Confidential report for:

EIT International

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Interim Report on:

Assessment of Bactiscan instrument for detection of food industry biofilms

Work performed by Campden BRI (Chipping Campden) Limited
Report number: MB/REP/ZCL/151662/1 ⬧ Issue date: 11th November 2020

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Content

1   SUMMARY

A study was conducted on a Bactiscan instrument as provided by EIT International, to assess the ability of the unit to detect Biofilms within food industry settings. The unit was compared against two commercially available units.

Stage 1 of the study focused on the detection of single species biofilms. Single species biofilms were generated for microorganisms in 9 categories representing important pathogens in the food, medical and veterinary industries on stainless steel discs. Biofilms were assessed using the Bactiscan, 2 competitor instruments and the presence and extent of biofilm formation was confirmed by fluorescent microscopy.

The Bactiscan performed the best at detection of single species biofilms, with all biofilms detected strongly. The presence of biofilms was visually evident as defined areas of turquoise/light green fluorescence when using the Bactiscan, whereas the other instruments returned a more diffuse blue fluorescence, which was more difficult to distinguish from reflected light/other debris. The Bactiscan was also able to pick up weaker biofilms, and in the case of the weakest biofilm formers, was able to pick up small intense specks of blue/green fluorescence, which on closer microscopic inspection revealed the presence of small areas of biofilm growth/microcolonies.

The presence of bacteria or biofilms are visually evident as a turquoise/green fluorescence on the surfaces using the Bactiscan. Determination of the presence of biofilm in practice within the Campden BRI microbiology process hall showed it to be highly effective at locating areas of contamination compared against other selected UV torches.

Stage 2 of the study used a strong biofilm forming strain, *Pseudomonas aeruginosa* PA01*.* This organism was grown under conditions of high shear a CDC bioreactor system to achieve dense, compact biofilm growth. Samples of the biofilm were taken at 5 stages during formation and analysed using the 3 instruments. The Bactiscan could detect laboratory biofilms at an early stage of development. By comparison, the competitor UV torches could only detect the stronger biofilms (UV torch) or none at all (Backlight). The Bactiscan was also able to detect biofilm after hot water immersion at 60°C for 5 minutes, PAA immersion at 200ppm for 20 minutes and sterilising at 121°C for 3 minutes. Total viable counts and direct live/dead fluorescent microscopy confirmed the presence of live bacteria after each of these treatments.

It can be concluded that the Bactiscan is able to detect biofilms from species typical of pathogens and other target microorganisms present in a multitude of environments from across many industry areas including food, medical and veterinary areas.

2   BACKGROUND

The customer approached Campden BRI to independently assess their Bactiscan instrument as a tool to detect biofilms within a food industry setting, against two UV detection products currently available on the market.

The study comprised the following stages:

- Stage 1. Detection of single species biofilms of relevant food industry microorganisms

- Stage 2: Detection of biofilms at different stages of development

3   SAMPLES/MATERIALS

The client provided Campden BRI with a Bactiscan unit, plus two other UV detection instruments for use within the study. These instruments were labelled as "backlight torch" and "UV torch, Budget". All microorganisms as used within the study were provided by Campden BRI.

4   METHODS

Culture of microorganisms

Cultures of the following organisms were obtained from the Campden BRI culture collection (Table 1).

Table 1. Microorganisms used in the study

Organism Category	Microorganism strain	Rationale for inclusion/areas of importance
Listeria monocytogenes	*Listeria monocytogenes* ATCC 11994	Food industry (all areas, particularly RTE)
	Listeria monocytogenes Scott A strain	Medical industry
	Listeria monocytogenes RA4377	
Gram positive cocci	Toxin producing *Staphylococcus aureus* RA 1224	Food industry (all areas)
	Methicillin-resistant *Staphylococcus aureus* ATCC 13143 (MRSA)	Medical industry Veterinary industry Hotel industry
	Vancomycin resistant *Enterococcus faecali*s (VRE) NCTC 12202	Public transport Cosmetics/homecare industries
Salmonella enterica	S. Typhimurium ATCC 14028	Food industry (all areas)
	S. Enteritidis PT30 ATCC BAA-1045	Veterinary industry Food service industry
	S. Montevideo FH/158	Hotel industry Public transport
Escherichia coli	*E. coli ATCC 25922*	Food industry (all areas) Veterinary industry Food service industry
	E. coli K12 ATCC 12435	Hotel industry Public transport
	Escherichia coli O157:H7 (non-toxigenic) ATCC 12900	Water industry Cosmetics/homecare industries
Pseudomonads	*Pseudomonas aeruginosa* PA01	Food Industry

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	Acinetobacter baumanii NCTC 12156	Medical industry
		Veterinary industry
	Aeromonas hydrophila	Water industry
		Cosmetics/homecare industries
Gram positive spoilage organisms	*Brocothrix thermosphacta* NCTC 10822	Food industry (meats/fish, ready meals, dairy)
	Micrococcus luteus H143	
	Carnobacterium divergens RA 16623	
Lactic acid bacteria	*Lactobacillus plantarum* RA 16658	Food industry (dairy, meats, ready meals, sauces)
	Lactobacillus paracasei RA 16659	
	Leuconostoc mesenteroides RA 5732	
Yeasts	*Candida albicans* NCTC 3179	Food industry (soft drinks, dairy, dessert sauces, fruit preparations, bakery)
		Medical industry (*C. albicans*)
	Pichia anomala RA 16331	Veterinary industry (*C. albicans*)
		Cosmetics/homecare industries (*C. albicans*)
		Hotel industry (*C. albicans*)
	Zygosaccharomyces rouxii RA 16630	Public transport (*C. albicans*)
Spore-forming bacteria	*Bacillus licheniformis* RA 16684	Food industry (dairies, commercially sterile products, pH-preserved ambient stable products)
	Clostridioides difficile RA 16629	Medical industry (*C. difficile*)
	Geobacillus stearothermophilus RA 16533	

Stage 1: Detection of single species biofilms of relevant food industry microorganisms

A culture of the organisms for the study were taken from frozen cryobead storage at -80°C, streaked onto either pre-poured Tryptone Soya Agar (TSA), Malt extract agar (MEA) or De Man, Rogosa & Sharpe (MRSA) agar plates and incubated at the optimum growth temperatures overnight. Plates were examined to confirm purity and colony morphology, and individual, well-isolated colonies were then transferred into a 100ml aliquot of the applicable nutrient media for that organism:

- *Listeria monocytogenes, Salmonella* spp, *Pseudomonas* spp*, Escherichia coli,* gram positive spore spoilage bacteria and spore forming bacteria – Tryptone soya broth (TSB*)*
- *Lactobacillus* spp- De Man, Rogosa & Sharpe broth (MRSB)
- Yeasts- Malt extract broth (MEB)

The nutrient broths were incubated for between 24 - 48 hours dependent on organism. For each microorganism, triplicate 304b stainless steel surfaces (2cm diameter, ~1.5mm thickness) were placed into the bottom of 3 x100ml containers, immersed in broth culture and left for 1 hour at ambient temperature to allow initial attachment of microorganisms to steel surfaces.

