	Other cell types (leukocytes, epithelial cells) (%)
Superiority of dry swab to Eswab	10/97 (10.3)	10/97 (10.3)	8/51 (15.7)
Dry swab equivalent to Eswab	43/97 (44.3)	24/97 (24.7)	35/51 (68.6)
Superiority of E swab to dry swab	44/97 (45.4)	63/97 (64.9)	8/51 (15.7)

Table 6: Comparison of Gram stain with Eswab and Copan dry swab for wounds

	No. of bacterial morphotypes (%)	No. bacteria/HPF (%)	Other cell types (leukocytes, epithelial cells) (%)
Superiority of dry swab to Eswab	2/52 (3.8)	1/52 (1.9)	1/45 (2.2)
Dry swab equivalent to Eswab	48/52 (92.3)	45/52 (86.5)	38/45 (84.4)
Superiority of E swab to dry swab	2/52 (3.8)	6/52 (11.5)	6/45 (13.3)

Clinical and organisational impact

The advantage of the Eswab system is the possibility of performing different laboratory tests (culture, PCR, rapid antigen testing) from a single collected sample [28, 30, 31,17].This implicates that one does not have to collect additional specimens from the patient for performing different tests. Moreover, both culture (MRSA screening) and PCR (Xpert MRSA assay) can be performed from the same sample, thereby reducing sampling bias [30].

Jones et al evaluated the Eswab in an automated system (AccuPAS, Dynacon, UK) for processing clinical swabs [36]. AccuPAS processing of Eswabs has proven to be very efficient in terms of bacterial recovery from a sample [36]. Bourbeau and Swartz likewise tested Eswab in a new automated microbiology plating instrument (WASP, Walk Away Specimen Processor). They found no cross-contamination with Eswab tubes and results of manually plated Eswabs were comparable with those worked up with the WASP [37]. The authors noted that specimen in the liquid phase are the only types of swab specimen usable with the WASP. Eswab is in this aspect the only swab with this capability [37].

Until present, there are no guidelines that have implemented this new type of swab transport system. The swab system was introduced onto the market in 2008, literature data are still very limited (6 publications, many posters).

Cost impact

All prices are BTW inclusive.

A dry Copan swab (FA264390) costs: 0.21 euro
A conventional swab with Amies transport medium (International Medical Products – FA570648) costs: 0.32 euro
A Copan ESwab (Liquid Amies medium) costs: 0.83 euro
A Double Copan swab (Venturi Transystem) for MRSA screening by means of PCR and culture costs: 1.37 euro
A Copan MRSA Eswab kit (Amies liquid) for MRSA screening by means of PCR and culture costs: 1.57 euro

Wound swabs:

- 1 dry swab and 1 swab in Amies transport medium: 0.53 euro
- 1 Eswab (Copan): 0.83 euro

MRSA screening includes:

- Swab specimens for culture
  - 2 dry Copan swabs, one from nose and one from the perineum: 0.42 euro
  - 1 Eswab MRSA collection kit (Copan): 1.57 euro
- Swab specimens for PCR + culture
  - 1 Double Copan swab Venturi Transystem: 1.37 euro
  - 1 Eswab MRSA collection kit (Copan): 1.57 euro

The Eswab system is more expensive than traditional wound swabs [37]. When we can reduce the number of swabs to be taken though (by for e.g. performing MRSA PCR and culture with the same swab), it could be possible that the resulting cost equals the present one. Moreover, the better performance of the Eswab would increase the MRSA detection rate. This would include a shorter length of stay and a reduced hospital cost for nosocomial MRSA infections. Therefore, the introduction of the expensive Eswab might be economical for the hospital overall.
The same is true for the wound swab. In case more potential pathogenic microorganisms are recovered with the Eswab, one can initiate proper antibacterial therapy sooner than when the bacterial load has to be higher for detection of that pathogenic microorganism with the conventional swab in gelatinous Amies transport medium. This possibly can reduce hospital stay and patient morbidity/mortality.

TO DO/ACTIONS

1. Finalisation of the study, the predefined number of samples has not been reached yet. This are preliminary results. The study has to be completed with the roll-plate method.
2. Implementation of the Eswab (for wounds and/or MRSA screening), depending on the possibility of a lower cost price of the swab system at MLS.

