SPECIMEN PROCESSING IN CLINICAL MICROBIOLOGY. WHAT’S NEW?

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Faculty Disclosure

The Association of Public Health Laboratories adheres to established standards regarding industry support of continuing education for healthcare professionals. The following disclosures of personal financial relationships with commercial interests within the last 12 months as relative to this presentation have been made by the speaker(s):

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Becton Dickinson - research
Objectives

• List the types of liquid specimens that are compatible with automated specimen processors.
• Describe the functions of automated specimen processors and relate how they can improve workflow in Clinical Microbiology.
• Discuss the use of sonication methods for culture of hardware implants.
What makes a specimen “good”?

1. Collection from appropriate patient
2. Sampling from infected site/portion of wound
3. Lack of contaminating flora
4. Volume sufficient to perform all tests
5. Transport that maintains pathogen w/o overgrowth of flora
6. Planting on proper media, separated colonies by streaking, correct incubation time & atmosphere
The best specimens are usually fluid and tissue

- Represent the infected patient
- Sufficient volume
- Fewer contaminants introduced
- Organism viability better maintained
- Homogeneous sample more likely
Specimen Collection with Swabs

- Swabs hold a very small volume compared to fluid or tissue.
  - 0.015 ml - NP swab
  - 0.150 ml – vaginal, throat, wound swab
- More likely to pickup contaminating flora.
- Swabs may trap microbes and cells in fibers & may not release them.
- Swabs used for gram stain & culture may give different results.
gloppy sputum

wound swab

Classical Processing

1 µl urine

fluid or ground tissue
New Paradigm: Liquid Specimens

- Liquid specimens in appropriate transport improve recovery by preserving & releasing cells & microbes
  - E-swab or other swab in saline
  - urine preservative
  - Cary Blair for stool
  - sputum in lysis solution
  - body fluids
  - ground tissue
- Liquid specimens are amenable to automated processors
“E” swab = elution

Swabs are placed in liquid transport medium to elute the cells and organisms.
Comparison of Automated Processing of Flocked Swabs with Manual Processing of Fiber Swabs for Detection of Nasal Carriage of S. aureus

- **Direct method:**
  - M40 charcoal Transystem swab
  - E Swab - 30 µl automated

- **Enrichment - salt broth method:**
  - 800 µl from E swab
  - 3 cm tip of M40 Trans Swab
  - After incubation 10µl planted

- Columbia BAP (w/abx) & ChromID MRSA agars

<table>
<thead>
<tr>
<th>Method</th>
<th># Pos</th>
<th>% Sens</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>E Swab direct</td>
<td>268</td>
<td>75.5</td>
<td>85.5</td>
</tr>
<tr>
<td>M40 direct</td>
<td>237</td>
<td>66.8</td>
<td>81.3</td>
</tr>
<tr>
<td>E Swab Enrich</td>
<td>325</td>
<td>91.6</td>
<td>94.5</td>
</tr>
<tr>
<td>M40 Enrich</td>
<td>295</td>
<td>83.1</td>
<td>89.5</td>
</tr>
</tbody>
</table>

Jones et al. (2011) JCM 49:2717-18
Flocked swabs act like a brush
### Improved Specimen Collection - Respiratory Virus Detection

Daly (2006) JCM 44:2265

<table>
<thead>
<tr>
<th>(No. pos)</th>
<th>Total cells/hpf</th>
<th>Infected cells/hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flocked</td>
<td>Rayon</td>
</tr>
<tr>
<td>FluA (20)</td>
<td>67.2</td>
<td>29.3</td>
</tr>
<tr>
<td>RSV (20)</td>
<td>51.7</td>
<td>19.6</td>
</tr>
<tr>
<td>DFA Neg</td>
<td>82.4</td>
<td>24.8</td>
</tr>
</tbody>
</table>
Barriers to implementation of E swab

- Cost
- Learning curve for practitioners
- Universal use vs. specific collection swabs per manufacturer
  - GCCT, Affirm, POC rapid tests, *S. aureus* or GAS PCR validated on specific swabs
- Use of incorrect swab leads to recollects, increased costs
- Lab validations
Barriers to receipt of a good urine specimen

Cup may sit in patient room or at nurses’ station.