After 1 hour, the inoculum was drained from each container and the stainless-steel surfaces were rinsed with sterile distilled water to remove non-attached cells and then immersed in dilute growth medium (1 :100 tryptone soya broth). Samples were then incubated at the optimum temperature for each organism for 4 hours. Post incubation, the surfaces were removed from the growth medium, rinsed in SDW, transferred to a petri dish and left to dry at ambient temperature for 2 hours.

Biofilms on each coupon were assessed visually using the Bactiscan and 2 competitor instruments and microscopically by fluorescent staining with acridine orange. Each instrument under assessment was shined onto each surface from ~20cm, minimising the distortion of the image as a result of background light.

The presence of microorganisms elicited a turquoise/green fluorescence on surfaces when assessed using the Bactiscan system. The 2 UV light instruments would show up the bacteria if present as a light blue UV hue.

The strength of response to the presence of biofilm observed with each system was assessed according to a points system:

- **Excellent** bacterial detection/visual. Strong light fluorescence observed **Score of 3**
- **Good** bacterial detection. Weaker light response observed **Score of 2**
- **Poor** bacterial detection. Very little light response observed. **Score of 1**.
- **No bacterial biofilm detection**- no visual seen. **Score of 0.**

Once the coupon biofilms had been visually inspected, they were subjected to an acridine orange staining procedure. The coupon was immersed in acridine orange solution using a Pasteur pipette and left for 10 minutes, dried in a flame for 2-3 seconds to fix the stain, then assessed using a fluorescent microscope with equipped with a mercury vapour lamp.

Negative control coupons were also assessed using the 3 instruments, to determine whether food debris or other material present within a factory would be detected by each system. 5 different substances were used as negative controls:

- Bovine serum albumen (protein soil used as standard for food, domestic, industrial, institutional, medical and veterinary areas)
- Skimmed milk (dairy residue)
- Sugar solution (sugar refineries)
- Pureed sterilised food
- Sheep erythrocytes (Used to simulate blood in meat processing facilities/medical/veterinary areas)

These substances were also placed into 100ml containers and the stainless-steel coupons immersed for 1 hour to attach in each solution and rinsed using sterile distilled water. Coupons were dried and then assessed.

Stage 2: Detection of biofilms at different stages of development

A model microorganism was selected based on data from stage 1- *Pseudomonas* aeruginosa PA01 was chosen as a strong biofilm former and used to seed a CDC bioreactor.

Biofilms of the *Pseudomonas aeruginosa* were grown on stainless steel coupons during batch and continuous phases within a bioreactor under conditions of high shear. 24 coupons were grown in total during the process (1 full bioreactor).

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Triplicate samples were taken at 5 timed intervals during biofilm formation:

- Batch phase + 4 hours

- Batch phase + 24 hours

- Continuous phase + 2 hours

- Continuous phase + 6 hours

- Continuous phase + 24 hours

Following the bioreactor growth above, the coupons at each of the 5 times stages were tested as follows:

- 1 coupon was assessed using the Bactiscan + 2 UV light products

- 1 coupon was assessed by plating to determine viable TVC count

- 1 coupon was assessed using fluorescent microscopy

For the 9 remaining coupons left following the full growth protocol, the biofilms were heat or chemical treated as follows:

- Immersed in water at 60°C for 5 minutes using test tubes pre- heated to 60°C

- Treatment with 200ppm peroxyacetic acid for 20 minutes

- Autoclaved (sterilised) at 121°C for 3 minutes.

After each treatment, coupons were again assessed using the Bactiscan + the 2 competitor products against the score system described above, fluorescent microscopy staining and TVC aerobic count plating. Results were compared and used to determine the extent to which damaged or dead biofilm is detected using Bactiscan.

5 RESULTS

Stage 1

Results- Detection of single species biofilms of relevant food industry microorganisms

Results obtained for single species biofilms and food residues on steel surfaces tested with Bactiscan and competitor systems are shown in table 2, below. A summary of the overall scores obtained for each detection system is shown in table 3.

Results showed that Bactiscan provided a much clearer indication of the presence of biofilm.

Table 2- Microorganism biofilm visual detection using 3 systems- Bactiscan, handheld backlight & UV handheld torch.

Organism Category	Microorganism strain/control	Bactiscan detection score	Backlight detection score	UV torch "Budget" detection score	Biofilm coverage observed with fluorescent microscopy
Listeria monocytogenes	*Listeria monocytogenes* ATCC 11994	2	0	1	Medium
	Listeria monocytogenes Scott A strain	3	0	1	Strong
	Listeria monocytogenes RA4377	2	0	1	Medium
Gram positive cocci	Toxin producing *Staphylococcus aureus* RA 1224	2	0	0	Medium
	Methicillin-resistant *Staphylococcus aureus* ATCC 13143 (MRSA)	3	0	1	Strong
	Vancomycin resistant *Enterococcus faecali*s (VRE) NCTC 12202	2	0	1	Strong
Salmonella enterica	S. Typhimurium ATCC 14028	2	1	2	Medium
	S. Enteritidis PT30 ATCC BAA-1045	2	1	1	Medium
	S. Montevideo FH/158	2	1	1	Medium
Escherichia coli	*E. coli* ATCC 25922	2	0	1	Medium
	E. coli K12 ATCC 12435	2	0	1	Weak-medium
	Escherichia coli O157:H7 (non-toxigenic) ATCC 12900	2	0	1	Medium
Pseudomonads	*Pseudomonas aeruginosa* H257	3	0	2	Very strong
	Acinetobacter baumanii NCTC 12156	3	0	2	Very strong
	Aeromonas hydrophila	3	0	2	Very strong
Gram positive spoilage organisms	*Brocothrix thermosphacta* NCTC 10822	3	0	2	Medium
	Micrococcus luteus H143	3	1	2	strong
	Carnobacterium divergens RA 16623	3	0	2	Very strong
Lactic acid	*Lactobacillus plantarum* RA 16658	2	0	1	Strong

bacteria	*Lactobacillus paracasei* RA 16659	2	0	2	Strong
	Leuconostoc mesenteroides RA 5732	2	0	2	Strong
Yeasts	*Candida albicans* NCTC 3179	3	0	2	Very strong
	Pichia anomala RA 16331	3	0	2	strong
	Zygosaccharomyces rouxii RA 16630	3	0	2	strong
Spore-forming bacteria	*Bacillus licheniformis* RA 16684	2	0	1	Strong
	Clostridioides difficile RA 16629	2	0	1	Medium
	Geobacillus stearothermophilus RA 16533	2	0	1	strong
Negative controls	Bovine serum albumen	0*	0	0*	Ok- no biofilm growth
	Skimmed milk	0*	0	0*	Ok- no biofilm growth
	Sugar solution	0*	0	0*	Ok- no biofilm growth
	Pureed sterilised food	0*	0	0*	Ok- no biofilm growth
	Sheep erythrocytes	0*	0	0*	Ok- no biofilm growth

