Test soils, cleaning indicators and protein residual tests in comparison


Austrian society for sterile supply
Hygiene institute University Vienna
MA 39 State Institute Vienna
AGES State Institute Vienna
Vamed Vienna
Hospital Hygiene Upper Austria (GESPAG)
Micromed Upper Austria
Institute for Applied Hygiene Styria
Hospital Hygiene Styria (KAGES)
Institute for Health Care Engineering Technical University Graz
Situation

- CEN ISO/TS 15883-5 is in revision
- Proposal: "Test rigs" to compare test soils

Figure 5: Spray test apparatus:
Publication in „Central service“* says that the test soils from England, Germany (egg yolk), „Browne TS“ (und „Browne Load Check“) are the most difficult ones

Tests in practice show exactly the opposite

Experts committee of the OEGSV decided to conduct a study

Content of the study

• Part A: Comparison of test methods of CEN ISO/TS 15883-5 under real conditions (WD with instruments)
• Part B: Comparison of cleaning indicators
• Part C: Protein residual tests in comparison (former work, updated)
Part A: Comparison of test methods under real conditions
Comparison of test methods

Questions:

1. Which test method is the most challenging in practice?
2. Are metal plates comparable to real instruments (clamps)?
Comparison of test methods

- Austria, Sweden, GB, Germany old (egg yolk), Germany new (blood)
  - WD: Miele 7836
  - Test objects:
    - Crile-clamps 14 cm straight,
    - Metal plates (50 x 50 mm, 0,2 mm, like given in the test rig proposal)
  - 5 baskets on 3 levels(2/2/1) each with 10 clamps and 2 metal plates
  - 6 parallel tests
## Test conditions

<table>
<thead>
<tr>
<th>15883 Annex</th>
<th>Test soil</th>
<th>Soiling</th>
<th>open/ close of the joint</th>
<th>Drying conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>reactivated sheep blood (Austria)</td>
<td>paint brush (joint only)</td>
<td>yes / no</td>
<td>room temperature 1 h</td>
</tr>
<tr>
<td>M</td>
<td>reactivated cattle blood (Sweden)</td>
<td>Immersion (whole test object)</td>
<td>yes</td>
<td>room temperature 2,5 h</td>
</tr>
<tr>
<td>N</td>
<td>defibrinated sheep blood, Mucin, egg yolk (GB)</td>
<td>paint brush (joint only)</td>
<td>no</td>
<td>room temperature 1 h</td>
</tr>
<tr>
<td>G</td>
<td>egg yolk (Germany „old“)</td>
<td>paint brush (joint only)</td>
<td>no</td>
<td>room temperature 1 h</td>
</tr>
<tr>
<td>-</td>
<td>reactivated sheep blood (Germany “new”)</td>
<td>Pipette (0,1 ml into the joint)</td>
<td>yes</td>
<td>45 °C / 1 h</td>
</tr>
</tbody>
</table>
### Test series 1:

**“Fail programme“**
according to the guideline of the DGKH, DGSV und AKI
(Annex 4: programme 2)

<table>
<thead>
<tr>
<th>Pre rinse 1</th>
<th>1 min (cold water)</th>
<th>2 min (cold water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre rinse 2</td>
<td>N. A.</td>
<td>5 min (40 °C, 0,3 % Mediclean forte)</td>
</tr>
<tr>
<td>Cleaning:</td>
<td>5 min (55 °C, 0,3 % Standard cleaner (DIN 10511)</td>
<td>10 min (60 °C, 0,8 % Mediclean forte)</td>
</tr>
<tr>
<td>Rinse 1:</td>
<td>2 min (1/3 cw- 2/3 ww)</td>
<td>2 min (1/3 cw- 2/3 ww)</td>
</tr>
<tr>
<td>Rinse 2:</td>
<td>1 min (deionised water)</td>
<td>2 min (deionised water)</td>
</tr>
</tbody>
</table>

### Test series 2

**“Pass programme“**

<table>
<thead>
<tr>
<th>Pre rinse 1</th>
<th>1 min (cold water)</th>
<th>2 min (cold water)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Rinse 1:</td>
<td>2 min (1/3 cw- 2/3 ww)</td>
<td>2 min (1/3 cw- 2/3 ww)</td>
</tr>
<tr>
<td>Rinse 2:</td>
<td>1 min (deionised water)</td>
<td>2 min (deionised water)</td>
</tr>
</tbody>
</table>
Results clamps*

*The results of the metal plates are not shown, because not a single one showed residuals.
Summary of the results

test series 1

Residuals test series 1
(mean values n=6)

%
Summary of the results

test series 2

Residuals (%) test series 2

%  0  5  10  15  20  25  30

A

GB

test no.

1  2  3
1. Which test method is the most challenging under real conditions?

**Strong**
- Sweden, Austria, Germany „new“

**Weak:**
- GB, Germany old (egg yolk)

Are metal plates comparable to real instruments (clamps)?

No!
Part B: Comparison of cleaning indicators
Questions:
- Which informative value do cleaning indicators have?
- Are the cleaning indicators comparable?

