

# Comparative Study of Four Floor Sanitizers for Biofilm Removal

## May 2019; Purdue University

### Objectives:

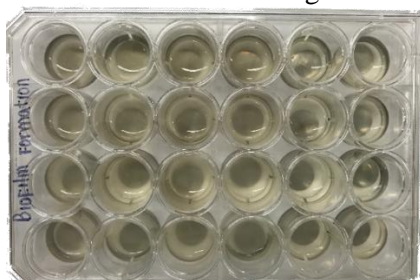
- Determine culture concentration for each sanitizer
- Biofilm formation on stainless steel coupons
- Sanitizer application and efficacy evaluation
- CryoSEM confirmation for presence of biofilms and impact on biofilm removal following treatment

### Culture concentration:

The strains of bacteria used in this study were *Salmonella enterica* s.v Typhimurium, *Listeria monocytogenes* and *E. coli* O157:H7. *E. coli* O157:H7 and *Salmonella* Typhimurium were grown in LB broth and *Listeria monocytogenes* was grown in BHI broth to a final concentration of  $10^8$  to  $10^9$  CFU/ml after 24 hours of incubation with rotation at 37°C. Liquid cultures were washed three times with 0.1 M phosphate buffer, pH 7.0 (PB) to remove the growing medium by centrifuging the samples (6,000 rpm, 5 min). The pelleted bacteria were resuspended in PB and mixed to form a cocktail of similar concentration of each bacterium for biofilm formation.

### Biofilm formation and detachment:

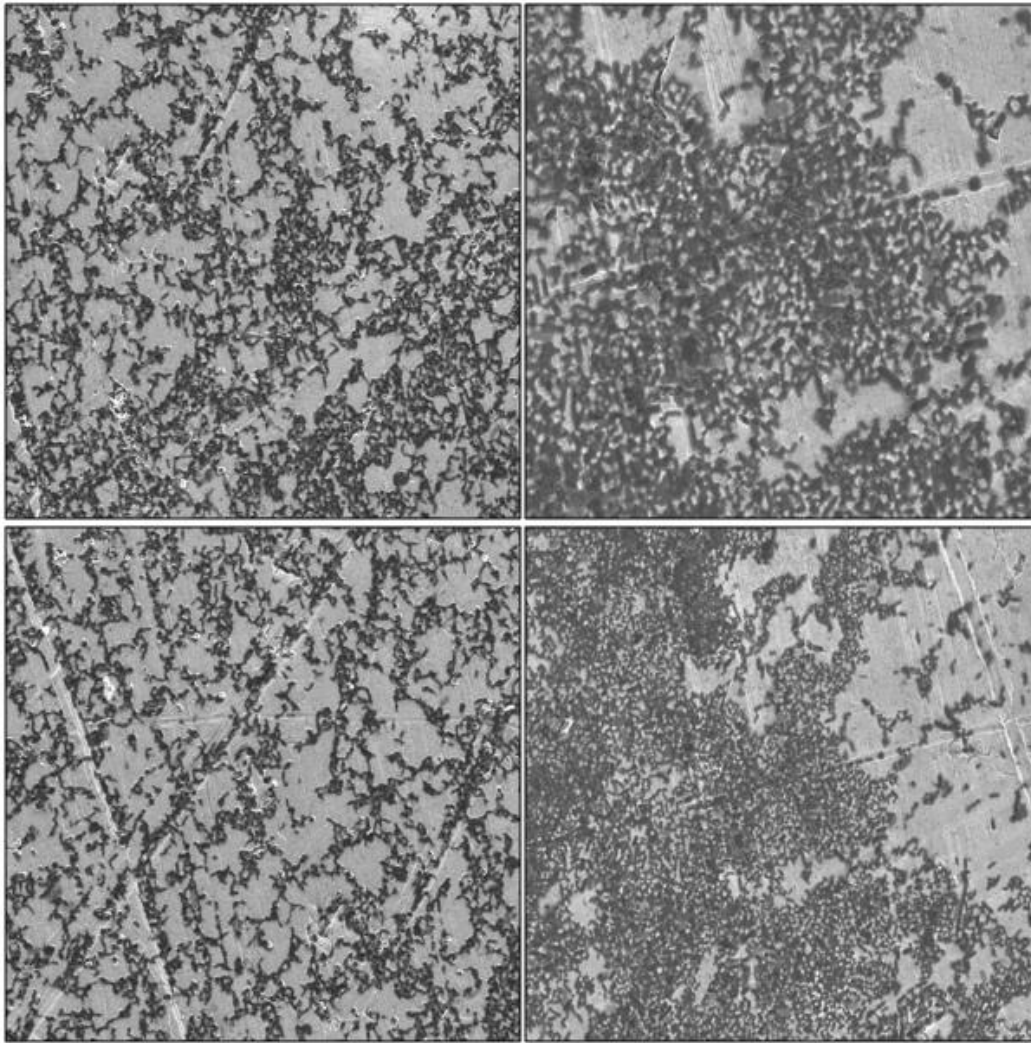
For biofilm formation on stainless steel coupons, multi-well plates were filled with 1 mL of inoculum mix culture and the stainless-steel coupons were placed into each well (Figure 1). Plates were incubated with shaking at 150 rpm at 32°C for 5 days, adding 0.5 mL of LB broth and 0.5 of BHI broth every 24 hours. The coupons were removed from the multi-well plate and washed with 1 mL of distilled water to remove any unattached cells. The coupons were then dried at room temperature for 5-10 minutes. To remove the biofilm from the stainless-steel coupons, each coupon was immersed into sterile tubes containing 1 mL neutralizer buffer, sonicated for 5 minutes, and then vortexed for 20 seconds. The samples were serially diluted using PB and spread plated on selective media for *E. coli* (MacConkey Agar), *Salmonella* (XLT4 Agar) and *Listeria* (Modified Oxford Medium). The bacteria were enumerated following incubation at 37°C for 48 hours.