Urine in cups should be kept cold.

Larger hospitals may use pneumatic tube

Some specimen cups arrive leaking
Specimen Collection & Transport for Urine Culture

- Usually highest volume specimen
- Mostly, clean catch mid-void in a sterile cup
- MCM:
  - 2 hrs, RT
  - 24 hrs, refrigerated
  - 48 hrs, RT in preservative tube
In 2 hours 1,000 CFU of *E. coli* could become >50,000 if urine is unrefrigerated & unpreserved.
Performance of the BD Vacutainer Urine C&S Preservative Tubes compared to non-preservative urine samples stored at 4°C and room temperature before culture

Eisinger AJCP 2013 & ASM abstract 2011

Blue = unpreserved, unrefrigerated
Red = refrigerated, unpreserved
Yellow = preserved
Implementation of Urine Preservative

**Benefits**
- Better specimen available for culture
- May improve CAUTI rate
- Do not need to maintain cold chain;
- Specimen good for 48 hrs
- No specimen sharing needed
- Easily handled by automated specimen processors

**Barriers**
- Someone has to transfer the specimen to tube
- Cost
- Need exact test requests
- Add-on testing not possible
Business Plan for Implementation of Urine Preservative

- # inpatient urines received in >2 hrs
- # specimens shared & time cost
- Cost savings for pour-off tube for other tests
- FTE savings for automated processing
- # tests cancelled due to leakage or lack of proper sharing
- Cost of transfer straw & preservative tubes for UA and Micro
- Cost of nursing assistant time for transfer to preservative
- Work with nursing to find best ordering & collection practices
- Cost sharing w/nursing
Specimen Collection & Transport for Stool Culture, EIA, PCR

MCM:

- Collect specimen from pt w/>3 loose stools in 24 hrs. Do not collect if inpt >3 days

- Unpreserved transport:
  - ≤1 hr if unpreserved at RT
  - 24 hr if refrigerated

- Cary Blair Preservative transport:
  - up to 48 hr at RT or refrigerated
Can sputum be liquefied?

Is liquefied sputum beneficial?

Will a liquid sample be suitable for an automated processor?
Liquid Solution for Sputum

- Facilitates homogeneous specimens
- Allows use of automated specimen processors
- Marketed as “Snot Buster”

www.copanusa.org
Analysis of the Copan SL Solution for Liquefaction of Respiratory Specimens Prior to Gram Stain and Culture

L. J. Doyle, G. S. Hall, A. C. Boss, and S. M. Harrington
ASM General Mtg Abstract 2012

<table>
<thead>
<tr>
<th></th>
<th>Routine &amp; SL</th>
<th>SL Solution</th>
<th>Routine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Num of Potential</td>
<td>76 (92%)</td>
<td>80 (96%)</td>
<td>79 (96%)</td>
</tr>
<tr>
<td>Pathogens (83)</td>
<td></td>
<td>4 only by SL</td>
<td>3 only by routine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Quantity of Organism on Plate</th>
<th>Routine = SL</th>
<th>SL Solution &gt; Routine</th>
<th>SL Solution &lt; Routine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Num of Potential Pathogens (83)</td>
<td>47 (57%)</td>
<td>31 (37%)</td>
<td>5 (6%)</td>
</tr>
</tbody>
</table>
Analysis of SL Solution for Liquefaction of Respiratory Specimens Prior to Gram Stain and Culture

L. J. Doyle, G. S. Hall, A. C. Boss, and S. M. Harrington
ASM General Mtg Abstract 2012

Gram Stain:

• Good agreement: PMNs and GNRs

• Quantity of PMNs: SL (n=28) > routine (n=8).

• Quantity of GNRs: routine (n=11) > SL (n=1)

• The presence of negatively staining debris in the SL prepared Gram stains may interfere with interpretation of Gram negative morphologies.
Why an automated specimen inoculator/processor?