ATTACHMENTS

Attachment 1

Table 1: Comparison of Eswab with other swab systems in bacterial recovery

Author	Swab systems	medium	Method of comparison	Results
Aerobic and anaerobic microorganisms				
Van Horn K et al [1, 9]	Eswab	Liquid Amies transport medium	CLSI M40-A quantitative elution method	▪ Acceptable recovery of *Haemophilus (H.) influenzae*, *Streptococcus (S.) pyogenes*, *S. pneumoniae*, *Pseudomonas (P.) aeruginosa* after 48 hrs at 4°C and at 24°C ▪ Acceptable recovery of *Neisseria (N.) gonorrhoeae* after 48 hrs at 4°C and after 24 hrs at 24°C ▪ Overgrowth of *P. aeruginosa* at 24 hrs and 48 hrs at 24°C ▪ Acceptable recovery of *Peptostreptococcus (P.) anaerobius*, *Bacteroides (B.) fragilis*, *Fusobacterium (F.) nucleatum* and *Propionibacterium (P.) acnes* after 48 hrs at 4°C and 24°C and *P. melaninogenica* after 48 hrs at 4°C ▪ No overgrowth of anaerobes at 24°C or 4°C after 48 hrs ▪ No recovery of *P. melaninogenica* after 24 – 48 hrs at room temperature
	Becton Dickinson CultureSwabMax V	Amies agar gel		▪ Acceptable recovery of *H. influenzae*, *S. pyogenes*, *S. pneumoniae*, *P. aeruginosa*, *N. gonorrhoeae* after 48 hrs at 4°C and at 24°C ▪ Acceptable recovery of *N. gonorrhoeae* after 48 hrs at 4°C and after 24 hrs at 24°C ▪ Overgrowth of *P. aeruginosa* at 24 hrs and 48 hrs at 24°C ▪ Acceptable recovery of *P. anaerobius*, *B. fragilis*, *F. nucleatum* and *P. acnes* after 48 hrs at 4°C and 24°C and *P. melaninogenica* after 48 hrs at 4°C ▪ No recovery of *P. melaninogenica* after 24 – 48 hrs at room temperature ▪ No overgrowth of anaerobes at 24°C or 4°C after 48 hrs
	Remel BactiSwab (RBS)	Amies agar gel		▪ Acceptable recovery of *S. pyogenes*, *S. pneumoniae*, *P. aeruginosa* except *H. influenzae* (max 6 hrs) after 48 hrs at 4°C and at 24°C ▪ Acceptable recovery of *N. gonorrhoeae* after 48 hrs at 4°C and after 24 hrs at 24°C ▪ Overgrowth of *P. aeruginosa* at 24 hrs and 48 hrs at 24°C ▪ Failure of recovery of *P. anaerobius* ▪ Acceptable recovery of *B. fragilis*, *F. nucleatum* and *P. acnes* after 48 hrs at 4°C and at 24°C ▪ No recovery of *P. melaninogenica* after 24 – 48 hrs at room temperature ▪ No overgrowth of anaerobes at 24°C or 4°C after 48 hrs

Van Horn et al [1, 20]	Eswab	Liquid Amies transport medium	CLSI M40-A roll-plate method	▪ Tenfold or greater recovery (release of microorganisms into the transport liquid) (%) of *H. influenzae, S. pyogenes, S. pneumoniae, P. aeruginosa, N. gonorrhoeae, B. fragilis, F. nucleatum, P. anaerobius, P. melaninogenica, P. acnes** from the Eswab liquid than from the rayon-tipped MaxV and BactiSwab swabs (range of recovery: 60.5 - 87.0%)
	Becton Dickinson CultureSwabMax V	Amies agar gel		▪ Recovery of 4.2 - 9.8 % of the viable organisms* to be released from the swab
	Remel BactiSwab (RBS)	Amies agar gel		▪ Recovery of 5.6 – 14.6 % of the viable organisms* to be released from the swab
Silbert et al [5]	Nylon Eswab (Copan)	Liquid Amies transport medium	Growth recovery of *H. influenzae* by culture (quadrant one and Kirby Bauer protocol)	▪ Eswab was superior to Transystem swab in number of colonies recovered at 0 hrs, 24 hrs and 48 hrs ▪ Recovery of *H. influenzae* up to 1 week at 4°C storage
	Copan Venturi Transystem swab (Copan)	Liquid Amies medium		▪ Recovery of *H. influenzae* up to 48 hrs at 4°C storage
Silbert et al [4]	Copan flocked swab (Copan Diagnostics)	Liquid Amies medium	CLSI M40-A roll-plate method	▪ Flocked swab maintains viability of *H. influenzae, S. pyogenes* and *N. gonorrhoeae* after 48 hrs at 4°C and at room temperature, excellent to maintain bacterial viability ▪ Optimum volume to inoculate: 100 µl
	Copan Venturi Transystem (Copan Diagnostics)	Liquid Amies medium		▪ Copan Liquid Amies swab was superior to Starswab for *H. influenzae, S. pyogenes* and *N. gonorrhoeae* ▪ Copan Liquid Amies swab maintains *N. gonorrhoeae* viability for 24 hrs at room temperature
	StarSwab (Starplex Scientific Inc.)	Liquid Amies medium		▪ Starswab does not maintain *N. gonorrhoeae* viability for 24 hrs at room temperature ▪ Bad recovery of *H. influenzae* after 48 hrs at 4°C and none after 24 hrs at room temperature (compared with Copan swabs)
Sarina et al [26]	Eswab Regular flocked applicator (Copan)	Liquid Amies transport medium	CLSI M40-A roll-plate method	▪ Eswab demonstrated significantly higher counts for all microorganisms (*S. pyogenes, S. pneumoniae, H. influenzae, N. gonorrhoeae, N. meningitidis, F. nucleatum, P. anaerobius, P. melaninogenica*) at 4°C and at room temperature up to 48 hrs storage (superior absorption and release capibility) ▪ Recovery of all tested microorganisms at 4°C and room temperature for 48 hrs
	BBL culture swab™ plus(Becton Dickinson)	Amies clear		▪ Low recovery of *N. meningitidis* and *N. gonorrhoeae* after 48 hrs at 4°C and at room temperature ▪ No recovery of anaerobes after 48 hrs at room temperature ▪ Recovery of *P. melaninogenica* after 48 hrs at 4°C
	StarSwab II (Starplex® Scientific Inc.)	Modified Amies clear		▪ No recovery of *N. meningitidis* and *N. gonorrhoeae* after 24 hrs at room temperature ▪ No recovery of anaerobes after 48 hrs at room temperature ▪ Recovery of *P. melaninogenica* after 48 hrs at 4°C
Condon et al [18]	Copan Eswab	Liquid Amies transport medium	CLSI M40-A roll-plate method	▪ Eswab showed better recovery of *H. influenzae, S. pyogenes, S. pneumoniae* compared with Transystem after 48 hrs at room temperature and of *N. gonorrhoeae* after 24 hrs at 4°C – room temperature ▪ Eswab overall had higher levels of growth than Transystem
	Copan Transystem Swab	Liquid Stuarts medium		▪ Transystem showed better recovery of *S. pneumoniae, S. pyogenes,* and *H. influenzae* after 24 hrs at 4°C