*red fluorescence observed

Table 3- Microorganism biofilm visual detection using 3 systems - score summary

Organism group	Overall score- Bactiscan detection	Overall score- backlight detection	Overall score- UV torch "Budget" detection
Listeria monocytogenes	7	0	3
Gram positive cocci	7	0	2
Salmonella enterica	6	3	4
Escherichia coli	6	0	3
Pseudomonads	9	0	6
Gram positive spoilage organisms	9	1	6
Lactic acid bacteria	6	0	5

|	9	0	6
Yeasts			
Spore-forming bacteria	6	0	3
Overall score/81	**65**	**4**	**38**

- From the visual scoring of biofilm detection, the Bactiscan unit scored 65/81, which indicates that it could detect biofilms on all bacterial groups well, including some weaker biofilm formers e.g *E. coli* K12.

- The Backlight system in comparison was very poor with only 2 visible responses detected in total.

- The UV torch, Budget system scored 38/81; it scored well on the strong biofilm formers, but responses were not as clear as seen with the Bactiscan- the light was more a light blue hue, which also picked up some other debris such as dust. Overall, the system appeared much less selective than Bactiscan.

- For the negative control samples (sterile food residues), the Bactiscan did produce a fluorescent response for all 5 components, but the response was red instead of the blue/green light hue as seen on the biofilms. On the staining images, there was no biofilm growth detected; any images were picking up other particles and could easily be identified from the biofilm images **(see Figure 1).**

- Therefore, it can be concluded that the Bactiscan unit can differentiate between biofilm and typical residues often found on environmental surfaces in a range of applications.

- The other two systems did not pick up any noticeable differences on the negative samples to the bacterial samples. Both showed a lower intensity light blue fluorescent response similar to the response seen for bacterial contamination.

**Figure 1:** Pureed food particles fluorescent stain image

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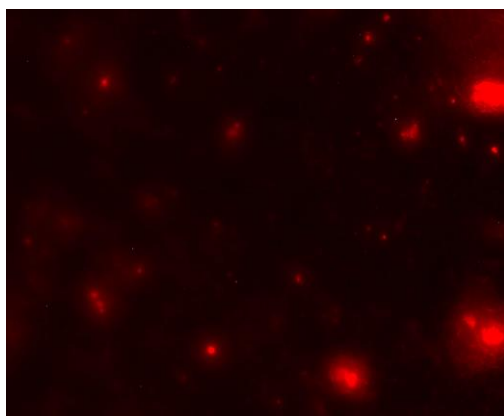
Example of **Excellent** (score of 3) biofilm visual- *Pseudomonas aeruginosa* picked up using Bactiscan unit (**Figure 2**) and UV torch (**Figure 3)**
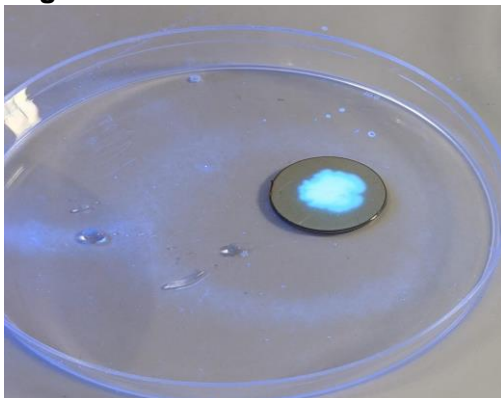
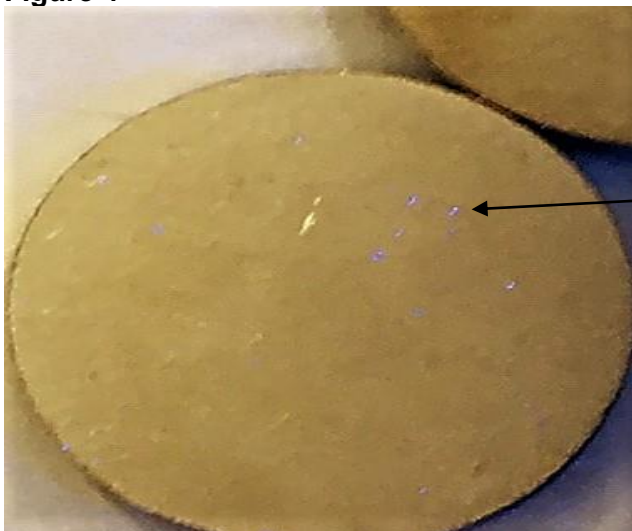**Figure 2**   **Figure 3**
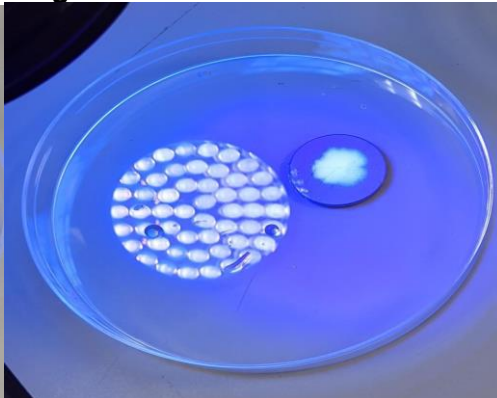
- Note using the UV torch, there was a shadow cast by the torch itself next to the discs as shown in **figure 3**, however the biofilm could be picked out on the disc.

- The good/poor biofilm visuals (scores of 2 or 1) could not be easily captured on camera- some of the "flecks" could not be captured as they were just too small; however, the visual match could be made against the staining if the biofilm was picked up using the instruments.

- **Figure 4** below illustrates the use of Bactiscan unit on single species biofilm of *Salmonella* Montevideo - small turquoise flecks of biofilm were detected by eye and could be captured by camera. Fluorescent staining of these coupons confirmed the presence of localised biofilm in these areas

Figure 4

Small turquoise light flecks picked up using Bactiscan unit

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Figures 5 & 6 below illustrate the Bactiscan & UV torch respectively on *E. coli* ATCC 25922, the Bactiscan image in figure 5 showing a more turquoise hue, the UV torch picked up the light as blue and detected other debris as blue in the dish