**Figure 1.** Multi-well plate used for biofilm formation of *E. coli*, *Listeria* and *Salmonella* on stainless steel coupons.

**Table 1.** Enumeration of bacteria (CFU/mL; SD = Standard Deviation) from stainless steel coupons in phosphate buffer.

<b>BIOFILM FORMATION ON STAINLESS STEEL COUPONS</b>			
<b>Replicate</b>	<b><i>E. coli</i></b>	<b><i>Salmonella</i></b>	<b><i>Listeria</i></b>
1	3.00E+08	2.00E+07	4.00E+06
2	1.00E+07	3.00E+07	1.00E+06
3	3.00E+07	2.70E+07	6.00E+05
4	4.00E+08	2.00E+07	4.00E+06
5	1.00E+07	8.00E+07	9.00E+05
<b>AVERAGE</b>	<b>1.50E+08</b>	<b>3.54E+07</b>	<b>2.10E+06</b>
<b>SD</b>	<b>1.86E+08</b>	<b>2.53E+07</b>	<b>1.74E+06</b>



**Figure 2.** Representative cryo-SEM Images of 5-day old biofilm from *E. coli*, *Listeria monocytogenes* and *Salmonella* Typhimurium formed on stainless steel coupons. Bacterial matrix is shown forming a consistent biofilm on the surface of the coupons.

**Table 2.** Enumeration of the bacteria in biofilms (CFU/mL; SD = Standard Deviation) from stainless steel coupons after **water** only application for 5 minutes.

<b>WATER TREATMENT CONTROL (5 minutes)</b>			
<b>Replicate</b>	<b><i>E. coli</i></b>	<b><i>Salmonella</i></b>	<b><i>Listeria</i></b>
1	3.00E+07	1.00E+06	2.00E+04
2	1.00E+07	7.00E+06	1.00E+05
3	2.00E+06	3.00E+07	2.00E+05
4	3.00E+06	4.00E+05	7.00E+05
5	9.00E+07	1.00E+06	6.00E+06
<b>AVERAGE</b>	<b>2.70E+07</b>	<b>7.88E+06</b>	<b>1.40E+06</b>
<b>SD</b>	<b>3.70E+07</b>	<b>1.27E+07</b>	<b>2.58E+06</b>
<b>LOG reduction</b>	<b>0.74</b>	<b>0.65</b>	<b>0.17</b>

Table 2 represents the concentration of bacteria after a **5-minute water** application to remove biofilm. The water treatment was ineffective on achieving reduction in the concentration of viable *Salmonella*, *E. coli* and *Listeria* cells that were present on the stainless-steel coupon.

**Table 3.** Enumeration of the bacteria in biofilms (CFU/mL; SD = Standard Deviation) from stainless steel coupons after **water** application for 10 minutes.

<b>WATER TREATMENT CONTROL (10 minutes)</b>			
<b>Replicate</b>	<b><i>E. coli</i></b>	<b><i>Salmonella</i></b>	<b><i>Listeria</i></b>
1	3.00E+07	2.00E+06	3.00E+06
2	9.00E+06	9.00E+05	2.00E+05
3	4.00E+06	2.00E+05	4.00E+04
4	1.00E+07	3.00E+06	2.00E+05
5	1.00E+06	2.00E+05	6.00E+04
<b>AVERAGE</b>	<b>1.08E+07</b>	<b>1.26E+06</b>	<b>7.00E+05</b>
<b>SD</b>	<b>1.13E+07</b>	<b>1.22E+06</b>	<b>1.29E+06</b>
<b>LOG reduction</b>	<b>1.14</b>	<b>1.45</b>	<b>0.48</b>

Table 3 represents the concentration of bacteria after a **10-minute water** application to remove biofilm. The water treatment was ineffective on achieving reduction in the concentration of viable *Salmonella*, *E. coli* and *Listeria* cells that were present on the stainless-steel coupon.