Siryani et al [38]	Copan Eswab	Liquid Amies transport medium	CLSI M40-A roll-plate method	▪ Eswab recovery was better than that of BD Culture swabMaxV(+) and Medical Wire & Equipment Transwab for *S. pneumoniae, H. influenzae, N. gonorrhoeae* and *N. meningitidis*
	BD culture swab MaxV(+) Transystem	Agar gel swab		
	Medical Wire & Equipment Transwab	Agar gel swab		
Allen et al [16]	Copan Eswab	Liquid Amies transport medium	CLSI M40-A quantitative elution method	▪ Recovery of anaerobes (*Clostridium* spp., *Bacteroides* spp., *Fusobacterium* spp., *Peptostreptococcus anaerobius, Finegoldia magna, Veillonella* sp., *Eggerthella lenta, Peptoniphilus asaccharolyticus, P .acnes)* was better after 24/48 hrs storage at 4°C than at room temperature with Eswab ▪ After 48 hrs at 4°C, Eswab (50%) had higher recovery of most anaerobes tested than PAC (28%) ▪ No/poor survival of certain clostridia (e.g. *C. difficile, C. clostridioforme*) and of fastidious anaerobic gramnegative rods (e.g. *Prevotella bivia, Porphyromonas asaccharolytica, Peptoniphilus asaccharolyticus*) both at 24°C and at 4°C
	BD Port-A-Cul (PAC)(Becton Dickinson)	Agar tube		▪ At room temperature after 48 hrs storage, PAC (34%) had better recovery than Eswab (22%) of most anaerobes tested.
Biggs C [25]	Copan Eswab – aerobes and anaerobes	Liquid Amies transport medium	CLSI M40-A roll-plate method	▪ Eswab had a higher recovery than BDS and STS and maintained viability for 24hrs at room temperature of all microorganisms tested (*S. pyogenes, H. influenzae, N. gonorrhoeae, P. anaerobius, P. melaninogenica*)
	Becton Dickinson Culture Swab (BDS) - aerobes	Liquid Stuarts medium		▪ Performance equivalent to Eswab
	Starplex Starswab (STS) - aerobes	Liquid Stuarts medium		▪ STS failed to recover *H. influenzae* after 24 hrs at room temperature
	Becton Dickinson Culture Swab Plus (BDA) - anaerobes	Amies gel		▪ Performance equivalent to Eswab
	Starplex Starswab (STA) - anaerobes	Amies gel		▪ STA failed to recover *P. anaerobius* and *P. melaninogenica* after 24 hrs at room temperature
Davidson et al [19]	Nylon Eswab (Copan)	Liquid Amies transport medium	% recovery with culture and real-time PCR	▪ Eswab allowed greater recovery than Dacron swab for *S. pyogenes* and *H. influenzae.* ▪ Eswab was equivalent in recovery of *N. meningitidis*, *N. gonorrhoeae*, *B. fragilis* and *P. anaerobius* ▪ Eswab was superior to Dacron swab for nucleic acid extraction for all tested microorganisms ▪ Both for Eswab and Dacron swab, *F. nucleatum* had a better survival after 24 hrs storage at 4°C in stead of 24°C
	Dacron Venturi Transystem (Copan)	Amies gel/liquid transport medium		▪ Dacron swab was superior for recovery of *S. pneumoniae* and *F. nucleatum* both at room temperature and at 4°C storage for 24/48 hrs. Remark that for these 2 microorganisms, the initial zero-time count was lower for Eswab than for the Dacron swab. This may have resulted in the lower survival with Eswab.

