Comparison of Eswab with Amies swab in maintaining viability of microorganisms

Comparison of Gram stain quality with Eswab versus dry swab

Veroniek Saegeman

GSO Clinical Biology, UHLeuven

19 may 2010

Eswab

- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab

- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab introduction

- To isolate and identify potential pathogens from clinical specimens
  - Appropriate collection of specimen
  - Maintenance of microorganism viability
- Specimens collected
  - Biopsy
  - Needle aspiration, drainage
  - Swab

Eswab introduction

- Swab
  - Not ideal
    - Toxic products/inactivating substances
    - Interference with identification methods
  - Frequently used
    - Patient comfort
    - Time saving

Eswab introduction

- Swab assessment
  - Swab tip
    - Cotton, Dacron, rayon
      - Possibility of toxicity (cotton)
      - Bacterial entrapment in dense fiber matrix
  - Transport medium
    - Protection of bacterial viability ↔ dry swab
    - Reducing substances for maintaining viability of anaerobes

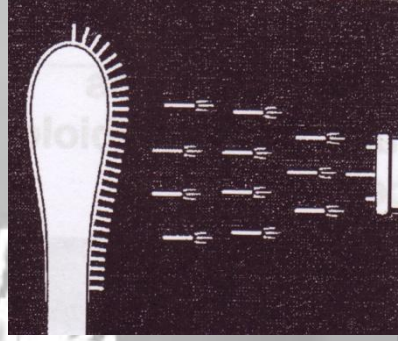
Eswab introduction

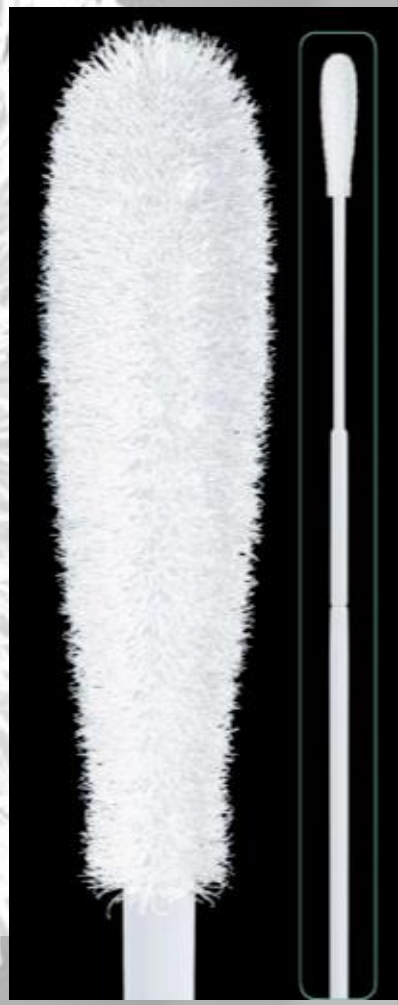
- Copan Eswab™ technique

– Screw-cap tube
– 1 mL liquid Amies medium
– Specimen collection swab
– Tip flocked with soft nylon fiber
→ better absorption and release of bacteria?[1]
– Storage at 4-8 C or at room temperature
– Delay of processing up to 48 hrs

[1] Van Horn et al, 2008

Eswab introduction





- Copan Eswab™ technique

– Screw-cap tube
– 1 mL liquid Amies medium
– Specimen collection swab
– Tip flocked with soft nylon fiber
→ better absorption and release of bacteria?[1]
– Storage at 4-8 C or at room temperature
– Delay of processing up to 48 hrs

[1] Van Horn et al, 2008

Eswab

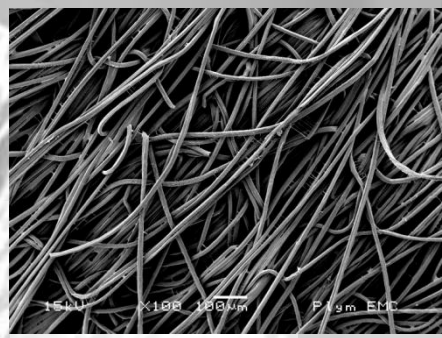
- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab Literature