- Need to do more with less technical staff
- Reduce repetitive tasks
- Fewer human errors
  - Transcription/labeling
  - Mixed-up specimens
- Allow technologists to do tasks appropriate for their education
- Consistency in depth of dipping into specimens – 1 µl urine subject to error
- Consistency in streaking
- Reduce contamination
- Reduce disposables
Beckton Dickinson: Innova
(Dynacon inocuLAB)

http://www.bd.com/
Becton Dickinson: InoquI A
(Kiestra)

- Processes any liquid specimens & non-liquid at (connected) manual bench
- Side labeling of plates amenable to connection to Total Lab Automation.

www.bd.com
• Vortexes, decaps & recaps tubes/cups
• Inoculates with pipette & streaks according to protocol
• Uses 10 µl for urine
• Can inoculate & streak 5 plates simultaneously
• Can make Gram stain & inoculate broths

Unique streaking:
• Rolling magnetic bead technology
• Plate is not opened
• Bead is removed

www.bd.com
Evaluation of Automatic versus Manual Inoculation

Antony Croxatto*, Klaas Dijkstra*, Guy Prod’hom*, Gilbert Greub*.
*Institute of Microbiology, Laboratory department, University Hospital Center and University of Lausanne, Lausanne, Switzerland.
* Center of Expertise Computer Vision, HNL University of Applied Sciences, Leeuwarden, The Netherlands.
bioMerieux – PREVI™ Isola

www.biomerieux-diagnostics.com/previ-isola
Comparison of PREVI™ Isola to manual processing

- Cxs w/more isolated colonies
  - 571 urines .......... 26%
  - 174 swabs in saline vs. dry ..................... 18%
  - 61 stools ............. 18%

23-44% more colonies/plate, depending on source

<table>
<thead>
<tr>
<th>Colony Count</th>
<th>Cx w/&gt;30 colonies in 3rd quadrant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 –10³</td>
<td>0/167</td>
</tr>
<tr>
<td>10³-10⁴</td>
<td>0/42</td>
</tr>
<tr>
<td>10⁴-10⁵</td>
<td>1/90</td>
</tr>
<tr>
<td>10⁵-10⁶</td>
<td>199/201</td>
</tr>
</tbody>
</table>

Glasson (2008) JCM 46:1281

Abstract C-06-4 F. Rice & A. Baruch, ASM Gen Mtg 2009
Copan WASP (Walk Away Specimen Processor)

- Continuous loading
- Any sized tube or cup
- Universal decapping/recapping
- Connects to Total Lab Automation

www.copanusa.com
WASP – Additional features

Automatic loop changer

Rotating warehouse – for KB or broth inoculation

Gram slide preparation

Sort out Stacker*

Side labeler*

MALDI TOF plate seeding

Dual loop streaker*

www.copanusa.com
Implementation of Automated Processing

- Determine specimen types
- Quantify volume of specimens
- Consider maintenance contracts
- Cost of disposables saved vs. purchased
- Quantify technical time saved
  - Receive/accession, label, inoculate, streak, sort
  - Factor in down time
- Determine FTE /salary savings for processing; repurposed effort
### Example Simplified Break Even Analysis – ROI

<table>
<thead>
<tr>
<th>Costs</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
<th>Year 6</th>
<th>Year 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purchase</td>
<td>350,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Service</td>
<td>0</td>
<td>10,416</td>
<td>31,250</td>
<td>31,250</td>
<td>32,812</td>
<td>32,812</td>
<td>32,812</td>
</tr>
<tr>
<td>Consummables</td>
<td>1,969</td>
<td>2,067</td>
<td>2,170</td>
<td>2,279</td>
<td>2,393</td>
<td>2,513</td>
<td></td>
</tr>
<tr>
<td><strong>Annual Total Cost</strong></td>
<td>12,385</td>
<td>33,317</td>
<td>33,420</td>
<td>35,091</td>
<td>35,205</td>
<td>35,325</td>
<td></td>
</tr>
<tr>
<td>Savings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>disposables</td>
<td>0</td>
<td>2,800</td>
<td>4,800</td>
<td>24,000</td>
<td>29,000</td>
<td>30,000</td>
<td>32,000</td>
</tr>
<tr>
<td>Labor</td>
<td>0</td>
<td>21,500</td>
<td>37,000</td>
<td>147,000</td>
<td>150,000</td>
<td>153,000</td>
<td>156,000</td>
</tr>
<tr>
<td><strong>Annual Total Savings</strong></td>
<td>24,300</td>
<td>41,800</td>
<td>171,000</td>
<td>179,000</td>
<td>183,000</td>
<td>188,000</td>
<td></td>
</tr>
<tr>
<td><strong>Annual Difference = Savings - Cost</strong></td>
<td>-350,000</td>
<td>11,915</td>
<td>8,483</td>
<td>137,580</td>
<td>143,909</td>
<td>147,795</td>
<td>162,675</td>
</tr>
<tr>
<td><strong>Cumulative savings</strong></td>
<td>11,915</td>
<td>20,398</td>
<td>139,978</td>
<td>283,887</td>
<td>431,682</td>
<td>594,357</td>
<td></td>
</tr>
</tbody>
</table>
Automation References

