Comparison of Eswab with Amies swab in maintaining viability of microorganisms

Comparison of Gram stain quality with Eswab versus dry swab

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Eswab

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

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- Literature
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- 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
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¹ Van Horn et al, 2008
Eswab

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Eswab Literature

- **Physical characteristics Eswab™**

<table>
<thead>
<tr>
<th></th>
<th>Flocked swab</th>
<th>Cotton/rayon swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle release</td>
<td>92% of initial inoculum</td>
<td>30% of initial inoculum</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Adhering by capillary action</td>
<td>Absorbed and enmeshed</td>
</tr>
<tr>
<td>Inoculum per plate</td>
<td>Constant</td>
<td>Decrease</td>
</tr>
</tbody>
</table>

1 Human and Jones, 2006
Eswab Literature

• Acceptance criteria

  – 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

Swab Elution Method

0.5 McFarland inoculum, 1:10 dilution to $10^7$ CFU/mL

Swab in 100 $\mu$L of $10^7$ inoculum, 10 seconds
Place swab in transport device 5 min/24 hr/48 hr

Place swab in 1 mL 0.85% saline, vortex 15 s ($\sim 10^6$ CFU/mL)

\[ \sim 10^6 \rightarrow \sim 10^5 \rightarrow \sim 10^4 \rightarrow \sim 10^3 \rightarrow \sim 10^2 \rightarrow \sim 10^1 \]

1 mL 900$\mu$L 900$\mu$L 900$\mu$L 900$\mu$L 900$\mu$L

Plate duplicate 100 $\mu$L aliquots
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 (~1.5x10^8 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:
    – 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)

<table>
<thead>
<tr>
<th></th>
<th>Pos Eswab</th>
<th>Pos Eswab</th>
<th>Pos Stuart</th>
<th>Neg Stuart</th>
<th>Neg Eswab</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All bacteria</td>
<td>259</td>
<td>62</td>
<td>38</td>
<td>35</td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>68</td>
<td>9</td>
<td>16</td>
<td>103</td>
<td></td>
<td>0.162</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>27</td>
<td>5</td>
<td>1</td>
<td>163</td>
<td></td>
<td>0.013</td>
</tr>
<tr>
<td>Haemolytic streptococci</td>
<td>14</td>
<td>2</td>
<td>4</td>
<td>176</td>
<td></td>
<td>0.414</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td>181</td>
<td></td>
<td>0.132</td>
</tr>
</tbody>
</table>
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™, 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

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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)
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

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<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Eswab +, Amies swab -</td>
<td>2</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Eswab -, Amies swab +</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
## 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

<table>
<thead>
<tr>
<th>Superiority of</th>
<th>No. bacterial morphotypes (%)</th>
<th>No. Bacteria/HPF (%)</th>
<th>Other cells (leukocytes, epithelial cells) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry swab</td>
<td>12/149 (8.1)</td>
<td>11/149 (7.4)</td>
<td>9/96 (9.4)</td>
</tr>
<tr>
<td>Eswab</td>
<td>46/149 (30.9)</td>
<td>69/149 (46.3)</td>
<td>14/96 (14.6)</td>
</tr>
</tbody>
</table>
Eswab study

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

→ Eswab superior Gram stain quality
Eswab

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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

<table>
<thead>
<tr>
<th>Wound swabs</th>
<th>MRSA screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 dry swab + 1 swab in amies medium</td>
<td>2 dry Copan swab</td>
</tr>
<tr>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>1 Eswab</td>
<td>1 Eswab</td>
</tr>
<tr>
<td>1 Eswab</td>
<td>1 Eswab</td>
</tr>
<tr>
<td>0.53 €</td>
<td>0.42 €</td>
</tr>
<tr>
<td>0.83 €</td>
<td>1.57 €</td>
</tr>
</tbody>
</table>

- 1 double Copan swab Venturi transystem

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<thead>
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<th></th>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>1.37 €</td>
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Costs:
- 0.53 €
- 0.83 €
- 0.42 €
- 1.57 €
- 1.37 €
- 1.57 €
Eswab conclusions

• Cost impact
  – Eswab: expensive swab system

BUT
  – Reduced No. of samples
  – Better performance $\rightarrow$ higher MRSA detection rate $\rightarrow$ shorter length of stay $\rightarrow$ hospital cost $\downarrow$
Eswab

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Eswab To do

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