Figure 5

Figure 6

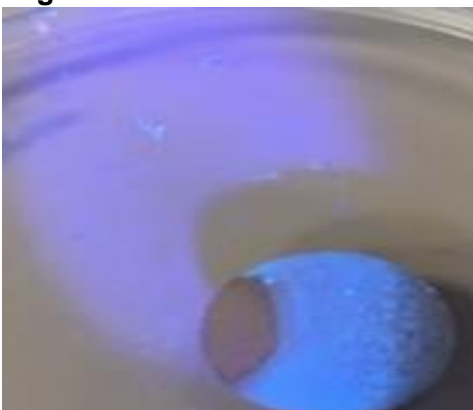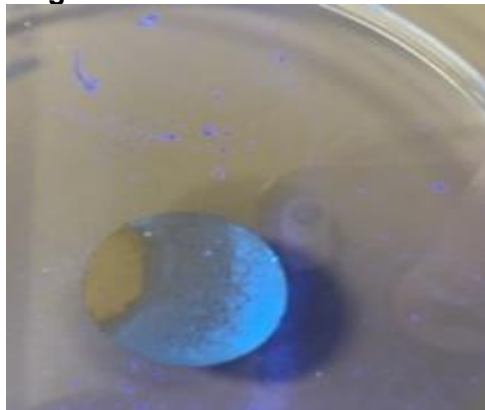**Figures 7 & 8** illustrate the Bactiscan & UV torch respectively on *Acinetobacter junii,* the Bactiscan image in figure 7 showing a strong turquoise hue, the UV torch picked up the light as blue but not so intense and also detected other debris as blue in the dish as red dots

Figure 7
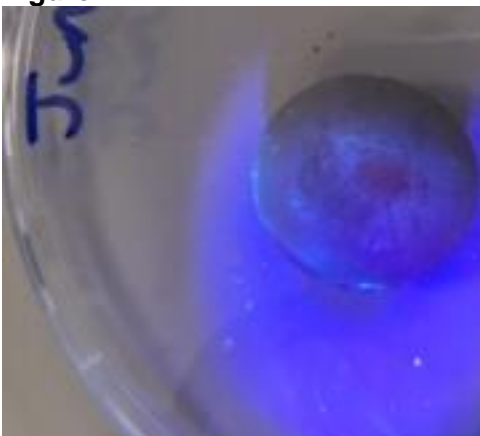
Figure 8
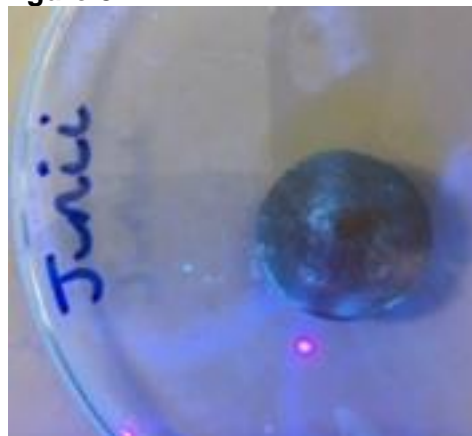
Figures 9 & 10 illustrate the Bactiscan & UV torch respectively on *Aeromonas hydrophila:* the Bactiscan image in figure 9 showing a more turquoise hue, the UV torch picked up the light as blue and detected other debris as a deep blue colour in the dish.

Figure 9

Figure 10

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The fluorescent staining images of all the biofilms can be found under **appendix 1**

The images show that the Bactiscan could detect all biofilms assessed, including those formed by less profuse biofilm formers, as shown by the lower levels observed through microscopic fluorescent staining (e.g. *E. coli* K12).

Determination of the presence of biofilm within the HRAD process hall facility

To further illustrate the relative detection capabilities of Bactiscan and the other UV devices, the Bactiscan was used to detect the presence of biofilm at three locations within the HRAD laboratory at Campden BRI. Swabs were taken from each area and analysed to determine total viable count.

Figures 11 & 12 show the overflow of a laboratory sink, visualised with Bactiscan and a UV torch. The Bactiscan (figure 11) showed the presence of biofilm as well-defined areas of green fluorescence, whereas the UV torch (figure 12) showed only a diffuse red/blue light which did not indicate the presence of bacteria. The TVC result for this swab area was 8,800 cfu/g.

Figure 11

Figure 12

Figures 13 & 14 show the underside of the handwash sink visualised using Bactiscan and a UV torch. The Bactiscan (figure 13) showed the presence of biofilm as well-defined areas of green fluorescence, whereas the UV torch (figure 14) showed only diffuse red/blue light. The TVC result for this swab area was 75 cfu/g, indicating the Bactiscan more reliably indicates the presence of biofilms, even at relatively low levels of culturable organisms.

Figure 13

Figure 14

Figures 15 & 16 show the spray head of the safety shower, the Bactiscan (figure 15) clearly shows the presence of biofilm around the spray jets as green fluorescent areas, the UV torch (figure 14) did not detect the presence of biofilm in these areas – showing only a diffuse red/blue fluorescent response light. The TVC result for this swab area was 560,000 cfu/g.

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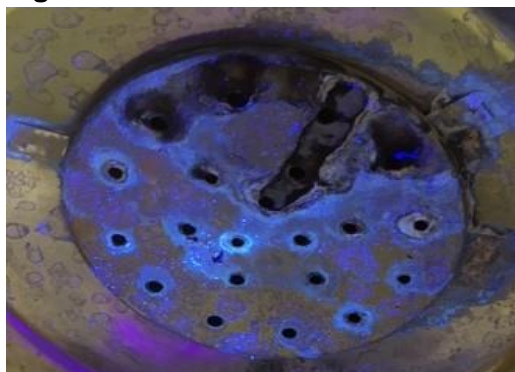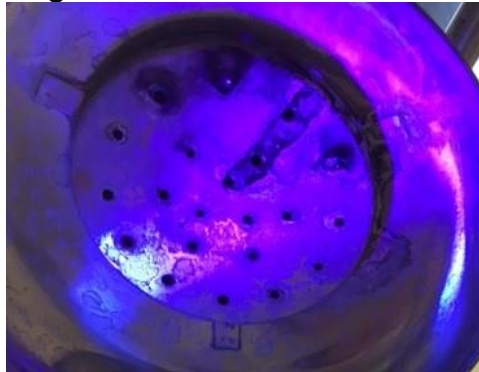
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Figure 15

Figure 16

These results show that in practice, the Bactiscan clearly indicated the presence of microorganisms at low medium and high levels of contamination, allowing easy identification of areas in which cleaning had not been effective. The cheaper competitor torch did not indicate contamination on any of these surfaces, despite the high levels of viable organisms on the sink overflow and safety shower spray head.

Stage 2 results- Detection of biofilms at different stages of development

Pseudomonas aeruginosa was selected for the stage 2 experiment, due to its known strong biofilm forming characteristics and the results shown for this organism in stage 1.

Table 4 shows the total viable count achieved on stainless steel CDC bioreactor coupons before an after various sublethal treatments, the fluorescent responses observed with Bactiscan and two competitor UV torches, and the fluorescent staining results.