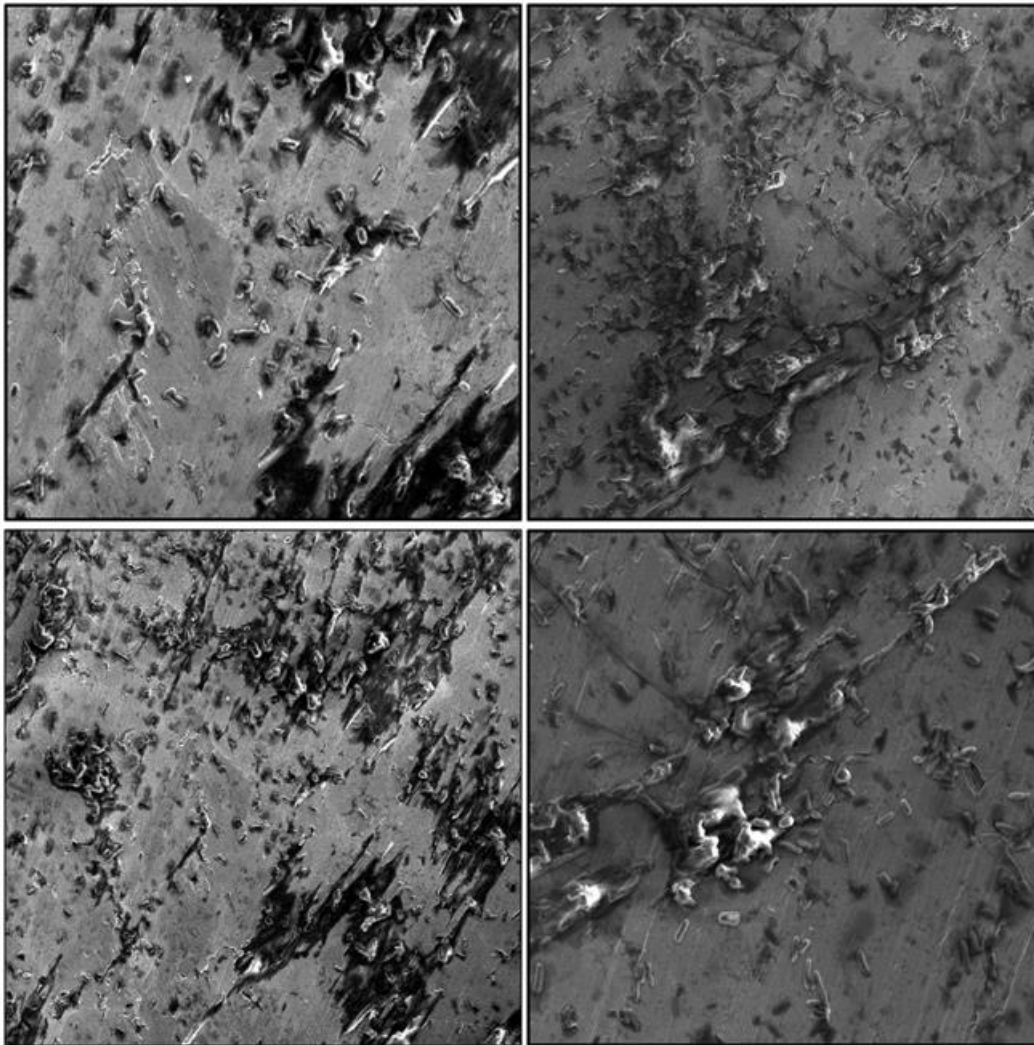
### Sanitizer Application

Preparation and application of the sanitizers was performed by following the company requirements. For the evaluation of the Floor Guard 1, PeraGuard (Enviro Tech), Ultra-Step (Sterilex) and Security Floor Sanitizer (DeVere), 37.5 grams of *each sanitizer* was dissolved in 1 liter of nanopure water. After biofilm formation, the stainless-steel coupons were immersed into each prepared sanitizer. Five replicates (different coupons) were used for the application of each sanitizers for 5 and 10 minutes of contact time. To remove the biofilm from the stainless-steel coupons following treatment, each coupon was immersed into sterile tubes containing 1mL neutralizer buffer and sonicated for 5 minutes and vortexed for 20 seconds. The samples were serially diluted using PB and spread plated on selective media for *E. coli* (MacConkey Agar), *Salmonella* (XLT4 Agar) and *Listeria* (Modified Oxford Medium). The bacteria were enumerated following incubation at 37°C for 48 hours.

**Table 4.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after Floor Guard 1 application for 5 minutes.

<b>Floor Guard 1 (5 minutes)</b>			
<b>Replicate</b>	<b><i>E. coli</i></b>	<b><i>Salmonella</i></b>	<b><i>Listeria</i></b>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	2.70E+01	2.00E+02	4.00E+01
4	6.00E+01	3.00E+01	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>1.74E+01</b>	<b>4.60E+01</b>	<b>8.00E+00</b>
<b>LOG reduction</b>	<b>6.94</b>	<b>5.89</b>	<b>5.42</b>
<b>LOG red Water</b>	<b>6.19</b>	<b>5.23</b>	<b>5.24</b>

Application of Floor Guard 1 for 5 mins achieved ~7, 6 and 5 log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.