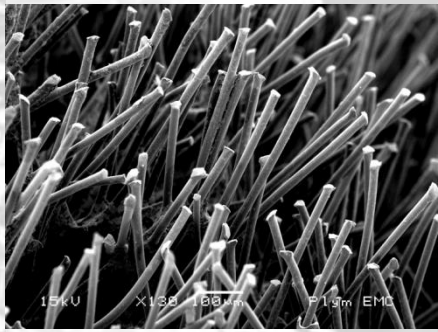
- Physical characteristics Eswab™

	Flocked swab	Cotton/rayon swab
Particle release	92% of initial inoculum	30% of initial inoculum
Bacteria	Adhering by capillary action	Absorbed and enmeshed
Inoculum per plate	Constant	Decrease

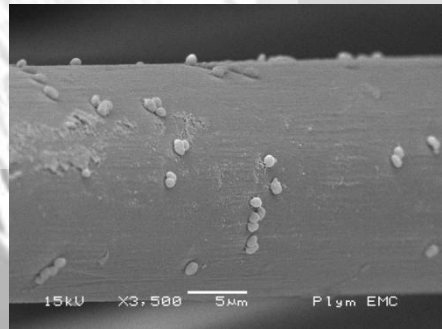
[1] Human and Jones, 2006



Cotton/rayon



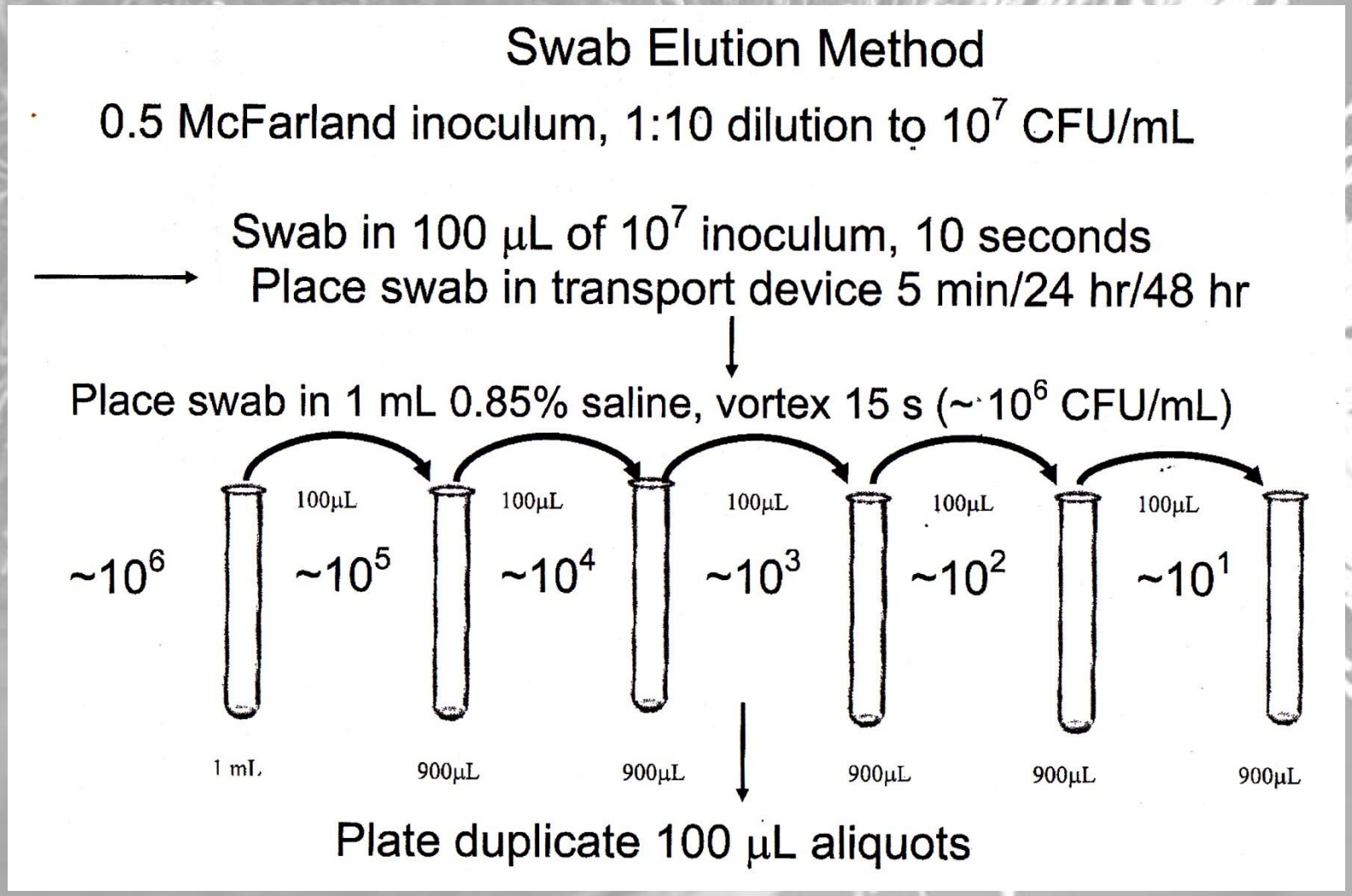
Flocked



Eswab Literature

- Acceptance criteria
  - CLSI standard M40-A'Quality control of microbiological transport systems' (2004)
  - Quantitative swab elution method
    - Ability of transport system to maintain organism viability
    - Acceptable: no more than a 3-$\log_{10}$ decline in CFU between the zero-time CFU count and the CFU count after the specific storage time

Eswab Literature



Eswab Literature

- Acceptance criteria
  - CLSI standard M40-A'Quality control of microbiological transport systems'
  - Qualitative roll-plate method
    - Include mechanical variables of the direct swabbing on a plate → influences release of the sample on the plate
    - Acceptable: ≥ 5 CFU at the storage time from the dilution that yielded zero-time plate counts closest to 300 CFU

Eswab Literature

Roll-Plate Method

0.5 McFarland inoculum (~1.5×10^8 CFU/mL)

↓

Serial 1:10 dilutions to ~10^5 – 10^4 organisms/mL

↓