Table 4. Bactiscan results on CDC bioreactor coupons at different stages of growth

Sample ID/condition	TVC Log cfu/g	Bactiscan detection score	Backlight detection score	UV torch detection score	Fluorescent staining image
Batch phase + 4 hours	4.41	2	0	2	Medium
Batch phase + 24 hours	7.46	3	0	2	Strong
Continuous phase + 2 hours	7.61	3	0	2	Strong
Continuous phase + 6 hours	7.96	3	0	2	Strong
Continuous phase + 24 hours	8.55	3	0	3	Very strong
Hot water immersion 60°C - 5 minutes	4.04	2	0	1	Medium (live/dead)
200ppm PAA 20 minutes	6.50	2	0	2	Strong (live/dead)
Autoclaving at 121°C 3 minutes	3.42	2	0	1	Medium (live dead)
Overall detection score/27	N/A	20	0	15	N/A

- The Bactiscan picked up the biofilm growth at all stages of the CDC bioreactor growth process, starting at +4 hours into the batch phase, with scores of either 2 or 3.
- The backlight again performed poorly and could not detect any biofilm growth on the discs, scoring 0.
- The UV torch did detect all stages of biofilm growth, but the visual wasn't quite as clear as with the Bactiscan, there were more flecks of light than the turquoise clear hue.
- The Bactiscan scored 20/27 for these tests against 15/20 for the UV torch and 0 for the backlight.
- The Bactiscan could also still detect the biofilm post-PAA, hot water and sterilising treatments, this matches up well to the live/dead staining images from the post treatment biofilm discs, which indicated that there was still viable biofilm growth on these discs. **See figures 17-20.**

Figure 17
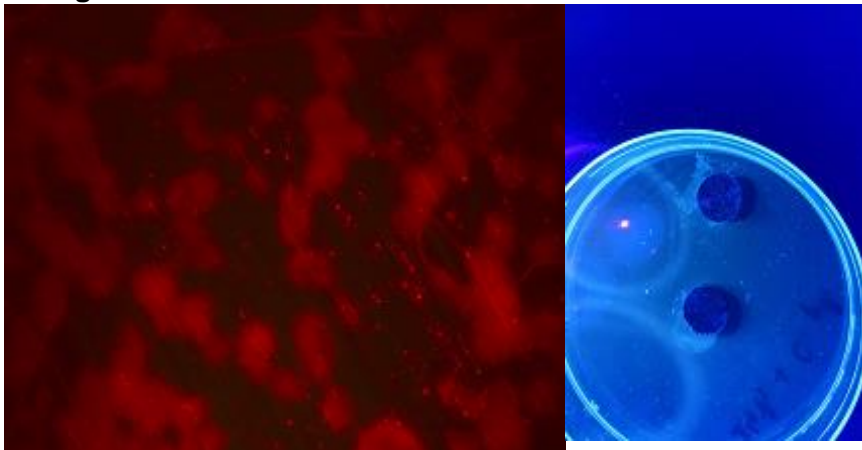
P.aeruginosa Continuous phase + 24 hours – fluorescent stain & Bactiscan detection

Figure 18

Bactiscan detection
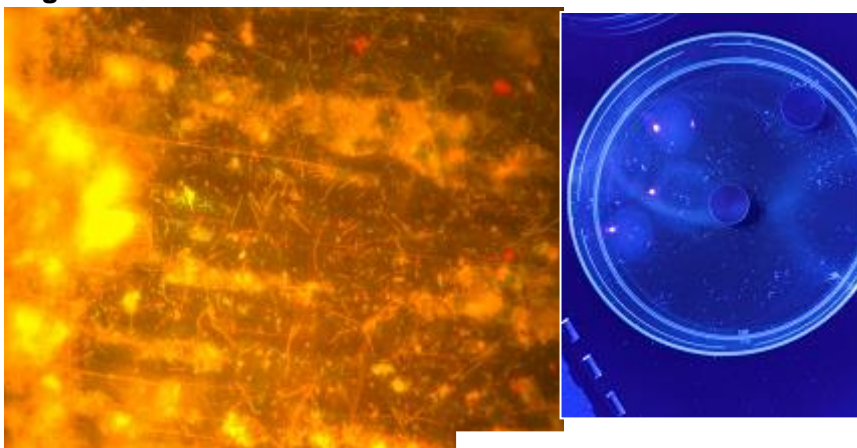
P.aeruginosa Autoclave Treatment – Live/Dead Stain

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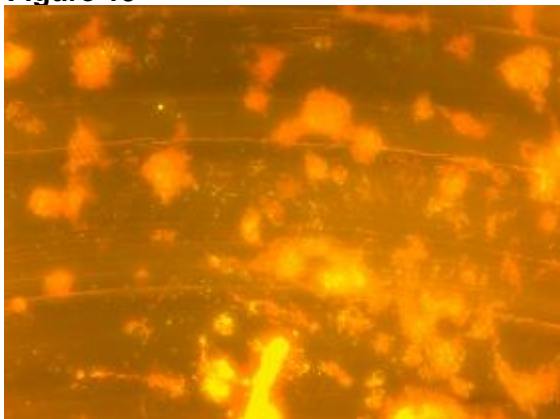
Figure 19

P.aeruginosa 200ppm PAA Treatment –
Live/Dead Stain

Bactiscan detection

Figure 20
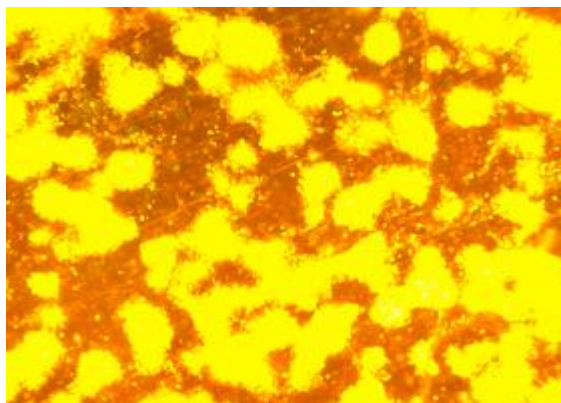
P.aeruginosa Hot Water
Treatment – Live/Dead Stain

Bactiscan detection

6. CONCLUSION/DISCUSSION

A study was conducted on a Bactiscan instrument as provided by EIT International, to assess the ability of the unit to detect Biofilms within food industry settings. The unit was compared against two commercially available units.

Stage 1 of the study focused on the detection of single species biofilms. Single species biofilms were generated for microorganisms in 9 categories representing important pathogens in the food, medical and veterinary industries on stainless steel discs. Biofilms were assessed using the Bactiscan, 2 competitor instruments and the presence and extent of biofilm formation was confirmed by fluorescent microscopy.

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pick up small intense specks of blue/green fluorescence, which on closer microscopic inspection revealed the presence of small areas of biofilm growth/microcolonies.

The presence of bacteria or biofilms are visually evident as a turquoise/green fluorescence on the surfaces using the Bactiscan. Determination of the presence of biofilm in practice within the Campden BRI microbiology process hall showed it to be highly effective at locating areas of contamination compared against other selected UV torches.

