

APPLICATION NOTE

Enumeration of waterborne pathogens by the ScanStation®

Utilization of membrane filtration

Introduction

The objective of this study was to evaluate the performance of the ScanStation® to count in real-time colonies on membrane filtration. Enumeration was performed on waterborne pathogens that have been linked to healthcare-associated infections (HAIs). Bacterial suspensions were passed through filtration membranes that were deposited on Petri dish. Colonies were manually counted and results were compared with automatic counts performed by the ScanStation®.

Materials

Bacteria strains

Lyophilized pellets are rehydrated and inoculated on agar TSA plates. The primary culture plates are incubated at 30°C during 24-48h according the strain. Colonies are transferred to sterile saline solution to making the primary suspension. Seven bacterial strains were used in this study: *Pseudomonas mosselii* (ATCC 49838), *Burkholderia cepacia* (ATCC 25416), *Ralstonia pickettii* (ATCC 27511), *Stenotrophomonas maltophilia* (ATCC 13637), *Sphingomonas paucimobilis* (ATCC 29837), *Bacillus licheniformis* (ATCC 12759), *Proteus mirabilis* (ATCC 25933).

Membranes tested

Bacterial suspensions were filtrated through 0.4 µm white polycarbonate membranes (FMFNL1050, PALL®) and through gridded 0.45 µm black polyethersulfone membranes (PN4808, PALL®).

Protocol

Serial dilutions of bacterial primary suspensions were performed, targeting a titer concentration around 50-300 CFU/mL. 1mL of each targeted microorganism suspension was filtrated on the 2 types of membranes. The membrane was then placed onto R2A agar. Additional plates are directly inoculated with 100µL of suspension (no membrane). All plates are loaded onto ScanStation® for incubation and automatic reading for 5 days at 32.5°C. Plates are counted manually and results are compared with that of ScanStation® (software v1.19).

Results

Automatic counts were performed with the Scanstation® on 2 types of membranes: white membranes w/o grid, black gridded membranes and directly on agar plates (Raw data are displayed in Table 1, annex I). Results show that in the case of gridded membranes (black), automatic counts are most of the time lower than that observed with the manual method (Figure 1B). These differences can be visualized on Figures 2B.

For the white membranes (w/o grid) and whatever the tested strains (n=7), automatic counts performed by the Scanstation® are in good agreement with manual counts (Figure 1A, 2A). In this case, two light configurations, white or black background, can be used to take pictures. The white background (light coming for bottom) is the one recommended, because of a better global illumination and a better contrast. Some pictures can be visualized in Figure 3 to Figure 8, in annex II. On agar plates, automatic and manual counts are quite similar (Figure 1C). Differences are quite low between the two methods as observed in Figure 2C.