Rivers et al [39, 40]	Eswab (Copan)	Liquid Amies transport medium	Comparison with gold standard: bedside inoculation of InPouch	▪ *Trichomonas vaginalis* viability: 85.3% sensitivity, 98.35% specificity, 96.7% PPV, 62.9% NPV compared with bedside inoculation ▪ *Trichomonas vaginalis*: No significant differences with UTM from the gold standard in the distribution of concordant and discordant results. ▪ A drop in sensitivity of Eswab culture compared with UTM medium: Eswab not as robust as UTM for use as collection and transport medium for *T. vaginalis*
	Universal transport medium UTM (Copan)			▪ *Trichomonas vaginalis*: 91.2% sensitivity, 97.0% specificity, 93.9% PPV, 95.5% NPV compared with bedside inoculation ▪ Sensitivity of Eswab 16% lower than that of UTM in reovery of *T. vaginalis*
Coleman et al [17]	Eswab (Copan)	Liquid Amies transport medium	Bacterial recovery with CLSI M40-A protocol: quantitative elution method	▪ Bacterial survival was consistently higher with Eswab for *S. pyogenes*, *N. gonorrhoeae*, *H. influenzae*
	Starswab II (StarplexScientific Inc.)			▪ With Starswab, there was no survival of *N. gonorrhoeae* after the zero time point at 4°C / room temperature and no survival of *H. influenzae* after the zero time point at room temperature
Friis-Moller et al [41]	Eswab (Copan)	Liquid Amies transport medium	% positive swabs with direct culture	▪ 128/196 (65%) sets: concordant results between Eswab and charcoal swab in Stuart medium ▪ 62 isolates exclusively isolated with Eswab
	Charcoal swab in Stuarts medium	Stuarts medium		▪ 38 isolates exclusively isolated with charcoal swab in Stuarts medium
Giambra et al [28]	Eswab (Copan)	Liquid Amies transport medium	Microbial viability after 24 hrs at room temperature and 72/96 hrs at 4°C with direct culture	▪ Eswab has a significant higher recovery % (average of 82%) than Amies agar gel transystem and therefore a better sensitivity for *P. anaerobius*, *S. pneumoniae*, *B. fragilis*, *S. agalactiae*, *S. pyogenes*, *H. influenzae*, *P. acnes*, *B. pertussis*, *N. meningitidis*, *S. aureus* (MRSA), *C. albicans*, *A. niger*, *C. sporogenes* ▪ Eswab was able to maintain all 15 strains viable up to 96 hrs storage at 4°C ▪ For 10/15 test strains, more than 50% of the starting inoculum was maintained ▪ *S. pyogenes*, VRE and MRSA showed an increase (< 1 log) in inoculum after 24 hrs storage
	Amies agar gel transystem			▪ Amies agar gel was able to maintain 11/15 stains viable up to 96 hrs storage ▪ For 4/15 test strains, more than 50% of the starting inoculum was maintained ▪ *C. albicans* showed an increase (< 1 log) in inoculum after 24 hrs storage
Human and Jones [14]	Flocked swab (Copan)	Liquid Amies medium	Bacterial recovery	▪ Better release of bacteria (*N. gonorrhoeae*, *P. anaerobius*, *H. influenzae*, *P. melaninogenica*, *S. pneumoniae)* with flocked swab (92%) than conventional M40 swab (30%), higher levels of bacteria on incubated plates ▪ Better recovery of all 5 strains when stored at 4-8°C than at 20-25°C ▪ Better bacterial survival of *N. gonorrhoeae*, *P. anaerobius*, *H. influenzae*, *P. melaninogenica* with flocked swab compared with M40 conventional swab

	M40 (Copan)	Gel medium		▪ Better bacterial survival of *S. pneumoniae* with M40 conventional swab compared with flocked swab after 48 hrs storage both at 20-25°C and at 4-8°C
Nys et al [15, 42, 42]	Eswab (480CE, Copan)	Liquid Amies transport medium	CLSI M40-A quantitative elution method	▪ Better recovery at room temperature of *S. agalactiae*, *E. coli* and *C. albicans* with Eswab (97-100%) compared with Copan Venturi Transystem (86-96%) at time point 0 h and 6 h. ▪ Beyond 6 h at RT, there was proliferation of *E. coli* and *C. albicans* in both transport systems. Therefore, preservation at room temperature is not recommended ▪ Higher recovery with Eswab (> 94%) as compared to Copan Venturi Transystem (77-94%) at 4°C for the three microorganisms tested (0 h – 48 h) ▪ Both transport systems stayed within the 3 $\log_{10}$ decrease limit defined by CLSI.
	Copan Venturi Transystem (108C, Copan Diagnostics)	Amies gel transport medium		
MRSA				
Milburn et al [43]	Eswab (Copan)	Liquid Amies transport medium	% of MRSA positive swabs with direct culture, centrifuged smple for culture and pre-enrichment culture	▪ 87.8% positive swabs for MRSA with neat Eswab medium ▪ 87.8% positive swabs for MRSA with centrifuged Eswab medium ▪ 92.2% positive swabs for MRSA with Eswab medium added to 1 mL selective broth and incubated overnight (but labor intensive, 1 day delay of result)
	Starplex II swab	Clear semisolid Amies medium		▪ 82.2% positive swabs for MRSA
Silbert S et al [29]	Eswab MRSA collection kit (Copan)	Liquid Amies transport medium	% positive swabs with direct culture, pre-enrichment culture, real-time PCR for *nuc* and *mecA* genes	▪ 100% concordance between both swab systems
	Amies agar gel traditional swab (Copan)	Amies agar gel		
Fontana et al [27]	Eswab MRSA collection kit (Copan)	Liquid Amies transport medium	Bacterial recovery with culture	▪ Microbial count with Eswab was 1 to 2 logs higher than with traditional swab (Stuart transystem) ▪ Fully concordant results for MSSA/MRSA with both swab systems
	Conventional Stuart swab system (Copan)	Stuart transystem		
Smismans et al [6]	Eswab (Copan)	Liquid Amies transport medium	CLSI M40-A quantitative elution method and roll-plate method and in vivo study	▪ MRSA recovery of 128% (roll-plate) – 172% (swab elution) after 48 hrs of incubation at 2-4°C ▪ MRSA recovery of 488% (roll-plate) – 468% (swab elution) after 48 hrs of incubation at 20-25°C (overgrowth) ▪ 3.6 – 6 fold higher recovery than the Venturi swab
	Venturi Transystem (Copan)	Amies gel transport medium		▪ MRSA recovery of 61% (roll-plate) – 95% (swab elution) after 48 hrs of incubation at 2-4°C ▪ MRSA recovery of 80% (roll-plate) – 103% (swab elution) after 48 hrs of incubation at 20-25°C
C. trachomatis* and *N. gonorrhoeae				
Castriciano et al [44]	Eswab (Copan)	Liquid Amies transport medium	% positive cultures vs positive PCR	▪ Fully concordant results with both swab systems for *C. trachomatis* (CT) and *N. gonorrhoeae* (NG) ▪ Analytical sensitivity same for Eswab and for PT (same minimal detection dilution: 10^{-6} for CT and 10^{-7} for NG)