Place swab in 100 µL of inoculum for 10 seconds

→ Place swab in transport device/5 min/24 hr/48 hr

↓

Streak plate in 3 planes following NCCLS guidelines

Eswab Literature

- Acceptance criteria Eswab
  - CLSI standard M40-A
  - Quantitative swab elution method and Qualitative roll-plate method
    - OK for *S. pyogenes*, *S. agalactiae*, *S. pneumoniae*, *E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *H. influenzae*, *C. albicans* at 4 C and 21 C for 48 hrs [1,2,3]
    - OK for *P. anaerobius*, *B. fragilis*, *F. nucleatum*, *F. necrophorum*, *P. acnes*, *P. melaninogenica*, *C. sporogenes*, *C. perfringens*, *Peptococcus magnus* at 4 C and 21 C for 48 hrs [1,2]
    - OK for *N. gonorrhoeae* at 4 C and 21 C for 24 hrs [1,2]

[1] Van Horn et al, 2008; [2] Eswab Copan product insert, 2006; [3] Nys et al, 2010

Eswab Literature

- Anaerobic microorganisms and Eswab
  - Survival of *P. melaninogenica* at room temperature:
    - Yes: Biggs, 2007; Sarina et al, 2009, Eswab Copan product insert, 2006
    - No: Van Horn et al, 2008
  - Poor/no survival of
    - certain clostridia (*C. difficile, C. clostridioforme*)
    - *Prevotella bivia*, *Porphyromonas asaccharolytica*, *Peptoniphilus asaccharolyticus* (Allen et al, 2009)

→ additional studies warranted for survival of Clostridia and fastidious anaerobic organisms