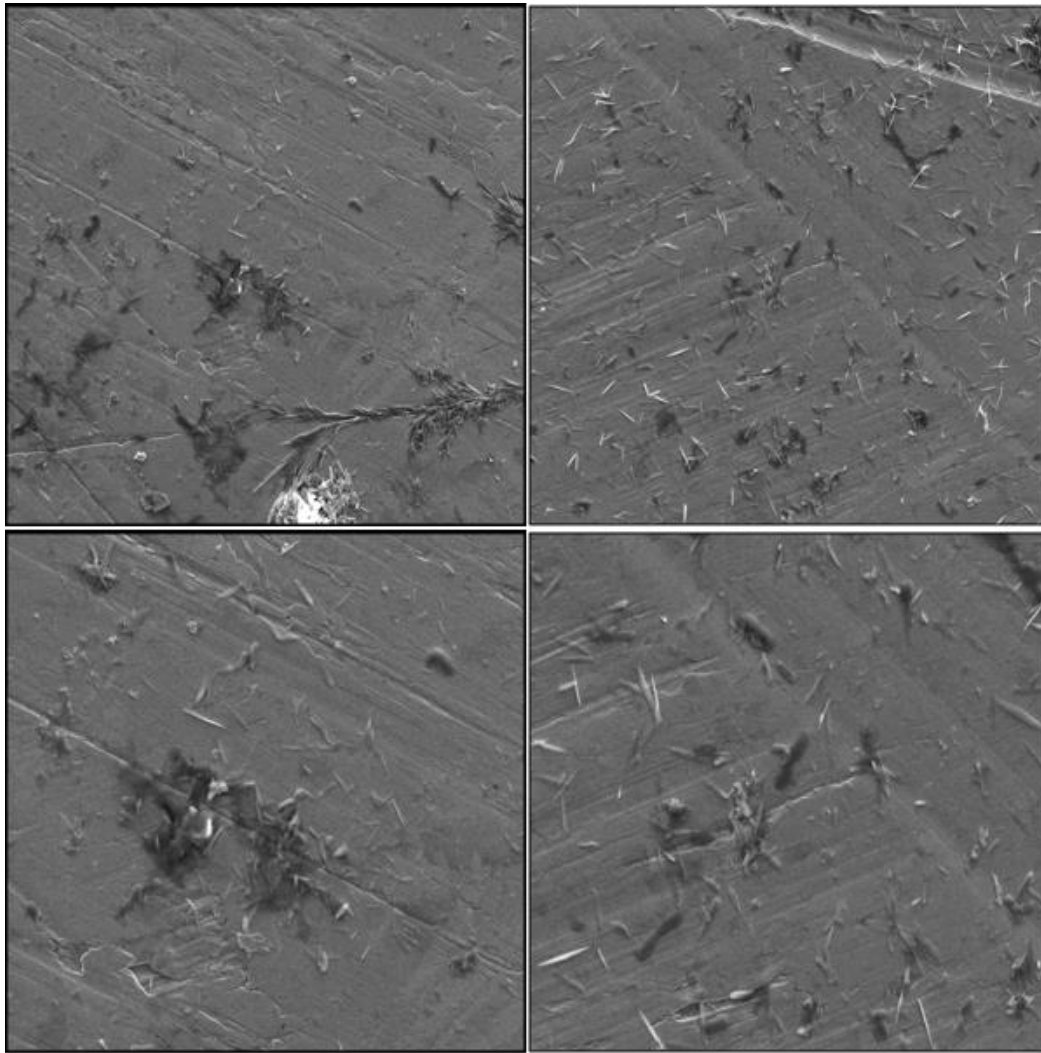


**Figure 3.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Floor Guard 1 for 5 minutes**. Bacterial debris is visible, however, total bacterial inactivation was achieved suggesting that the attached cells were disrupted because of the action of the sanitizer

**Table 5.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **PeraGuard** application for 5 minutes.

<i>PeraGuard (5 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.43</b>	<b>6.90</b>	<b>6.15</b>

Application of **PeraGuard for 5 mins** achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.

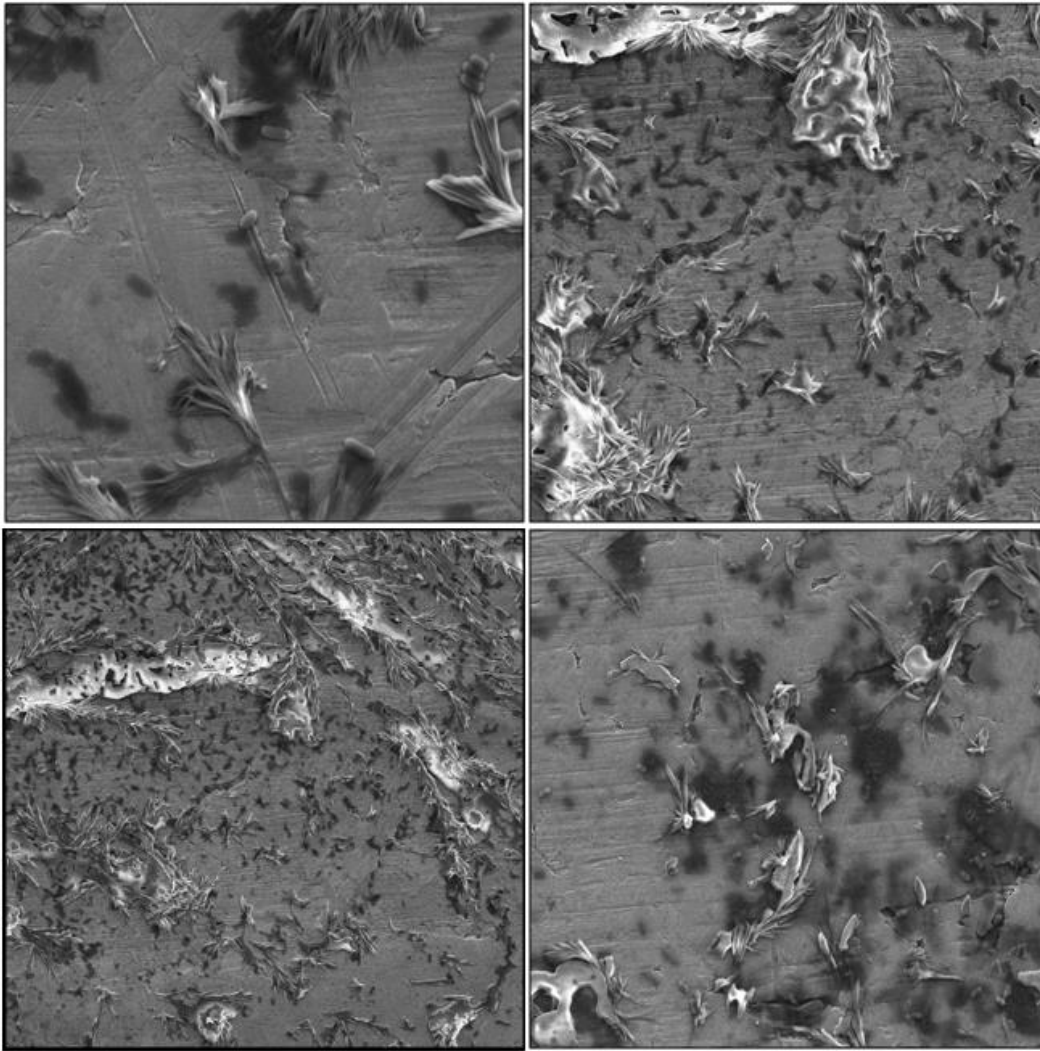


**Figure 4.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **PeraGuard for 5 mins**. Some crystallization of the sanitizer is observed on the surface of the coupon. A few inactivated bacterial cells were found on the surface. Very good removal of biofilm overall.