	BD ProbeTec™ swab (PT) – nucleic acid amplification			
Chernesky et al [45]	Eswab (Copan)	Liquid Amies transport medium	% positives in APTIMA Combo 2 assay (Gen-Probe) – nucleic acid amplification	▪ Fully concordant results with both swab systems for *C. trachomatis* and *N. gonorrhoeae* (100% sensitivity and 100% specificity for the three swab systems) ▪ Same analytical sensitivity in APTIMA Combo 2 media and Eswab media (same minimal detection dilution 10^{-6} for CT and for NG)
	APTIMA swab (Gen-Probe)	APTIMA buffer		
	Flocked swab into ACM (Copan)	APTIMA buffer		
Hoffman and Berthelsen [46]	Eswab (Copan)	Liquid Amies transport medium	% recovery with culture	▪ No substantial difference in recovery of *N. gonorrhoeae* between Eswab and CSTM, neither after 24 hrs or 48 hrs (ratio of Eswab/CSTM recovery 0.08-64 at 24 hrs) ▪ Considerable and immediate decrease in viability count (DD absorption problem?)
	Charcoal impregnated cotton swab (CSTM)	Modified Stuart's transport medium (Statens Serum Institut)		

Attachment 2

Study protocol UH Leuven 09/2009-05/2010

Materials and methods

Among 50 patients of the unit Septic Orthopaedics (E231) with positive wound culture results, swabs will be taken of these wounds with both swab systems: the Eswab and the combination red Copan swab (Sterilin – Copan) and the conventional blue swab with Amies transport medium (International Medical Products). At the Burn Care Unit (E519), 200 consecutive swabs will be taken in double (same way as for Septic Orthopaedics) of wounds without previous knowledge of the colonisation status of the wound. In this manner, a potential difference can be made between the two swab systems in terms of the minimal detection limit of bacterial load
At the microbiology laboratory, the records were screened each day in search for patients with a positive MRSA screening. Included in the study were those hospital wards with most MRSA screenings: E455 (Geriatrics Medicine, UH Leuven), E640 (Geriatrics Medicine, UH Leuven), E641 (Geriatrics Medicine, UH Leuven), E230 (Geriatrics Medicine, UH Pellenberg), E454 (General Internal Medicine, UH Leuven). Among 200 patients of these wards, a MRSA Eswab and two conventional red Copan swab were taken. At the Burn Care Unit (E519, UH Leuven), 50 consecutive patients were sampled both with the MRSA Eswab and the conventional red Copan swab without previously knowing the patient's MRSA colonisation status. Remark that one patient could participate several times in this study.

Selection of patients
Concerning E231, E640, E641, E455, E230, E454:
As mentioned, these patients are selected with a previous positive wound culture or positive MRSA screening. We tried to include patients with wound cultures positive for a variety of microorganisms: MRSA, CNS, MSSA, gram-negatives, and when possible anaerobic microorganisms. This means that the particular hospitalisation ward should be alerted as soon as possible to take new swabs with both swab systems.
Concerning E519: consecutive patients were selected without previous knowledge of their MRSA/wound colonisation/infection status.

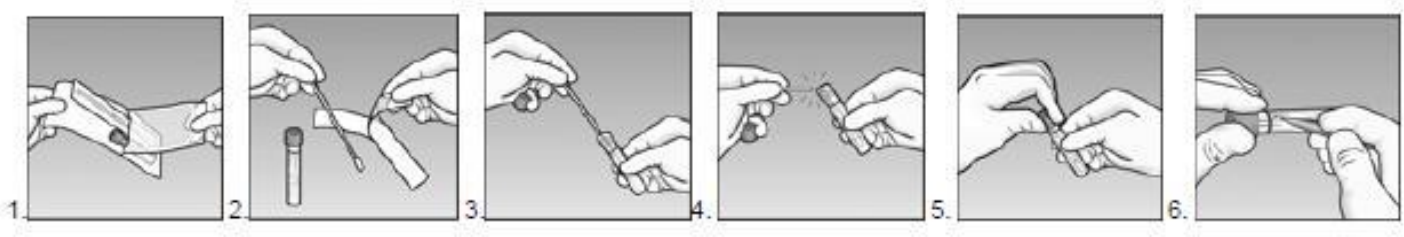
Practical method at the hospital ward for taking the swab

Concerning wound fluids:

- Alternate, the Eswab or the combination -red and blue- swab will be used first to sample the infected/colonised body region
- The three swabs are identified with a patient identification sticker
- On an Excel file, one registers 1) the day and time of sampling and 2) which swab was used first: E (Eswab) or B (Blue swab)

Instructions for specimen collection with Eswab system (cfr Fig.):

- Open the Eswab sample collection pouch and remove the tube and the swab
- Collect the sample from the patient
- Aseptically unscrew and remove the cap from the tube
- Insert the swab into the tube and break the swab at the breakpoint indicated by the colored line marked on the swab shaft. Discard the broken handle part of the swab shaft.
- Replace cap on the tube and secure tightly
- Apply patient identification label on the tube
- Register on Excel file/paper: 1) day and time and 2) swab first used: E swab (E) or blue swab (B)



Instructions for specimen collection with conventional swab systems:
When taking a sample from a wound with the conventional red and blue swabs, one uses a standard aseptic technique. This means that after rinsing the wound, one first uses the dry swab (for Gram stain) followed by the blue swab (for culture). These swabs are taken either immediately before or immediately after the Eswab, and so at nearly the same time.