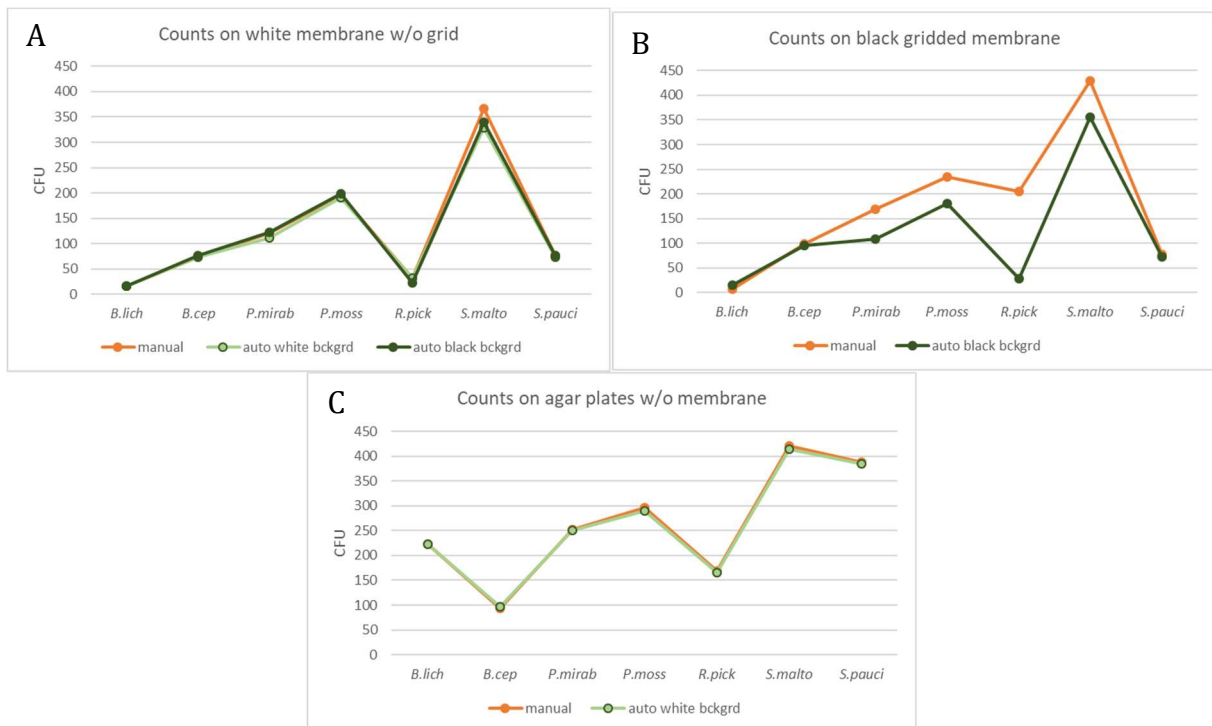


Figure 1. CFU counts of 7 bacterial strains on different types of membranes (A: white membrane w/o grid, B: black gridded membrane) and on agar plate without membranes (C). The orange lines are for manual plate counts ("reference") and green lines are for automatic counts performed by the ScanStation®. For automatic counts, two configurations for light to take pictures were tested: black bckgrd (black background) and white bckgrd (white background). List of bacteria tested= B.lich.: Bacillus licheniformis, B.cep: Burkholderia cepacian, P.mirab: Proteus mirabilis, P.moss: Pseudomonas mosselii, R.pick: Ralstonia pickettii, S.malto: Stenotrophomonas maltophilia, S.pauci.: Sphingomonas paucimobilis.