Eswab Literature

- MRSA Eswab collection kit™
  - Pooled samples of nares, (throat) and perineum
    → multiple tests: culture, PCR
    → ↓ sampling bias
    → ↓ costs
  - higher MRSA recovery than with conventional swab systems (Venturi Transystem, Copan: Smismans et al, 2009; Stuart liquid transystem, Copan: Giambra and Castriciano, 2007 and Fontana et al, 2008)

Eswab Literature

- Wounds: Eswab vs charcoal swab in Stuart transport medium (Friis-Moller et al, 2008)

	Pos Eswab Pos Stuart	Pos Eswab Neg Stuart	Pos Stuart Neg Eswab	Neg Stuart Neg Eswab	P-value
All bacteria	259	62	38	35	0.016
S. aureus	68	9	16	103	0.162
P. aeruginosa	27	**5**	**1**	163	0.013
Haemolytic streptococci	14	2	4	176	0.414
anaerobes	4	8	3	181	0.132

Eswab Literature

- General remarks Eswab
  - Reliable for molecular testing: nucleic acids stable up to 15 days at RT (Moore et al, 2008)
  - *Trichomonas vaginalis* viability maintained, although lower sensitivity than UTM Copan (Rivers et al, 2007)
  - *Neisseria gonorrhoeae* and *Chlamydia trachomatis* same sensitivity as conventional swabs (BD Probe Tec^{TM}, APTIMA swab) for NAT (Castriciano et al, 2009; Chernesky et al, 2009)

Eswab Literature

- Gram stain with Eswab
  - Eswab superior quality versus rayon swab in Amies gel (Copan) (Fontana et al, 2009)
    - More bacterial morphotypes visualised
    - More distinguishable bacterial morphology, i.e. shape, colour… (diplococci)
    - Better detail and higher number of human cells (epithelial cells, leucocytes, red blood cells)
    - No influence of
      - volume used (50 µl – 100 µl)
      - time delay for microscopy (2h – 24h – 72h)

Eswab

- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab study

- Copan Eswab™ versus
  - Blue swab in Amies transport medium (Int. Medical Products)
    - Minimal detection limit; bacterial recovery (CFU/mL); species recovery
  - Red dry Copan swab
    - Gram stain
- Wound swabs
  - Hospital wards: Septic Orthopaedics (E231), Burn Care Unit (E519)

Eswab study

- Copan Eswab™ versus
  - Blue swab in Amies transport medium (Int. Medical Products)
    - Minimal detection limit; bacterial recovery (CFU/mL); species recovery
  - Red dry Copan swab
    - Gram stain
- Method: CLSI M40-A
  - Quantitative swab elution method (125 samples)
  - Qualitative roll-plate method (125 samples)

Eswab study

- Copan MRSA Eswab™ versus
  - Red dry Copan swabs (1 of nares / 1 of perineum)
    - Minimal detection limit; bacterial recovery (CFU/mL); species recovery; gram stain

- MRSA screening of nose and perineum
  - Hospital wards: Burn Care Unit (E519), Geriatric Medicine (E640, E641, E455, E230), General Internal Medicine (E454)

Eswab study

- Copan MRSA Eswab™ versus
  - Red dry Copan swabs (1 of nares / 1 of perineum)
    - Minimal detection limit; bacterial recovery (CFU/mL); species recovery; gram stain
- Method: CLSI M40-A
  - Quantitative swab elution method (125 samples)
  - Qualitative roll-plate method (125 samples)

Eswab study

- Interfering parameters?
  - Time interval from sample collection to processing: room temperature storage
  - Type of swab used first

Eswab study

- Minimal detection limit?
  - Eswab vs dry swab
    - 9-fold higher recovery with Eswab
  - Eswab vs Amies gel swab
    - 6-fold higher recovery with Eswab

→Inocula on dry swab/Amies gel swab must be 9/6 times higher to reach similar detectable growth as with Eswab