**Table 6.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **Ultra Step application for 5 minutes**.

<i>Ultra Step (5 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.43</b>	<b>6.90</b>	<b>6.15</b>

Application of Ultra-Step for 5 mins achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.

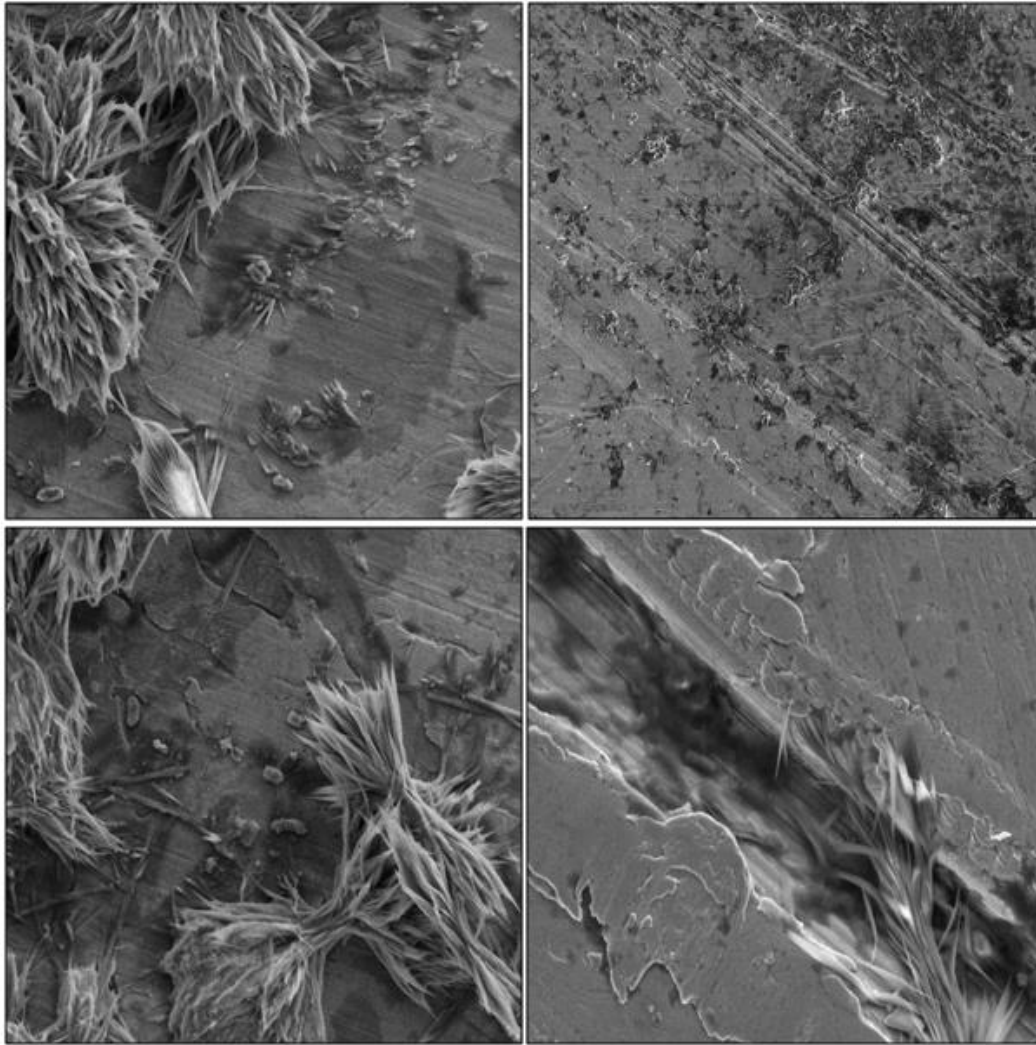


**Figure 5.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Ultra-Step for 5 mins**. Moderate debris from the sanitizer and/or biofilm were visualized on the surface of the stainless steel coupons.

**Table 7.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **Security Floor Sanitizer application for 5 minutes**.

<i>Security Floor Sanitizer (5 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG reduction Water</b>	<b>7.43</b>	<b>6.90</b>	<b>6.15</b>

Application of **Security Floor Sanitizer for 5 mins** achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.