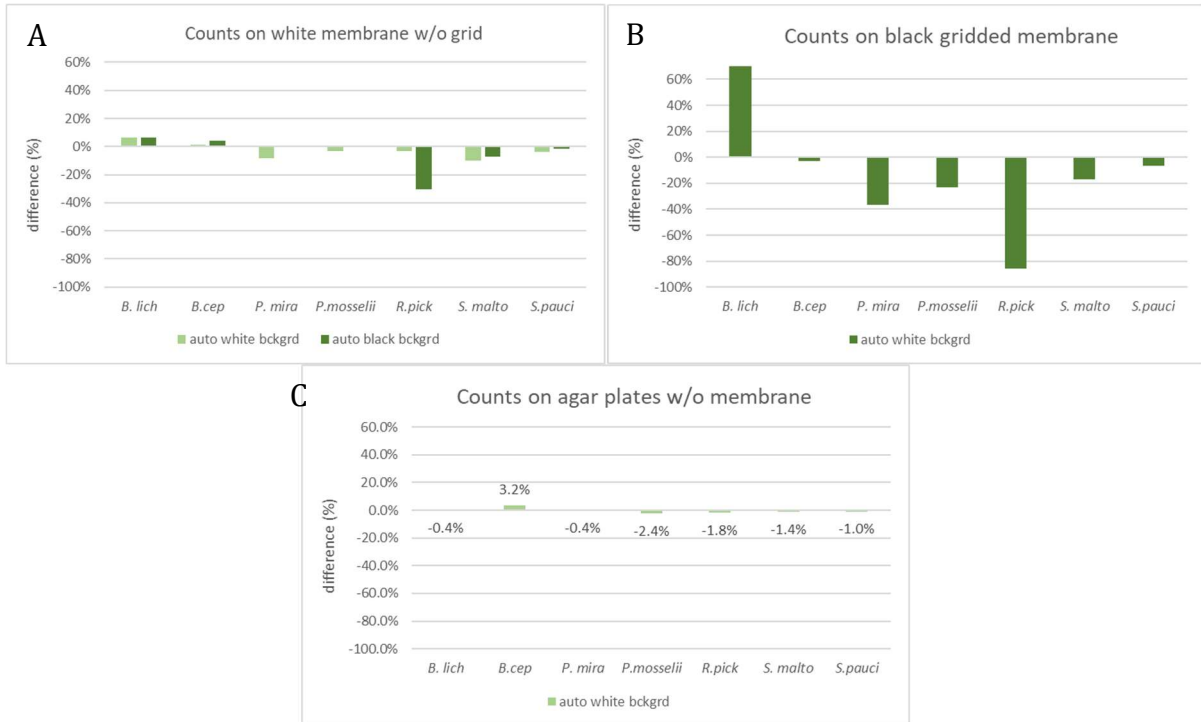


Figure 2. Differences between manual and ScanStation® automatic counts of CFU on different types of membranes (A: white membrane w/o grid, B: black gridded membrane) and on agar plate without membranes (C). $\text{Difference} = ((\text{CFU automatic counts} - \text{CFU manual counts}) / \text{CFU manual counts}) * 100$. For automatic counts, two configurations for light to take pictures were tested: black bckgrd (black background) and white bckgrd (white background). List of bacteria tested= *B.lich.*: *Bacillus licheniformis*, *B.cep*: *Burkholderia cepacian*, *P.mirab*: *Proteus mirabilis*, *P.moss*: *Pseudomonas mosselii*, *R.pick*: *Ralstonia pickettii*, *S.malto*: *Stenotrophomonas maltophilia*, *S.pauci.*: *Sphingomonas paucimobilis*.

Conclusions

The Scanstation® shows good performance to count in real-time colonies on filtration membranes. For the seven tested strains, automatic and manual counts are similar when filtration of bacterial suspensions is performed on white polycarbonate membranes (w/o grid). To obtain better automatic counts in this case, the light configuration that is advised is the white background (light coming for the bottom). This study shows that bacterial colonies can be efficiently enumerated with the Scanstation®.

Annex I

Table 1. Raw data table, automatic and manual counts of CFU and difference between the two methods.

Strains	CFU counts			Difference (%)	
	manual counts	automatic counts		automatic versus manual counts	
		Camera configuration		Camera configuration	
		white bckgrd	black bckgrd	white bckgrd	black bckgrd
white membrane w/o grid					
<i>Bacillus licheniformis</i>	16	17	17	6%	6%
<i>Burkholderia cepacia</i>	73	74	76	1%	4%
<i>Proteus mirabilis</i>	121	111	122	-8%	1%
<i>Pseudomonas mosselii</i>	196	190	198	-3%	1%
<i>Ralstonia pickettii</i>	33	32	23	-3%	-30%
<i>Stenotrophomonas maltophilia</i>	366	329	339	-10%	-7%
<i>Sphingomonas paucimoblis</i>	77	74	76	-4%	-1%
black gridded membrane					
<i>Bacillus licheniformis</i>	7	-	15	-	114%
<i>Burkholderia cepacia</i>	99	-	96	-	-3%
<i>Proteus mirabilis</i>	170	-	108	-	-36%
<i>Pseudomonas mosselii</i>	235	-	181	-	-23%
<i>Ralstonia pickettii</i>	206	-	29	-	-86%
<i>Stenotrophomonas maltophilia</i>	429	-	355	-	-17%
<i>Sphingomonas paucimoblis</i>	78	-	73	-	-6%
agar plate					
<i>Bacillus licheniformis</i>	223	222	-	-0.4%	-
<i>Burkholderia cepacia</i>	93	96	-	3.2%	-
<i>Proteus mirabilis</i>	252	251	-	-0.4%	-
<i>Pseudomonas mosselii</i>	296	289	-	-2.4%	-
<i>Ralstonia pickettii</i>	168	165	-	-1.8%	-
<i>Stenotrophomonas maltophilia</i>	420	414	-	-1.4%	-
<i>Sphingomonas paucimoblis</i>	388	384	-	-1.0%	-

Annex II.