Stage 2 of the study used a strong biofilm forming strain, *Pseudomonas aeruginosa* PA01. This organism was grown under conditions of high shear a CDC bioreactor system to achieve dense, compact biofilm growth. Samples of the biofilm were taken at 5 stages during formation and analysed using the 3 instruments. The Bactiscan could detect laboratory biofilms at an early stage of development. By comparison, the competitor UV torches could only detect the stronger biofilms (UV torch) or none at all (Backlight). The Bactiscan was also able to detect biofilm after hot water immersion at 60°C for 5 minutes, PAA immersion at 200ppm for 20 minutes and sterilising at 121°C for 3 minutes. Total viable counts and direct live/dead fluorescent microscopy confirmed the presence of live bacteria after each of these treatments.

It can be concluded that the Bactiscan is able to detect biofilms from species typical of pathogens and other target microorganisms present in a multitude of environments from across many industry areas including food, medical and veterinary areas.

7 STATEMENTS

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Unless otherwise expressly agreed in writing and signed by a duly authorised representative of Campden BRI, the services to which this report pertains are subject to the Campden BRI Standard Terms and Conditions of Contract a copy of which is available upon request or can be downloaded from our website at **http://www.campdenbri.co.uk/campdenbri/CampdenBRISupplyTerms.pdf**

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8. Appendix 1- Fluorescent staining images from organisms assessed in Stage 1, Figures A- Y

Figure A: Acinetobacter baumanii
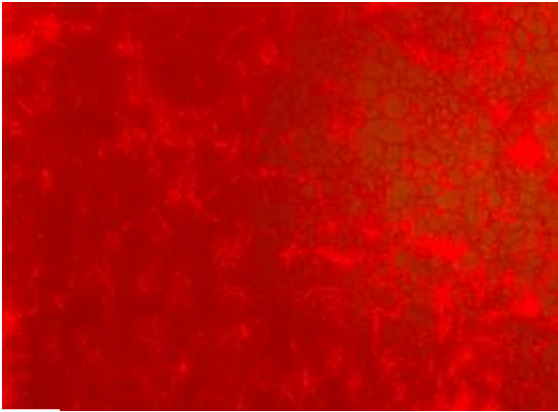
Figure B: Aeromonas hydrophila
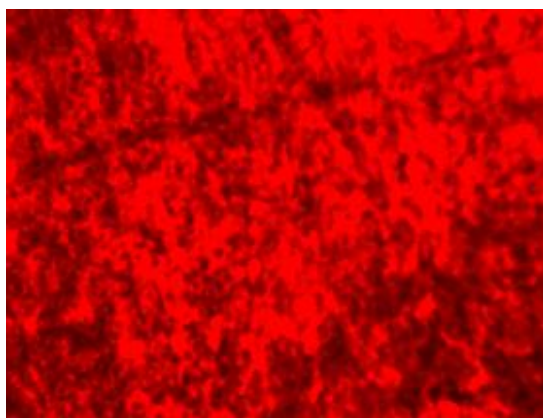
Figure C:Bacillus.licheniformis
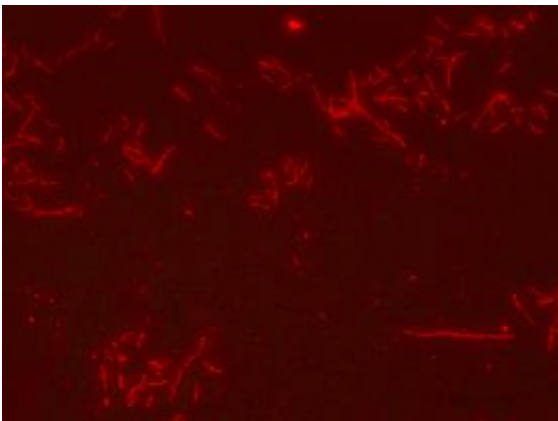
Figure D: B.thermosphacta
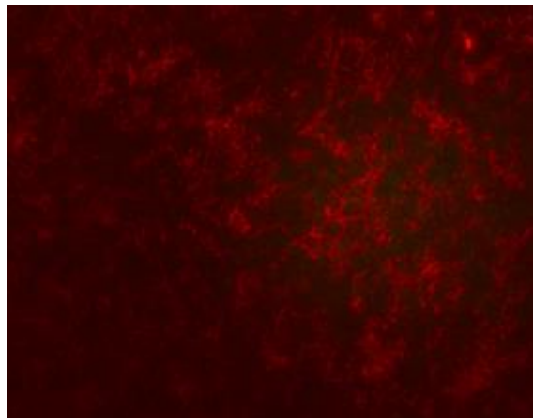
Figure E: C.albicans
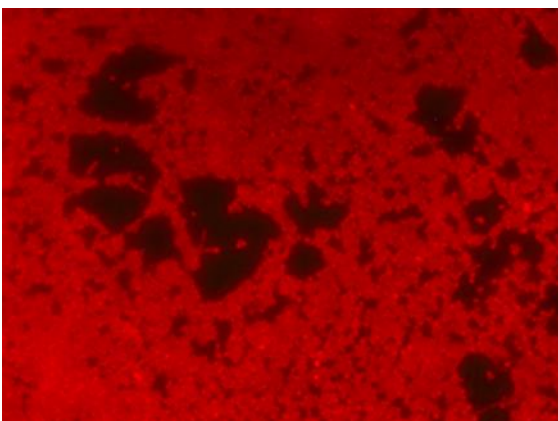
Figure F: *Clostridium dificile*

