TRANSFER AND REGROWTH TEST FOR THE EVALUATION OF ANTI-BIOFILM ACTIVITY OF CLINELL® SPORICIDAL WIPE

Work carried out at Cardiff University, School of Pharmacy & Pharmaceutical Sciences, Pharmaceutical Microbiology Laboratory, under the supervision of Dr Katarzyna Ledwoch.

Client: GAMA Healthcare Ltd

Period of testing
Date of delivery: 13/04/18
Dates of tests: 16/01/18 - 13/04/18
Date of report: 15/05/2018

Tests performed

Dry biofilms
Dry biofilms of Staphylococcus aureus (NCTC 10788) were produced by inoculating $10^6$ CFU/ml S. aureus overnight culture in 1 mL of TSB with 5% w/v glucose and 3 g/L BSA on stainless steel disc AISI 430 (0.7±0.07 mm thickness; 10±0.5 mm diameter) placed in 24 wells plate. Biofilms were grown for 12 days in subsequent wet and dry cycles (2 days per each cycle). For the dry cycle, the media was drained out from each well plate and the samples were incubated at 37°C. For the wet cycle, 1 mL of TSB with 3 g/L BSA was dispensed to each well and the well plate was placed on rotary shaker at room temperature.

Wiping:
ASTM E2967-15 test modified for dry biofilm testing. Coupons harbouring dry biofilms were wiped on both sides for 10 seconds at 500 g pressure.

Transfer test:
Transferability determines how well pathogens from dry biofilm can transfer to other surfaces post-wiping. 24h after wiping, test surfaces were pressed with 100g pressure against DE agar surface and incubated overnight in 37°C; 36 consecutive adpressions were performed. Transferability was measured by dividing the number of positive (growth) contacts by the total number of adpressions (i.e. 18/36=50%).

Regrowth test:
Regrowth test measures the time needed for a treated biofilm to regrow. Treated biofilms were placed into DE broth (neutraliser) and incubated at 37°C. Change in broth turbidity and change in colour from purple to yellow indicated bacterial growth.

Log reduction test:
Reduction (expressed in $\log_{10}$ (CFU/mL)) gives the number of bacteria that are removed following wiping. Following wiping and incubation at room temperature, inoculated test surfaces are vortexed in neutralising broth with 100 µg/mL proteinase K to recover viable bacteria. The number of colony forming units that survived treatment is determined by
measuring the log_{10} difference between the number of bacteria recovered from untreated (reference control) and treated surfaces.

**Test products**
- See table 1
- Clinell® Sporicidal wipes lot number SA411217

**Validation of test**
- Preparation of GAMA Sporicidal wipes followed standard operating procedure no. R&D SOP-022.
- For neutralization of disinfectant activity, the Dey-Engley neutraliser was used.

**Neutralising formulations**
- **Dey-Engley neutralising broth** - Glucose 10 g/L, lecithin 7 g/L, sodium thiosulphate 6 g/L, tween® 80 5 g/L, tryptone 5 g/L, sodium bisulphite 2.5 g/L, yeast extract 2.5 g/L, sodium thioglycolate 1 g/L, bromocresol purple 0.02 g/L.

**Test results**
Each test was carried out in 2-4 replicates.

**Experimental conditions**
**Test strains:** *Staphylococcus aureus* NCTC 10788
**Wiping time:** 10 seconds
**Contact times:** 2 minutes
Test temperature: 25° C

**Interfering substance:** Biofilms were grown in 3 g/L BSA

**Results**
Following the modified ASTM E2967-15 test, the Clinell® sporicidal wipe reduced the transferability of *S. aureus* from dry biofilms to 33%. Regrowth of dry biofilm was prevented for 5 days following treatment using the Clinell® Sporicidal wipes (Table 1).

NaDCC-based products treatment resulted in 50% to 100% transferability of bacteria from dry biofilms. Biofilm regrowth was prevented only from 1 to 2 days following NaDCC-based products treatments. Chlorine dioxide-based product and NaOCl 1,000ppm did not reduce transferability of test bacteria. Regrowth was observed after 1 and 3.9 days for chlorine dioxide and NaOCl 1,000ppm, respectively (Table 1).