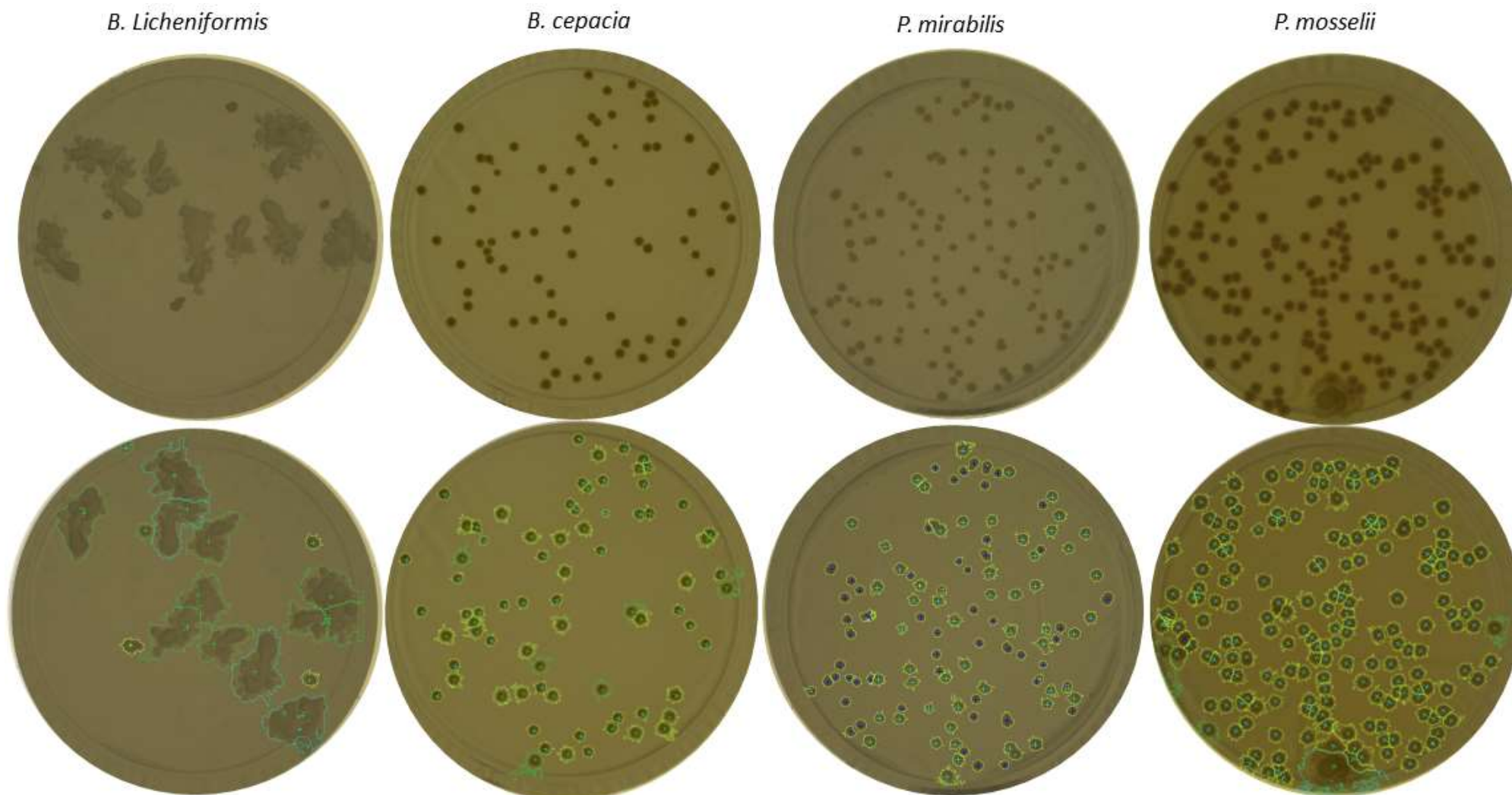


Figure 3. Top pictures show images of the white membranes (w/o grid) taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is white background.