Both swabs (Eswab, red and blue swab) are sent to the microbiology laboratory UHLeuven (addressed to Veroniek Saegeman). Swab transport is organised by means of the pneumatic transport system or the pendle service (samples from UHPellenberg).

Concerning MRSA screening:
Instructions for specimen collection with Eswab system:
Specific Eswabs are available for MRSA screening. Such MRSA Eswab collection kits comprises of three regular size flocked swabs: one for the nose, one for the throat and one for the perineum. At UH Leuven, only nose (both of the nares, white swab) and perineum (pink swab) are sampled for MRSA screening. One of the swabs accordingly will have to be eliminated from the collection kit. After collecting the perineal pink swab, the swab is dipped and stirred gently for 5 seconds in the liquid Amies medium. Afterwards, the swab is lifted up and swirled against the tube walls to allow release of the sample from the flocked fibre. This perineal swab is removed. The white swab (nose) is taken last and is broken into the tube before recapping the tube.

Instructions for specimen collection with conventional swab systems:
The conventional red Copan swabs are taken as in routine: one swab of both nares and one swab of the perineum.

- Alternate, the Eswab or the red swabs will be used first to sample the infected/colonised body region
- The three swabs are identified with a patient identification label
- On an Excel file, one registers 1) the day and time of sampling and 2) swab first used: E (Eswab) or R (Red swabs)

Both swabs (Eswab and red swabs) are sent to the microbiology laboratory UH Leuven (addressed to Veroniek Saegeman). Swab transport is organised by means of the pneumatic transport system or the pendle service (samples from UH Pellenberg).

Test procedures

Gram staining
At the microbiology laboratory, a Gram stain was performed of both swabs (Eswab and red dry swab). Gram stains of the Eswab were performed according to the manufacturer's instructions (Copan) [7]:

- Vortex mix the ESwab tube containing the swab sample for 5 seconds to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the Liquid Amies transport medium
- Unscrew the Eswab cap and using a sterile pipette, transfer 1-2 drops of Liquid Amies medium on a glass slide
- Allow the specimen on the slide to air dry
- Smears are fixed with heat
- The Gram stain is performed with a Mirastainer® colouring instrument (Merck KGaA)(run 1)

For the preparation of a Gram stain of the wound swabs, the red dry swab is used. This swab is suspended shortly in 1 mL of sterile physiologic saline 0.85% and is streaked onto the surface of a glass slide. In case only a blue swab with Amies transport medium is available, one makes the Gram stain with this swab in the way as was described for the red swab without suspending it in physiologic saline.

Both swabs will be compared (semi)quantitatively according to the 'Swab elution method' on the one hand and the 'roll plate method' on the other hand (CLSI M40-A) [12]. The swab elution method allows a quantitative measurement of the ability of a transport system to maintain viable organisms [12]. The roll plate technique takes into consideration some mechanical variables of the direct swabbing action that exist in the clinical laboratory, and which can influence the release of the sample onto culture plates [12].

Quantitative 'swab elution method'

Quantitative 'swab elution method' for 250 Eswabs (125 MRSA Eswabs and 125 wound Eswabs) and 250 corresponding conventional swabs (125 red dry swabs and 125 blue swabs in Amies transport medium)

In order to include pre-analytical patient variables, we did not inoculate the swabs with a previously known bacterial load (CFU/mL).

The time interval between sampling and processing of the sample is not under our control, this variable was considered in the analysis.

Since swabs taken during the day in general are not stored in the refrigerator, we limited this study to an evaluation of swabs stored at room temperature (20-25°C).

Practical method for quantitative evaluation of swabs:

- The Eswab is suspended in 1mL of Liquid Amies transport medium, vortex mix for 10-15 seconds.
- The conventional blue swab is suspended in a gelatinous Amies transport medium or exists out of two dry red swabs (nose and perineum, MRSA screen). After arrival at the laboratory, the swab(s) is/are suspended in 1 mL of sterile physiologic saline 0.85% during 10-15 seconds by means of vortex mixing. The swab(s) is/are swirled well against the wall of the hemolysis tube (rotating it on the inside of the tube).
- From both suspensions, three tenfold serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) are performed in 1.0 mL of 0.85% physiologic saline (100 µL added to 900 µL), resulting in dilution concentrations of approximately 10^4, 10^3, 10^2, and 10^1CFU/mL.
- Of each of these dilutions, 50 µL is inoculated on a Mannitol Salt agar (MSA) and a MRSA screen agar (dilution 10^{-1}) for the MRSA screening tests and on a blood agar plate (all dilutions), a MSA (dilution 10^{-1}), a MacConkey agar (dilution 10^{-1}), a chocolate agar (dilution 10^{-1}) and a blood agar plate for anaerobic incubation (dilution 10^{-1}) for the wound swabs. Plates are incubated at36°C during 24-48 hours. Remind to vortex mix the diluted suspensions before offering them to the spiral plater (Spiral Biotech, autoplate 4000).
- The total CFU/mL is counted with the IUL Countermat Flash 4.2 automatic reader system.