*S. aureus* were transferred from dry biofilms in all contacts after water treatment or when no treatment was applied (control). Regrowth was observed within 24 h (Table 1).
Table 1: Bacteria transferability and regrowth following treatments. The most effective treatment results in the lowest transferability, as less touches of treated surface result in pathogens' transfer. The least effective treatment is indicated by the highest transferability; 100% transferability indicates that all contacts resulted in bacterial transfer and surface is not safe to patients and hospital staff. The most effective treatment prevents the regrowth of pathogens for a longer time. The longer the regrowth time, the better the treatment. Fast regrowth (within 1-2 days) indicates ineffective treatment; as bacteria recover quickly; frequent cleaning/disinfection would be required to maintain the surface safe. Log$_{10}$ reduction indicates how many bacteria were killed following the treatment. Effective treatment is characterised by high log$_{10}$ reduction number.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Transferability</th>
<th>Regrowth</th>
<th>Log$_{10}$ reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinell® Sporicidal wipes</td>
<td>33%</td>
<td>5 days</td>
<td>&gt;3</td>
</tr>
<tr>
<td>NaOCl 1,000ppm on wipe*</td>
<td>100%</td>
<td>3.9 days</td>
<td>&gt;3</td>
</tr>
<tr>
<td>1.6-3.3 g/L NaDCC, 0.3-0.6 g/L adipic acid, 0.2-0.3 g/L arylsulfonates, 0.03-0.08 g/L sodium fatty acid sarcosides on wipe*</td>
<td>100%</td>
<td>2 days</td>
<td>&gt;3</td>
</tr>
<tr>
<td>0.6-1.8 g/L NaDCC, 0.6-1.8 g/L adipic acid, 0.3-0.6 g/L sodium toluene sulphonate, 0.06-0.3 g/L sodium n-lauroylsarcosinate on wipe*</td>
<td>78%</td>
<td>2 days</td>
<td>&lt;3</td>
</tr>
<tr>
<td>0.6-1.8 g/L NaDCC, &lt; 0.2 g/L sulfonic acid wipes</td>
<td>75%</td>
<td>1.5 days</td>
<td>&lt;3</td>
</tr>
<tr>
<td>1-2g/L NaDCC, 0.3-1g/L adipic acid, 0.03-0.2g/L sodium carbonate on wipe*</td>
<td>78%</td>
<td>1.3 day</td>
<td>&lt;3</td>
</tr>
<tr>
<td>1-1.6 g/L NaDCC, 0.7-1 g/L sodium hydrogen carbonate, 0.3-0.6 g/L adipic acid, 0.1-0.3 g/L sodium toluenesulphonate, 0.03-0.1 g/L sodium N-lauroyl sarcosinate, 0.03-0.1 g/L pentasodium tripophosphate, 0.003-0.03 g/L silicon dioxide on wipe*</td>
<td>100%</td>
<td>2 days</td>
<td>&gt;3</td>
</tr>
<tr>
<td>0.2 ml/ L chlorine dioxide on wipe*</td>
<td>100%</td>
<td>1 days</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Water on wipe*</td>
<td>100%</td>
<td>1 day</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Reference (no treatment)</td>
<td>100%</td>
<td>&lt;1 day</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*Wipe material: Disposable Microfiber Cloth (Rubbermaid Commercial), 1.5 ml disinfectant per 0.6 g wipe
SUMMARY

Measuring transferability and time for regrowth of *S. aureus* NCTC10788 from dry biofilms are essential indicators for determining the effectiveness of treatments against dry biofilms. These indicators provide information on microbial prevalence on dry surfaces. To ensure patients and staff safety, it is indicated that the transfer of pathogens from dry biofilms is decreased following treatment and that treatment prevent the regrowth of dry biofilms on surfaces. Clinell® sporicidal wipes (lot number SA411217) were three times more effective in reducing the transferability of pathogens from dry biofilm compared to 1,000ppm NaOCl, 0.2 ml/L chlorine oxide and two NaDCC-based products. None of the other products were able to decrease transferability of *S. aureus* NCTC10788 below 50% level. In addition, Clinell® sporicidal wipes prevented the regrowth of dry biofilm for 5 days. This is a better result in comparison to all NaDCC-based products and chlorine dioxide tested. Clinell® sporicidal wipe was able to kill more than 99.9% of bacteria from dry biofilm. Log_{10} reduction achieved by Clinell® sporicidal was better than chlorine dioxide-based product and several NaDCC-based products.

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Professor of Pharmaceutical Microbiology
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