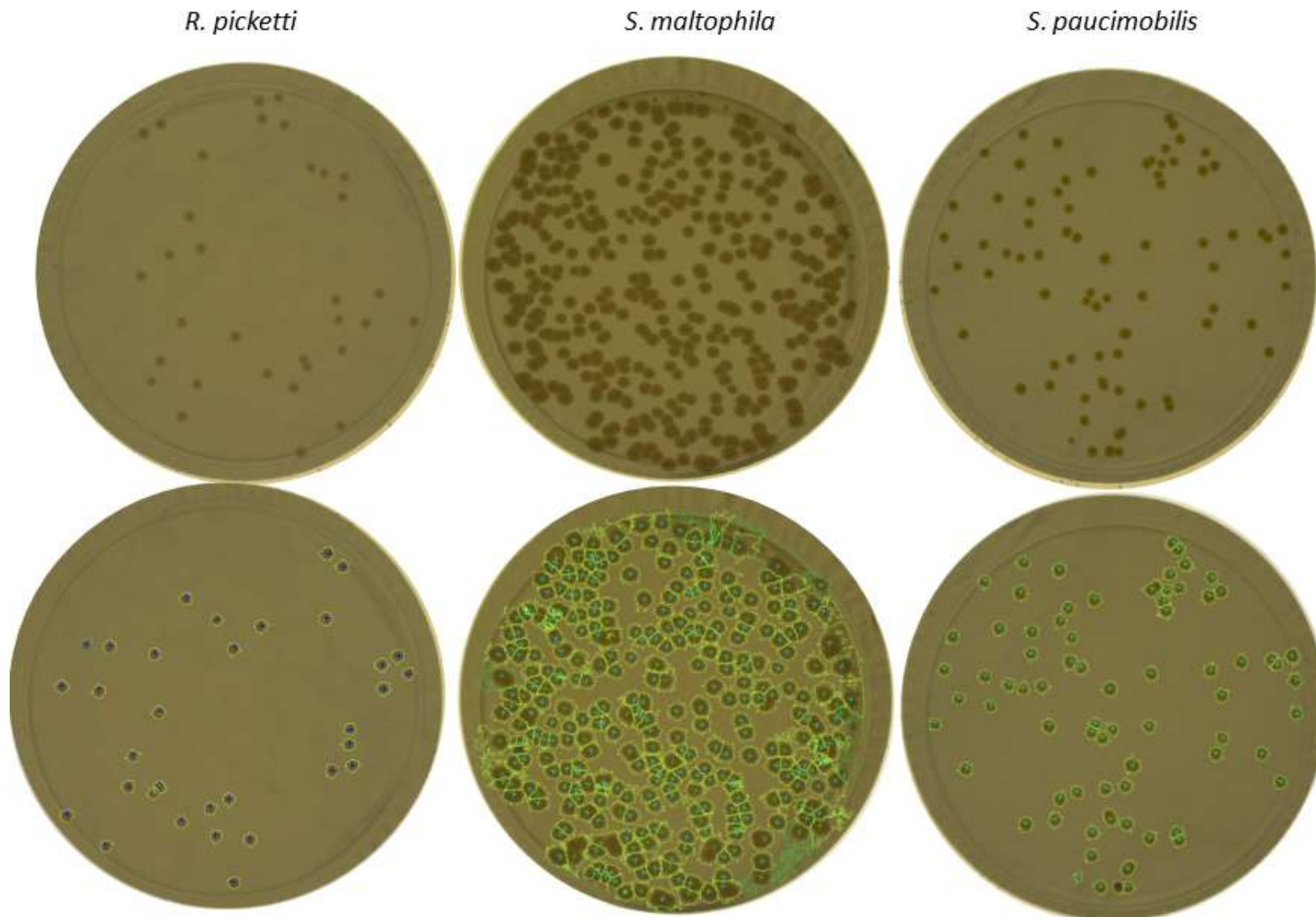


Figure 4. Top pictures show images of the white membranes (w/o grid) taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is white background.