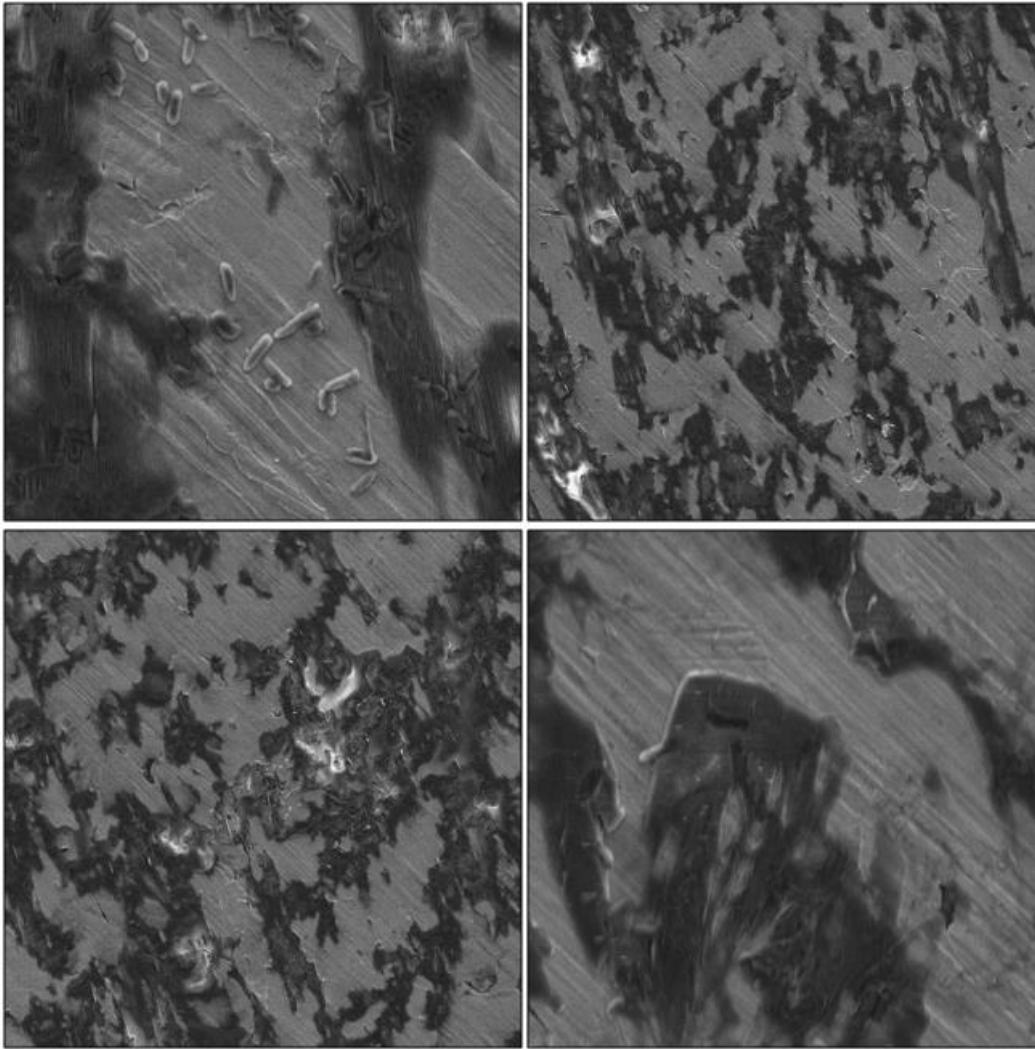


**Figure 6.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Security Floor Sanitizer for 5 minutes**. Debris both from the bacterial cells, biofilm and crystallization of the sanitizer were found on the surface of the stainless steel coupons.

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**Table 8** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **Floor Guard 1 application for 10 minutes**.

<i>Floor Guard 1 (10 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.03</b>	<b>6.10</b>	<b>5.85</b>

Application of **Floor Guard 1 for 10 mins** achieved complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.



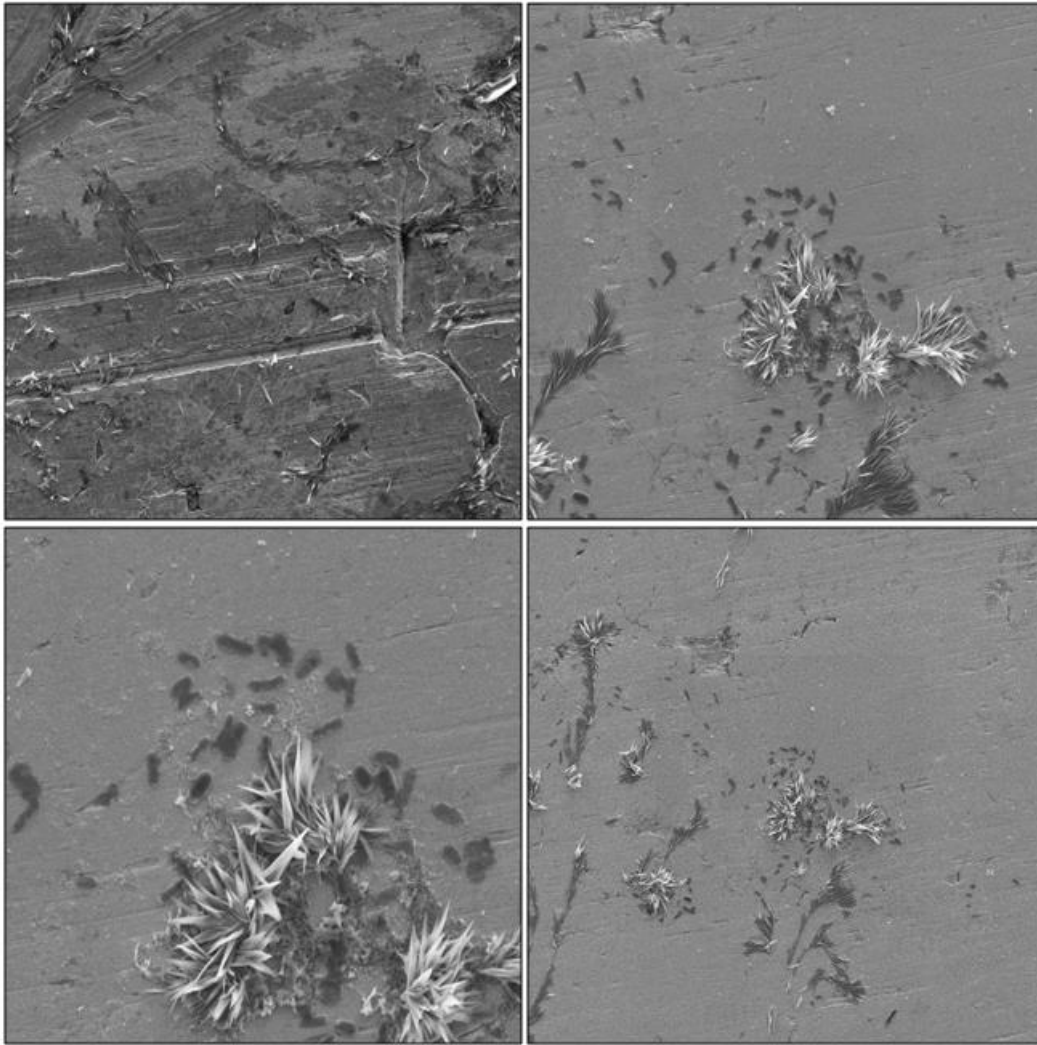
**Figure 7.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Floor Guard 1 for 10 mins**. Modest debris from bacterial cells were observed. However, the microbiological assay shows a complete log reduction, indicating that attached bacterial cells were inactivated.