Semiquantitative 'roll plate method'

For an evaluation of the semiquantitative 'roll plate method', the following procedure is followed for the 250 Eswabs (125 MRSA Eswabs and 125 wound Eswabs) and the 250 corresponding conventional swabs (125 dry swabs and 125 swabs in gelatinous Amies transport medium).

For this semiquantitative evaluation, the CLSI protocol was modified because we expect an overgrowth when streaking a swab over the entire surface of an agar plate.

Plating of the Eswab was performed according to the manufacturer's recommendations [7]:

- Vigorously mix the Eswab tube using a vortex mixer for 5 seconds to release the sample from the swab tip and evenly disperse and suspend the patient specimen in the liquid transport medium
- Unscrew the Eswab cap and remove the swab applicator
- Roll the tip of the Eswab applicator onto the surface of one quadrant of the culture media plate to provide the primary inoculum
- If it is necessary to culture the swab specimen onto a second culture media plate, return the Eswab applicator to the transport medium tube for two seconds to absorb and recharge the applicator tip with transport medium
- Make further streaks starting from the primary inoculum over the second, third and fourth quadrant of the plate
- The total CFU/mL is judged semiquantitatively (-/+/++) after 24-48 hours of incubation at 36°C

The conventional swab system will also be streaked over the first quadrant of the culture plate (MRSA swabs: MSA and MRSA screen agar / wound swabs: Blood agar plate, MSA, MacConkey, chocolate agar, and blood agar plate for anaerobic incubation). With the aid of a sterile bacteriology loop, the primary inoculum is streaked over the second, third and fourth quadrant of the culture agar . After incubation at 36°C for 24-48 hours, the total CFU/mL is judged semiquantitatively (-/+/++).

Analysis

Results of the Gram stains were compared in a semi-quantitative manner.
Culture results were compared both in a qualitative (MRSA positive/negative), semiquantitative and quantitative manner to evaluate the bacterial recovery with both methods. McNemar Chi square (paired qualitative data) and paired t-tests (paired quantitative data) were used for these respective analyses. A p-value < 0.05 was considered significant.
Time interval from sampling up to culture processing, and 2) swab taken first are two factors possibly influencing the results. The influence of these factors on the results will be evaluated with correlation analysis.

Versie 080820: aanpassing sjabloon
Revisie 090827: geen wijzigingen

RELEVANT EVIDENCE/REFERENCES

Reference List

(1) Van Horn K, Audette C, Tom B. Evaluation and comparison of three swab collection and transport systems tested by the CLSI M40-A method. 2006.

(2) Verhaegen J, Vandeven J, Pyckavet M. Pathologische specimens. *Medische microbiologie voor laboratoriumtechnologen, deel 1*. 2 ed. Leuven: ACCO; 2008. p. 4-14.

(3) Osterblad M, Jarvinen H, loonqvist K et al. Evaluation of a new cellulose sponge-tipped swab for microbiological sampling: a laboratory and clinical investigation. *J Clin Microbiol* 2003;41:1894-900.

(4) Silbert S, Pereira A, Monteiro J, Marques F, Fernandes T, Pignatari A. Comparative study of the ability of a new and novel swab transport system to maintain viability of clinically important aerobes at room and refrigerator temperatures. 2006.

(5) Silbert S, Pereira A, Paschoal L, Monteiro J, Pignatari A. Recovery of *Haemophilus influenzae* (HI) using the new EswabTM transport system. 2008.

(6) Smismans A, Verhaegen J, Shuermans A, Frans J. Evaluation of the Copan ESwab transport system for the detection of methicillin-resistant *Staphylococcus aureus*: a laboratory and clinical study. *Diagn Microbiol Infect Dis* 2009;65(2):108-11.

(7) Copan Innovation. Eswab collection and transport system, Product insert and How to Use Guide. 2008. Italy.

(8) Copan. Why flocked swabs are superior to fiber wrapped swabs and foam swabs and how they can improve infectious disease diagnosis. 2010. Brescia, Italy, Copan Innovation.

(9) Van Horn K, Audette C, Sebeck D, Tucker K. Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. *J Clin Microbiol* 2008;46(5):1655-8.

(10) Dhondt E, Duerinckx R, Laes I, Schuermans A. Impact of a MRSA search and destroy policy in a tertiary care emergency department. 2006.

(11) Schuermans A. MRSA incidence. 2010.

(12) Body B, Arbique J, Bourbeau P et al. Quality Control of Microbiological Transport Systems; Approved Standard. Pennsylvania, USA; 2003 Dec. Report No.: M40-A, volume 23, Number 34.

(13) Fontana C, Favaro M, Limongi D, Pivonkova J, Favalli C. Gram-stain by a new specimen collection system. 2009.

(14) Human R, Jones G. A new concept or transporting clinical material on flocked swabs in liquid Amies medium. 2006.

(15) Nys S, Vijgen S, Magerman K, Cartuyvels R. Comparison of the Copan eswab system with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*. 2009.

(16) Allen S, Kedra J, Siders J. Evaluation of a flocked nylon swab transport system (Copan Eswab) for maintaining viability of anaerobes. 2009.

(17) Coleman S, Shah-Khan M, Sautter R, Bahrani-Mougeot F. Comparative study of the ability of new Copan Eswab (Liquid Amies transport system) with another swab transport system for maintaining viability of clinically important aerobic bacteria. 2007.