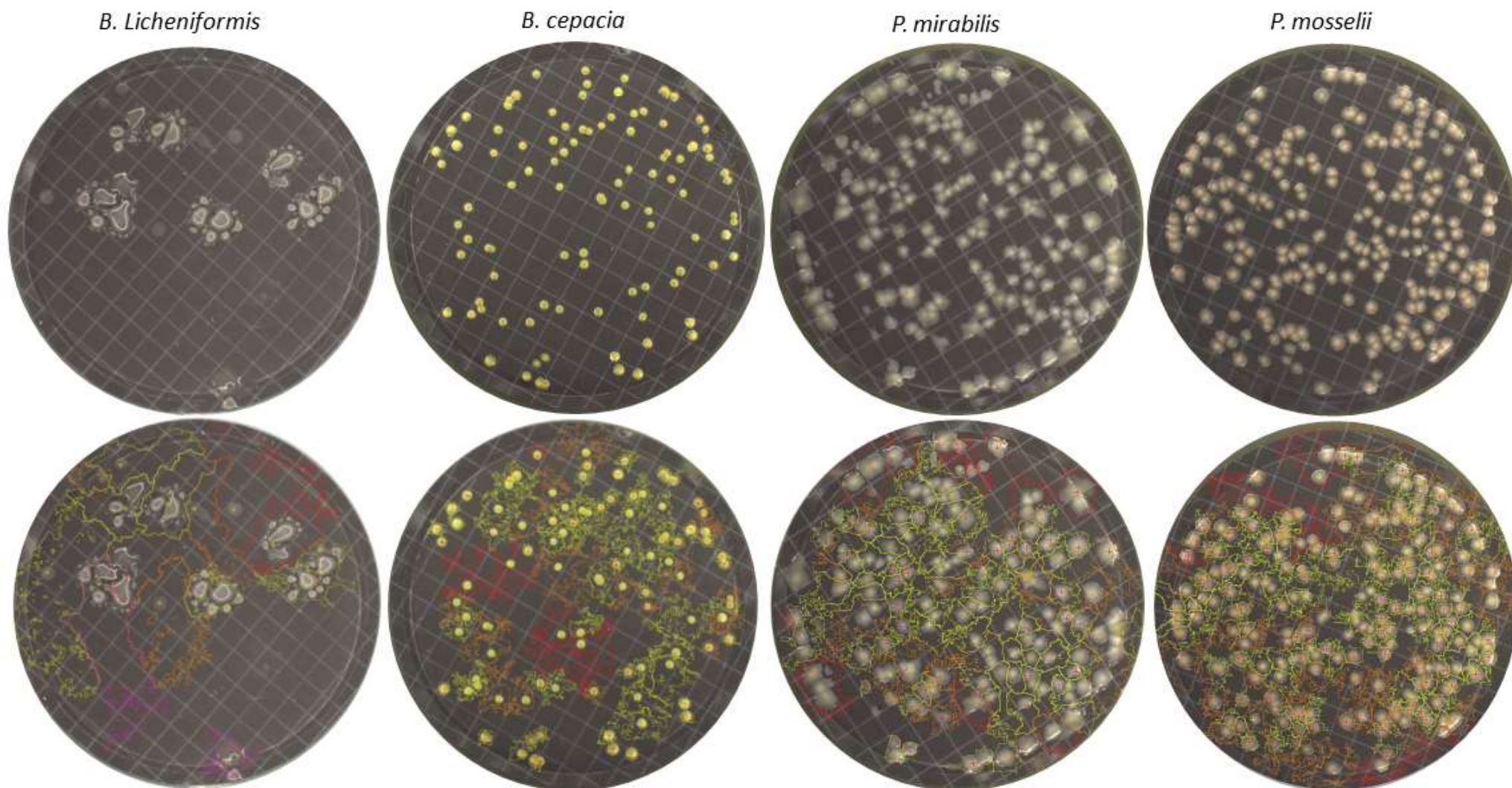


Figure 5. Top pictures show images of the black gridded membranes taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is black background.

R. picketti



S. maltophila



S. paucimobilis