**Table 9.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **PeraGuard application for 10 minutes**.

<i>PeraGuard (10 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.03</b>	<b>6.10</b>	<b>5.85</b>

Application of **PeraGuard for 10 mins** achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.



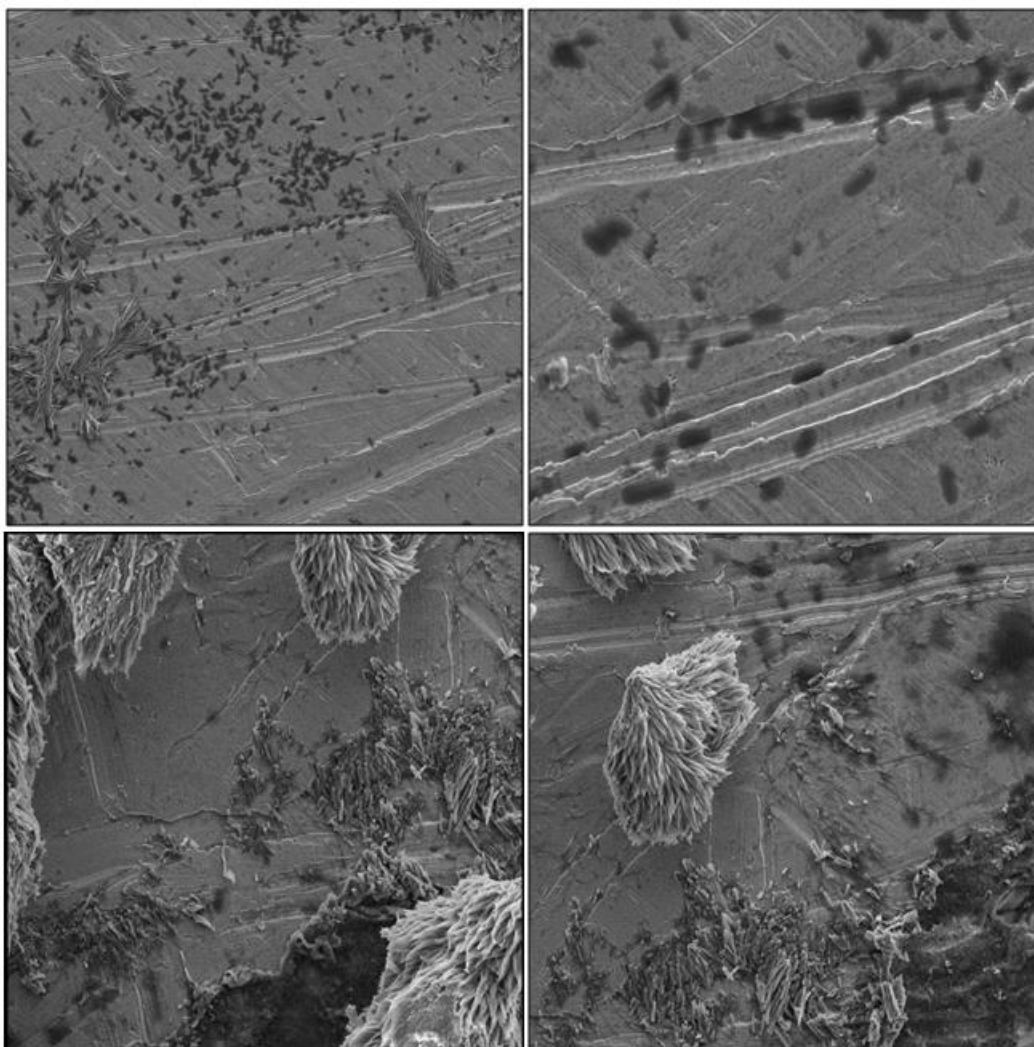


**Figure 8.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **PeraGuard for 10 mins**. After 10 minutes minor bacterial and crystalized sanitizer debris were found on the surface of the stainless steel coupons. Very good biofilm removal overall.

**Table 10.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **Ultra Step** application for 10 minutes.

<i>Ultra Step (10 minutes)</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.03</b>	<b>6.10</b>	<b>5.85</b>

Application of **Ultra Step for 10 mins** achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.