(18) Condon S, Ginocchio C. Comparison of the Copan Transystem swab and the Copan Eswab for the preservation and recovery of fastidious bacteria. 2007.

(19) Davidson J, DeFields M, Bergstrome A, Pienaar C, Martinez L, Kelly M. Evaluation of flocked nylon swabs (Copan Eswab) for detection of bacterial pathogens by culture and PCR. 2007.

(20) Van Horn K, Audette C, Tucker K, Sebeck D. Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria. *Diagn Microbiol Infect Dis* 2008;(62):-471.

(21) Giambra A, Castriciano S, Paroni R. Copan Eswab, the first liquid based microbiology device, preserves microbial viability up to 96/120 hrs. 2009.

(22) Moore C, Corden S, Sinha J, Jones R. Dry cotton or flocked respiratory swabs as a simple collection technique for the molecular detection of respiratory viruses using real-time NASBA. *J Virol Methods* 2008;153:84-9.

(23) Copan. Eswab collection and transport system? Product insert and how to use guide. 2006. Brescia, Italy.

(24) Human R, Jones G. Evaluation of swab transport systems against a published standard. *J Clin Pathol* 2004;57:762-3.

(25) Biggs C. Evaluation of Eswab a new multipurpose liquid swab transport system for aerobic and anaerobic bacteriology. 2007.

(26) Sarina M, Frost M, Middleton R. Comparative evaluation of three transport swabs potential to maintain viability of clinically important aerobic and anaerobic organisms. 2009.

(27) Fontana C, Favaro M, Martora P et al. Comparison of the Eswab (Copan) to the traditional swab for screening patients for methicillin-resistant *Staphylococcus aureus* (MRSA) colonization by culture and by GeneOhmTM MRSA assay (Becton & Dickinson). 2008.

(28) Giambra A, Castriciano S. Evaluation of the ESwab for MRSA screening with real-time PCR, BD GeneOhm and culture. 2007.

(29) Silbert S, Carballo Menezes L, Monteiro J, Celle Ribeiro M, Pereira S, Pignatari A. Screening of MRSA: a comparison between one and multiple sites sample collection using the new Eswab MRSA collectiion kit. 2009.

(30) Martens K, De Beenhouwer H, Frans J, Van den Abeele A, Cartuyvels R, Coppens G. Evaluation of eSwab™ for surveillance of MRSA by Xpert MRSATM and culture on pooled samples. 2009.

(31) Silbert S, dos Santos Pereira A, Marques da Silva F et al. Complete MRSA nasal screening using a single, new and novel swab transport system. 2007.

(32) Fontana C, Favaro M, Limongi D, Pivonkova J, Favalli C. Comparison of the eSwab collection and transportation system to an amies gel transystem for Gram stain of clinical specimens. *BMC Research Notes* 2009;2(244).

(33) Daley P, Castriciano S, Chernesky M, Smieja M. Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients. *J Clin Microbiol* 2006;44:2265-7.

(34) Loens K, Van Heirstraeten L, Malhotra-Kumar S, Goossens H, Ieven M. Optimal sampling sites and methods for detection of pathogens possibly causing community-acquired lower respiratory tract infections. *J Clin Microbiol* 2009;47(1):21-31.

(35) Abu-Diab A, Azzeh M, Ghneim R et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. *J Clin Microbiol* 2008;46:2414-7.

(36) Jones G, Matthews R. An evaluation of an automated system for processing clinical swabs. 2008.

(37) Bourbeau P, Swartz B. First evaluation of the WASP, a new automated microbiology plating instrument. *J Clin Microbiol* 2009;47(4):1101-6.

(38) Siryani I, Ghneim R, Abu-Rayan A, Kanaan M, Hindiyeh M. Evaluation of Eswab against BD CultureSwab MaxV(+) and Medical Wire & Equipment Transwab for the maintenance of fastidious aerobic bacteria. 2009.

(39) Rivers C, Schwebke J. Viability of *Trichomonas vaginalis* in Copan Universal Transport Medium and eSwab transport medium. *J Clin Microbiol* 2008;46(9):3134-5.

(40) Rivers C, Barrientes F, Meltzer M, Schwebke J. Evaluation of expanded use of Copan diagnostics' UTM viral transport media and Eswab for the transportation, culture, and nucleic acid detection of *Trichomonas vaginalis* from genital samples. 2007.

(41) Friis-Moller A, Schou Andersen A, Engberg J, Jorgensen B. Comparison of the Copan Eswab system with traditional charcoal swab in Stuarts transport medium. 2008.

(42) Nys S, Vijgen S, Magerman K, Cartuyvels R. Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans* . *Eur J Clin Microbiol Infect Dis* 2010;29:453-6.

(43) Milburn S, Reyes R, John M et al. Comparison of Copan ESwab™ and Starplex Starswab II™ for the detection of methicillin-resistant *Staphylococcus aureus* from nasal and perianal specimens. 2009.

(44) Castriciano S, Giambra A, De Maria D, Ravarino D, Latino A. Comparison between the Eswab collection kit and the ProbeTec™ collection system for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the ProbeTec™ assay. 2009.

(45) Chernesky M, Jang D, Portillo E, Feng Y, Smieja M, Castriciano S. Diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with APTIMA Combo 2 testing of self-collected vaginal samples using flocked swabs and an Eswab collection and transport system. 2009.

(46) Hoffmann S, Berthelsen L. Recovery after 24 hours and 48 hours of gonococci from Eswab and from charcoal impregnated swabs in Stuart's transport medium. 2007.