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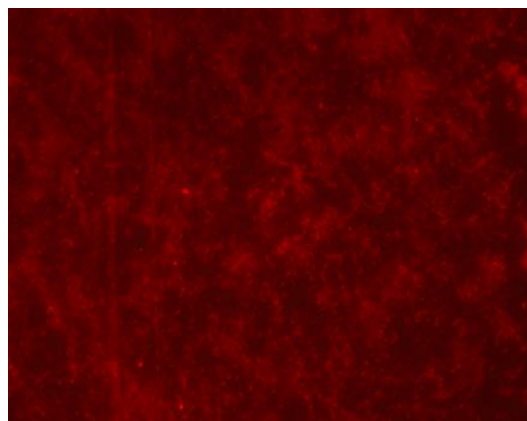
Figure G: C.divergens
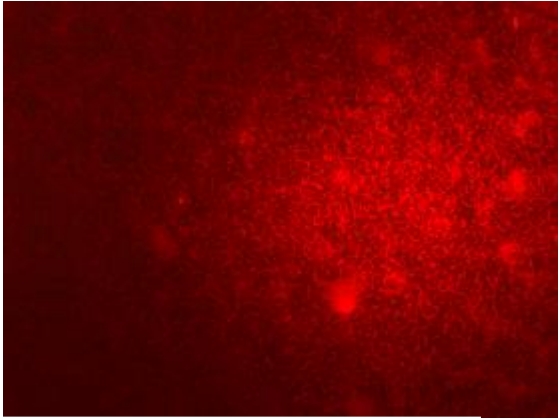
Figure H: E.coli ATCC 25922
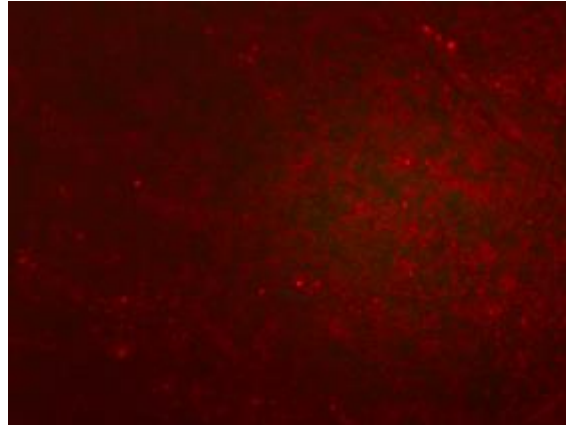
Figure I: E.coli K12
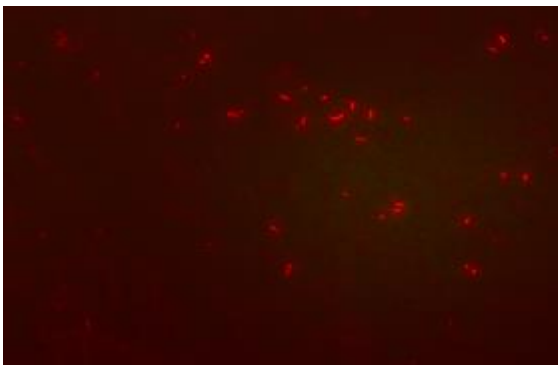
Figure J: G.stearothermophilus
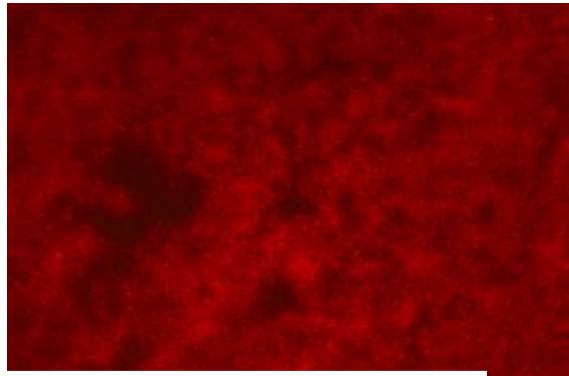
Figure K: Leuconostoc.mesenteroides
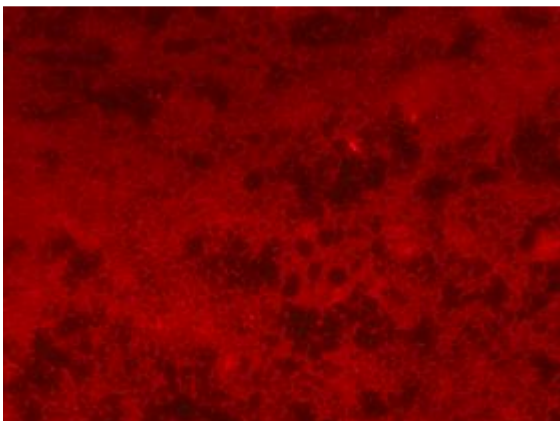
Figure L: Listeria monocytogenes 3754
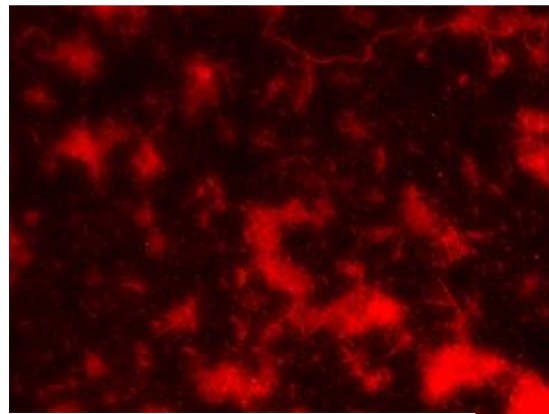
Figure M L.monocytogenes 4377
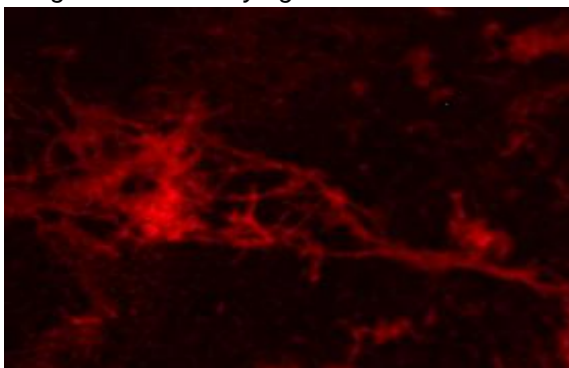
Figure N: L.monocytogenes 11994
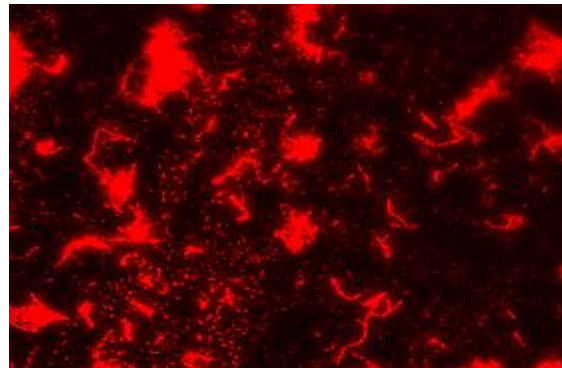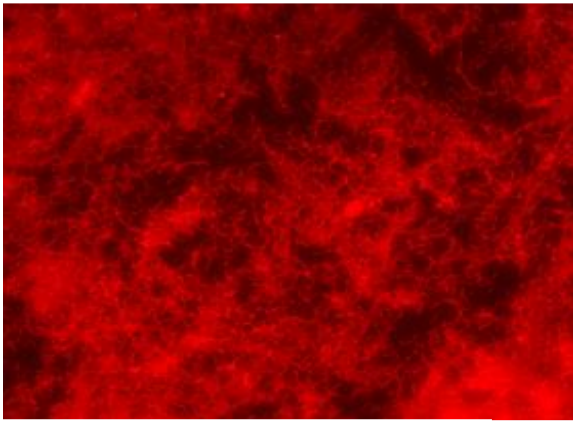
Figure O: Lactobacillus.paracasei
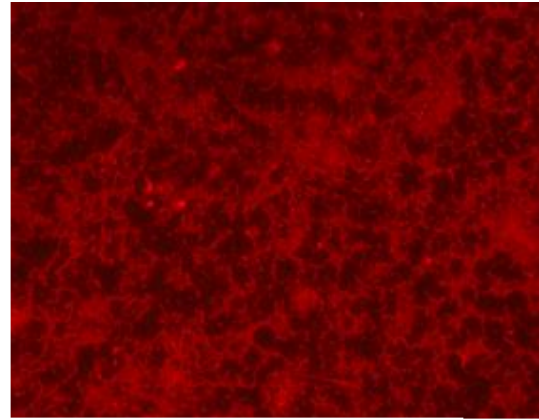
Figure P: Lactobacillus.planatrum