- Rice. (2009) ASM Abstract C-064 F
- Glasson, JH. Evaluation of an automated instrument for inoculating and spreading samples on to agar plates (2008) JCM 46:1281
Hardware & Sonication

- Increasing need to diagnose biofilm-related infections on implanted devices.
- Is the hardware the best specimen?
- Is there a standard protocol?
- How are results to be interpreted?
- Balance between detection of pathogen in biofilm vs. reporting contamination.
CRBSI: Central Venous Catheters
Roll-plate (Maki) vs. Sonication?

**Roll-plate**
- 5 cm tip rolled on BAP
- ≥ 15 CFU (correlate to bld cx)

**Sonication**
- Sonicate tip in broth
- ≥100 or 1000 CFU (correlate to bld cx)
- Also assesses intraluminal biofilm

- Either technique recommended by CDC and IDSA (2009) CID 49:1-45
- Sensitivity & specificity variable in literature
- Many patients receive abx prior to cx
- Combine with other diagnostic methods
- Don’t culture unless suspected infection

Diagnosis of CIED Infection:

- 2 sets of blood cxs prior to abx
- Cx of generator pocket tissue and lead-tip
- Pos blood cxs (or suspect endocarditis): TEE
- If FUO, pt should see cardiologist or ID specialist
- Do not perform percutaneous aspiration of generator pocket
Diagnosis of CIED Infection

- Exact culture methods vary
- Most literature: Sonicate cultures of leads & devices provide highest sensitivity with some reduction in specificity
  

  **See also Viola JCM (2009) 47:4168**

- CIED device, leads, or tissue can be colonized in asymptomatic patients (~33%). Colonization doesn’t always predict infection.

  Baddour Circulation 121:1686
• Multi-center study in Europe
• Compared sonication cx of (14) VP drains & (13) VP shunts to CSF cx for diagnosis of meningoventriculitis.
• Sonication of VP drain improved dx
• Sonication of VPS corroborated CSF cx, but did not improve sensitivity; 5/13 FP, two with significant CFU.

http://www.totalhealth.co.uk/clinical-experts/mr-christopher-chandler/treating-hydrocephalus/

http://www.chw.org/medical-care/neuroscience/conditions/hydrocephalus/
Prosthetic Joint Infections - PJI

Criteria for Diagnosis of PJI
Del Pozo (2009) NEJM 361:787

- Elevated ESR/CRP
- Pre-operative joint fluid – PMNs & pos cx
- Acute inflammation on histologic exam (frozen sections)
- Sinus tracts
- Low organism burden Microbiology:
  - Isolation of same microbe from ≥2 cxs
  - ≥ 20 CFU/10ml of cx of sonicate fluid
- Intraoperative Gram stain is NOT recommended by AAOS.
PJL – Common Microbes; biofilm associated
Del Pozo (2009) NEJM 361:787

• Gram positive cocci (~65%)
  • Coag Neg Staph**
  • S. aureus*
  • Streptococcus
  • Enterococcus

• Polymicrobial (~20%)
• Culture Neg (~7%)
• Aerobic Gram Neg Rods* (~6%)
• Anaerobes (~4%)
  • Propionibacterium**
  • Peptostreptococcus
  • Finegoldia
Diagnosis of PJI by Microbiologic Culture
Atkins (1998) JCM 36:2932-2939

Collect 5-6 samples; Dx of PJI correlates to 2-3 pos cxs.
How long should cultures be incubated?