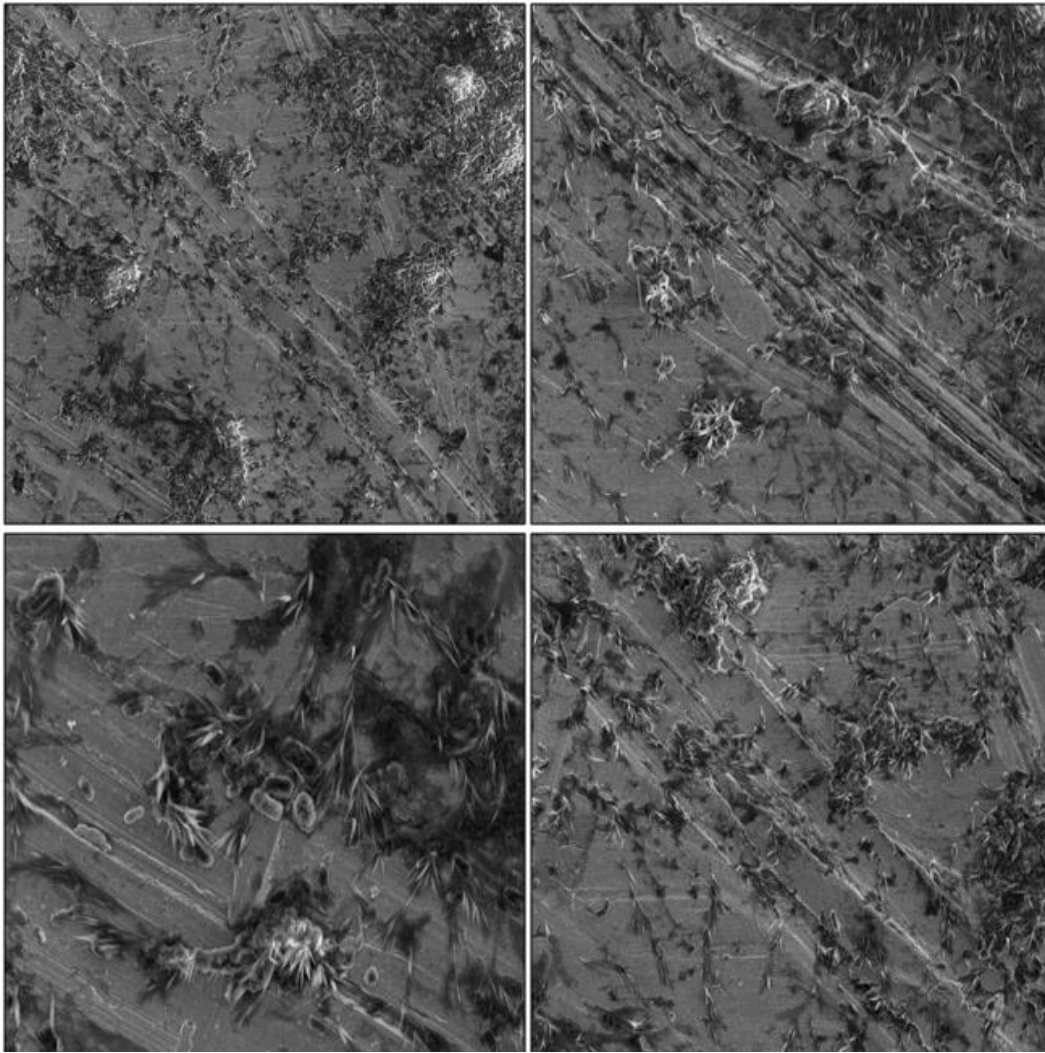


**Figure 9.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Ultra Step for 10 mins**. Moderate debris from the sanitizer and some individual inactivated bacterial cells were visualized on the surface of the stainless steel coupons, indicating only modest biofilm removal properties.

**Table 11.** Enumeration of the bacteria in biofilms (CFU/mL) from stainless steel coupons after **Security Floor Sanitizer** application for 10 minutes.

<i>Security Floor Sanitizer</i>			
<i>Replicate</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Listeria</i>
1	0.00E+00	0.00E+00	0.00E+00
2	0.00E+00	0.00E+00	0.00E+00
3	0.00E+00	0.00E+00	0.00E+00
4	0.00E+00	0.00E+00	0.00E+00
5	0.00E+00	0.00E+00	0.00E+00
<b>AVERAGE</b>	<b>0.00E+00</b>	<b>0.00E+00</b>	<b>0.00E+00</b>
<b>LOG reduction</b>	<b>8.18</b>	<b>7.55</b>	<b>6.32</b>
<b>LOG red Water</b>	<b>7.03</b>	<b>6.10</b>	<b>5.85</b>

Application **Security Floor Sanitizer for 10 mins** achieved a complete log reduction of *Salmonella*, *E. coli* and *Listeria* present on stainless steel coupons after immersion in neutralizer broth.



**Figure 10.** Representative cryo-SEM Images of biofilm on stainless-steel coupons, after application of **Security Floor Sanitizer for 10 mins**. Moderate crystalized sanitizer debris and inactivated cells was found on the surface of the stainless steel coupons, indicating only modest biofilm removal properties at best.

### **Summary**

In terms of bacterial efficacy after 5 minutes contact time, the following are the rankings from left to right, best to worst:

**PeraGuard (Enviro Tech) = Ultra Step (Sterilex) = Security (DeVere) > Floor Guard I (Enviro Tech)**

After 10 minutes all performed equally as well.

In terms of biofilm removal after 10 minutes, the following are the rankings from left to right, best to worst:

**PeraGuard (excellent) > Ultra Step (good) > Floor Guard I (moderate) > Security (modest)**

**Note:** Although all products were used at the same application rates (page 3), the ‘Security’ from DeVere was used at much lower rates than the labeled use directions.