Figure Q: Micrococcus.luteus
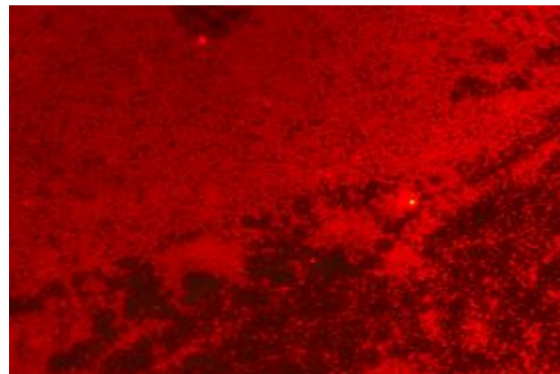
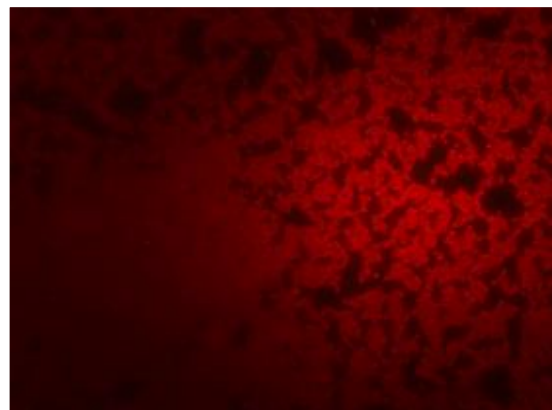Figure R: MRSA S. *aureus* 13143
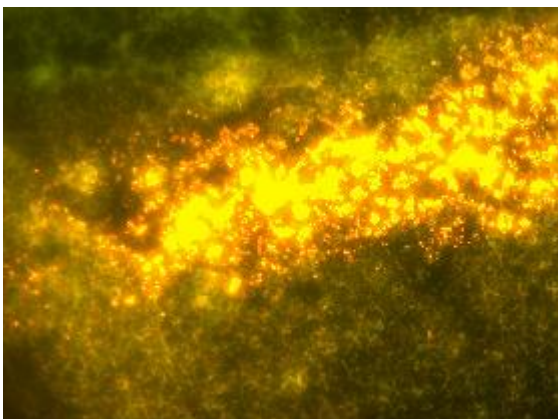
Figure S: Pseudomonas.aeruginosa

Figure T: Pichia.anomala
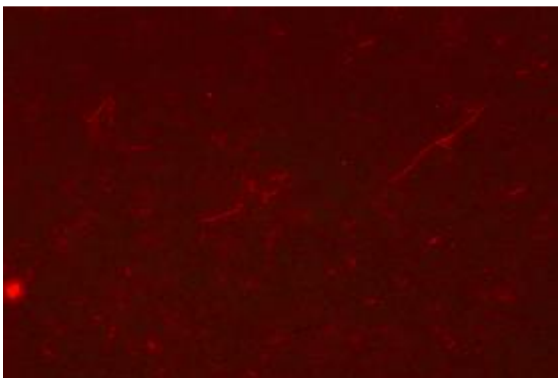
Figure U: Staphylococcus.aureus
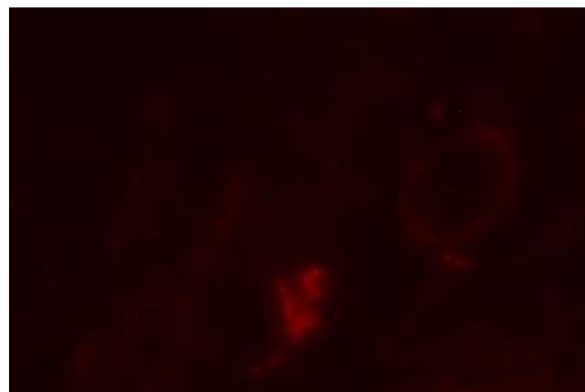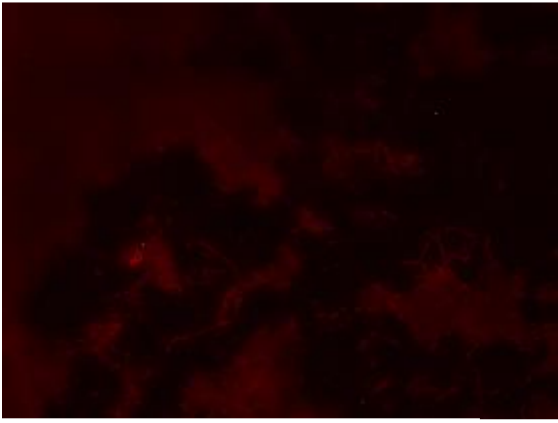

Figure V:Salmonella.montevideo

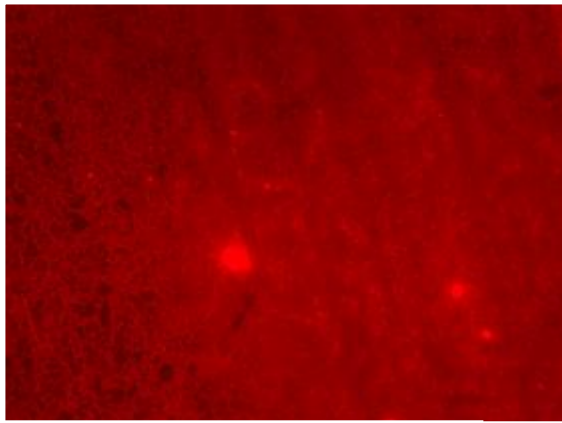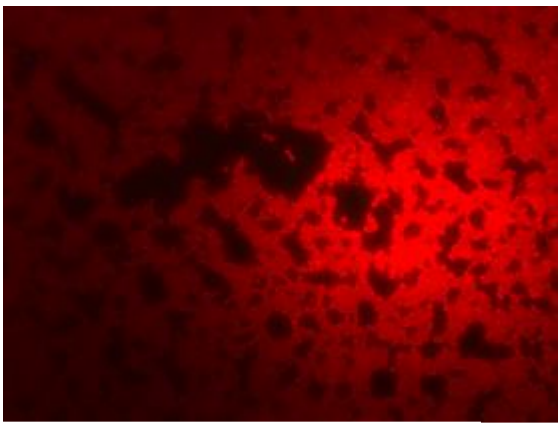Figure W: *Salmonella.typhimurium*

Figure X: VRE- Vancomycin resistant *Enterococcus*

Figure Y: *Zygosaccharomyces rouxii*