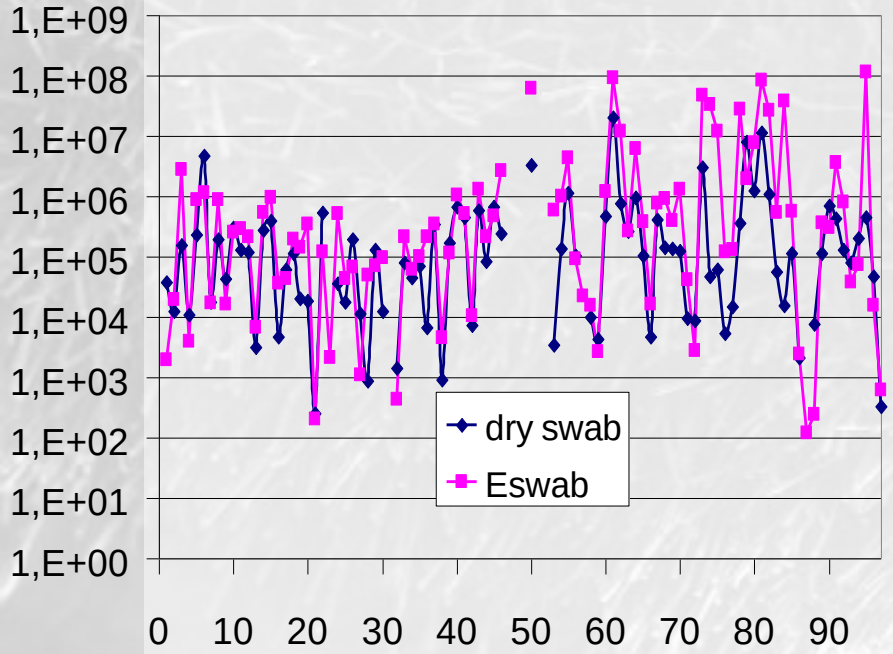
Eswab study

- Minimal detection limit?
  - 1 Eswab vs 4 dry swabs without growth
  - 20 Eswabs vs 25 Amies gel swabs without growth

→ Eswab lower minimal detection limit

Eswab study

- Bacterial recovery? MRSA Eswab

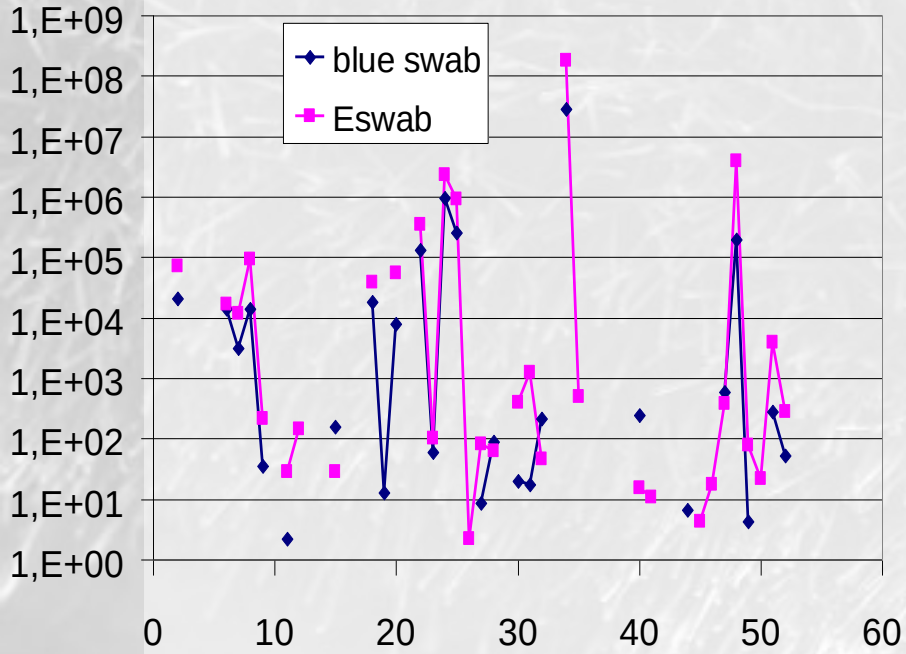


Eswab significant higher recovery than dry swab (paired t-test, $p < 0.01$)