13 days

Schafer (2008) CID 47:1403

Butler-Wu (2011) JCM 49:2490
Orthopaedic Implant Cultures
Sonication Method

Trampuz (2007) NEJM 357:654

- Prosthesis is placed in large sterile container for transport to lab
- Add 400 mls of Ringer’s salt solution
- Vortex 30 sec
- Sonicate for 5 min (40 kHz; 0.22 W/cm²)
- Vortex 30 sec
- Plant 500 ul aliquots to aerobic & anaerobic blood agar plates.
- Centrifuge 200 ml of sonicate fluid and perform gram stain on pellet.
Orthopaedic Implant Cultures
Sonication Method

Trampuz (2007) NEJM 357:654

- 331 patients with total hip and knee prosthesis;
- 79 PJI

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovial Fluid Culture</td>
<td>56.3</td>
<td>98.1</td>
</tr>
<tr>
<td>Tissue Culture: ≥ 2 cx pos</td>
<td>60.8</td>
<td>99.2</td>
</tr>
<tr>
<td>Sonicate-fluid Cx</td>
<td>78.5</td>
<td>98.8</td>
</tr>
<tr>
<td>Gram stain of sonicate fluid</td>
<td>44.7</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Summary for Microbiology of PJI

- Greater need for diagnosis
- Small numbers of organisms form biofilm
- Microbiologic diagnosis is challenging:
  - Collect 5 – 6 tissue or joint fluid cultures
  - ≥2 positive cultures correlates with PJI
  - Hold cultures about 2 weeks, esp to detect anaerobes (*P. acnes*)
  - Culture of fluid from sonication of implants has good sensitivity & specificity
  - Intraoperative gram stains have low sensitivity for diagnosis of PJI
Spinal Implants
Sampedro Spine (2010) 35:1218

- 112 subjects
  - 22 with spinal infection
  - 76 aseptic failure
  - 14 with chronic antimicrobial suppression
Spinal Implants
Sampedro Spine (2010) 35:1218

- Tissue cx:
  - 73% sens; 93% spec
- Sonication:
  - 91% sens; 97% spec

Table 3. Microbiology Results of Subjects With Infection of Spinal Implants

<table>
<thead>
<tr>
<th>Case</th>
<th>Peri-Implant Tissue</th>
<th>Concordant Microbiology*</th>
<th>Sonicate Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VGS</td>
<td>Actinomyces odontolyticus</td>
<td>Veillonella species</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Propionibacterium acnes</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>P. acnes</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>P. acnes</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>P. acnes</td>
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<tr>
<td>6</td>
<td></td>
<td>P. acnes</td>
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<tr>
<td>7</td>
<td></td>
<td>P. acnes</td>
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<tr>
<td>8</td>
<td></td>
<td>P. acnes</td>
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</tr>
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<td>9</td>
<td></td>
<td>P. acnes</td>
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</tr>
<tr>
<td>10</td>
<td></td>
<td>CNSt</td>
<td></td>
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<tr>
<td>11</td>
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<tr>
<td>12</td>
<td></td>
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<tr>
<td>13</td>
<td></td>
<td>CNSt*</td>
<td></td>
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<tr>
<td>14</td>
<td></td>
<td>CNSt</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>P. acnes</td>
<td></td>
</tr>
<tr>
<td>17</td>
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<td></td>
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<td>18</td>
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<tr>
<td>22</td>
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</tbody>
</table>
For further study…

• Standardized culture methods: sonication, vortex in broth, incubate in broth after sonication/vortex?
• Sonication parameters: mHz, W/cm² and time
• Volume of fluid for sonication, amount to plant
• Correlation of CFU to clinical parameters. Assessment of specificity

• Need larger, controlled studies
Thank you!

Robert J. Tomsich Pathology & Laboratory Medicine – in Spring!