- A: Wash Check (Dr. Früh)
- B: TOSI (Pereg)
- C: Simicon RI (Simicon) prior to and after improvements
- D: Load Check (Browne)
Tested cleaning indicators
Evaluation scheme

- 0 clean
- 1 small residuals
- 2 large residuals
- 3 very large residuals
- 4 test soil not removed at all
1. „Immersion Test Rig“ (50 °C softened water, standard cleaner 0,5 %, Mediclean forte 0,5 and 1 %)
2. Test programme WD with only cold water (1-10 min)
3. Indicators used during comparison of test methods
Immersion-Test-Rig
Results Test Rig

- Deion. water (10 Min)
- Mediclean forte 0,5% (5 Min)
- Mediclean forte 1% (5 Min)
- Standard cleaner 0,5% (10 Min)

Dr. Früh
Tosi
Simicon
Load Check
### Results immersion Test Rig

<table>
<thead>
<tr>
<th>min</th>
<th>Softened water / 50 ºC</th>
<th>min</th>
<th>Mediclean forte 0,5 % / 50 ºC</th>
<th>min</th>
<th>Mediclean forte 1,0 % / 50 ºC</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td><img src="image1" alt="Image" /></td>
<td>3</td>
<td><img src="image2" alt="Image" /></td>
<td>1</td>
<td><img src="image3" alt="Image" /></td>
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<tr>
<td>10</td>
<td><img src="image4" alt="Image" /></td>
<td>5</td>
<td><img src="image5" alt="Image" /></td>
<td>5</td>
<td><img src="image6" alt="Image" /></td>
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<tr>
<td>10</td>
<td><img src="image7" alt="Image" /></td>
<td>5</td>
<td><img src="image8" alt="Image" /></td>
<td>5</td>
<td><img src="image9" alt="Image" /></td>
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<tr>
<td>10</td>
<td><img src="image10" alt="Image" /></td>
<td>10</td>
<td><img src="image11" alt="Image" /></td>
<td>5</td>
<td><img src="image12" alt="Image" /></td>
</tr>
</tbody>
</table>
Results WD (H₂O)

- Dr. Früh
- Tosi
- Simicon
- Load Check

Time: 1 min, 2 min, 3 min, 4 min, 5 min, 10 min
Results WD (H$_2$O)
Results WD („Fail“ programme*)
% of indicators which show residuals

* Austrian test method: 78 % residuals

„Pass“ programme**
None of the indicators showed residuals

** Austrian test method: 23 % residuals
Results WD „Fail“ programme
1. Which informative value do the tested indicators have?

Very low: Load Check, Simicon RI (prior to improvements), Tosi

Acceptable (by changing the evaluation scheme): Wash Check (Dr. Früh)

Acceptable (after improvements): Simicon RI

2. Are the indicators comparable?

No, not at all!
Part C: Protein residual tests
Test Kits

- HyLite (ATP)
- HemoCheck-S (Pereg)
- Test-Kit for Protein Detection (Miele)
- Protect-M (Biotrace)
- BCA Protein Assay Kit (Pierce)
HY-Lite (VWR)

• ATP-detection
  – Adenosintriphosphate in cells
  – reaction with Luciferin/Luciferase
  – the beamed light is measured in a luminometer (RLU)
• Only ATP, not protein is detected!
Results HY-Lite

- It was not possible to create a calibration curve
- i.e. the unit RLU could not be correlated with the amount of protein

<table>
<thead>
<tr>
<th>Blood</th>
<th>BCA µg/ml</th>
<th>RLU 1 10 sec</th>
<th>RLU 2 30 sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:100</td>
<td>130</td>
<td>&gt;&gt;&gt;</td>
<td>&gt;&gt;&gt;</td>
</tr>
<tr>
<td>1:1000</td>
<td>12</td>
<td>23000</td>
<td>73000</td>
</tr>
<tr>
<td>1:10000</td>
<td>0</td>
<td>18000</td>
<td>12000</td>
</tr>
<tr>
<td>1:100000</td>
<td>0</td>
<td>2000</td>
<td>1300</td>
</tr>
<tr>
<td>1:1000000</td>
<td>0</td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td>1:10000000</td>
<td>0</td>
<td>23</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Instruments</th>
<th>µg/ml</th>
<th>RLU</th>
</tr>
</thead>
<tbody>
<tr>
<td>clean</td>
<td>20-50</td>
<td>12-110</td>
</tr>
<tr>
<td>Fibrin</td>
<td>50-100</td>
<td>16-310</td>
</tr>
<tr>
<td>bloody</td>
<td>&gt;&gt;100</td>
<td>980-10000</td>
</tr>
<tr>
<td>clean/dry</td>
<td>&gt;&gt;100</td>
<td>44</td>
</tr>
<tr>
<td>clean/wet</td>
<td></td>
<td>190</td>
</tr>
<tr>
<td>touched</td>
<td></td>
<td>920</td>
</tr>
<tr>
<td>BSA</td>
<td>2000</td>
<td>79</td>
</tr>
<tr>
<td>water</td>
<td></td>
<td>77</td>
</tr>
</tbody>
</table>

Conclusion: NOT suitable for medical devices!
HemoCheck-S (BAG)

- Test tubes
  - Activator
  - Indicator
- Swab

+ very sensitive (0.1 µg blood)
- Detects blood only
  - BSA: negative
  - fibrin: negative

BSA blood

Conclusion: NOT suitable for medical devices!
Test-Kit for Protein Detection (Miele)

- Reagents A - C
- Rinse solution
- 1 ml pipette
- Colour label (reflectometer)

+ Sensitivity ~ 5 µg protein
+ quick
- rather laborious (time consuming)

Conclusion: suitable for medical devices
Protect-M (Biotrace)

- Test tube (with reagents, colour label and swab)

+ easy handling
- not suitable for narrow cavities
- rather expensive

Conclusion: Limited suitability for medical devices
BCA Protein Assay Kit (Pierce)

- Reagent A
- Reagent B
- Albumin standard ampules

+ easy handling (through modification)
+ rather low priced
+ suitable for nearly all MDs
- cross reaction with certain metals (copper)

Conclusion: suitable for medical devices

See poster!
Hy-Lite: ATP does not correlate with protein

HemoCheck-S: only detects blood (~ 0.1µg)

Miele: quickest protein detection (but labourious handling)

Protect-M: easiest handling

BCA (modified): most flexible method
Thank you

For your attention!