Dry swab:

Eswab study

- Bacterial recovery? Eswab wounds



Eswab significant higher recovery than Amies gel swab (paired t-test, $p < 0.01$)

Amies gel swab:

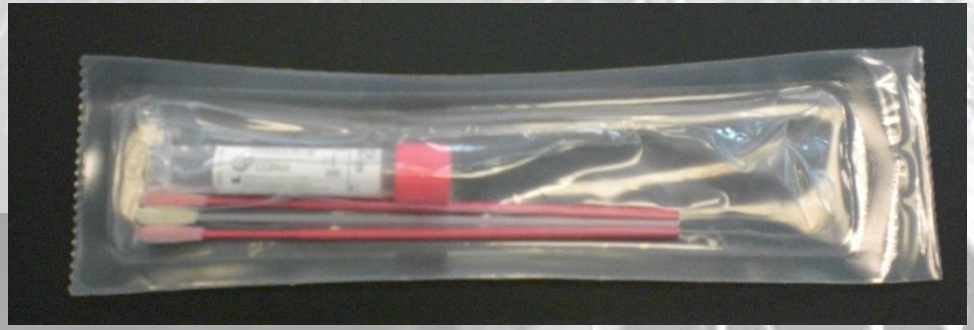
Eswab study

- Bacterial recovery?
  - Parameters – MRSA screen
    - No influence of time delay at room temperature until processing
    - No influence of swab type used first

Eswab study

- Species recovery?
  - MRSA screen: Eswab vs dry swab
    - Dry swab missed 4 MRSA strains
    - Eswab missed 3 MRSA strains

→ more MRSAs with Eswab ($p > 0.05$)



Eswab study

- Species recovery?
  - Wounds: Eswab vs Amies gel swab: more species with Eswab

	Gram-negatives	*Staphylococcus* spp./*Micrococcus* spp.	*Streptococcus* spp./ *Enterococcus* spp.	*Corynebacterium* spp.
Eswab +, Amies swab -	2	10	7	0
Eswab -, Amies swab +	3	1	1	1

Eswab study

- Gram stain: Eswab vs dry swab
  - 1 drop of vortexed Eswab Amies medium vs rolling dry swab on slide
  - MRSA screening and wound swab

Superiority of	No. bacterial morphotypes (%)	No. Bacteria/ HPF (%)	Other cells (leukocytes, epithelial cells) (%)
Dry swab	12/149 (8.1)	11/149 (7.4)	9/96 (9.4)
Eswab	46/149 (30.9)	69/149 (46.3)	14/96 (14.6)

Eswab study

- Gram stain: Eswab vs dry swab
  - MRSA and wound swab

→ Eswab superior Gram stain quality

Eswab

- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab conclusions

- Superiority of Eswab compared with dry swab/Amies gel swab in terms of
  - ‘Minimal detection limit’
  - Bacterial recovery (CFU/mL)
  - Species recovery
  - Gram stain quality

Eswab conclusions

- Clinical-organisational impact
  - Several lab tests with a single sample (rapid antigen testing, culture, PCR)
  - Suitable for automated swab processing systems (AccuPAS, Dynacon; WASP, Copan)

Eswab conclusions

- Cost impact
  - Eswab: expensive swab system

Wound swabs	MRSA screening	
	culture	PCR + culture
1 dry swab + 1 swab in amies medium ↕ 1 Eswab	2 dry Copan swab ↕ 1 Eswab	1 double Copan swab Venturi transystem ↕ 1 Eswab
0,53 €	0,42 €	1,37 €
0,83 €	1,57 €	1,57 €

Eswab conclusions

- Cost impact
  - Eswab: expensive swab system

  BUT
  - Reduced No. of samples
  - Better performance → higher MRSA detection rate → shorter length of stay → hospital cost ↓

Eswab

- Introduction
- Literature
- Study UHLeuven
- Conclusions
- To do / actions

Eswab To do

- Finalisation of swab study
- Introduction of Eswab depending on price?