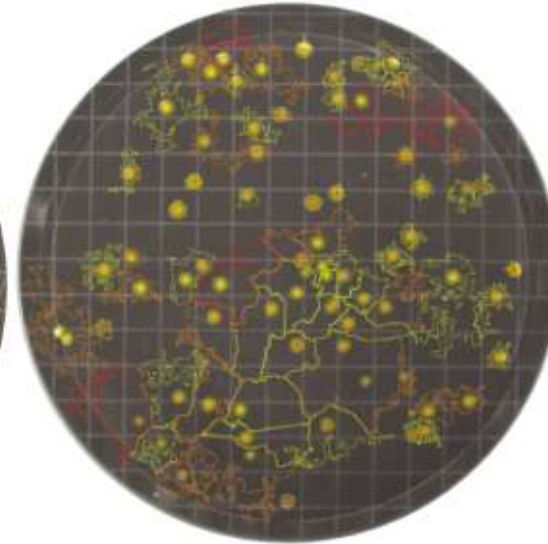
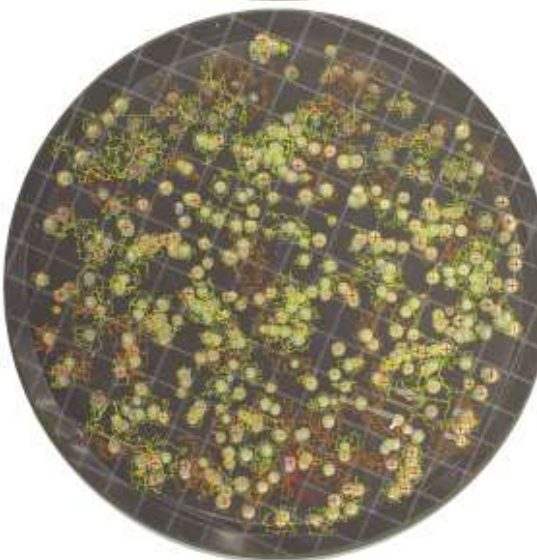
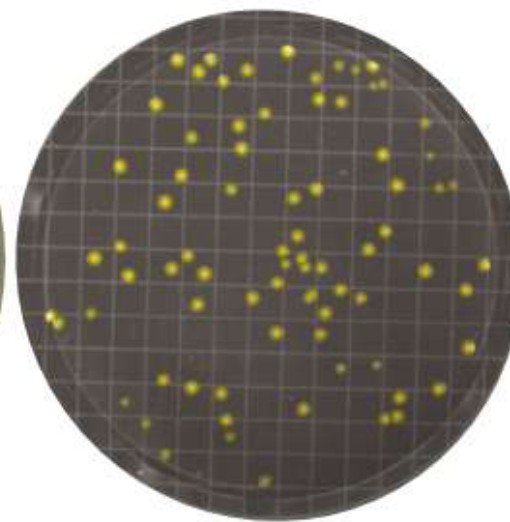


Figure 6. Top pictures show images of the black gridded membranes taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is black background.

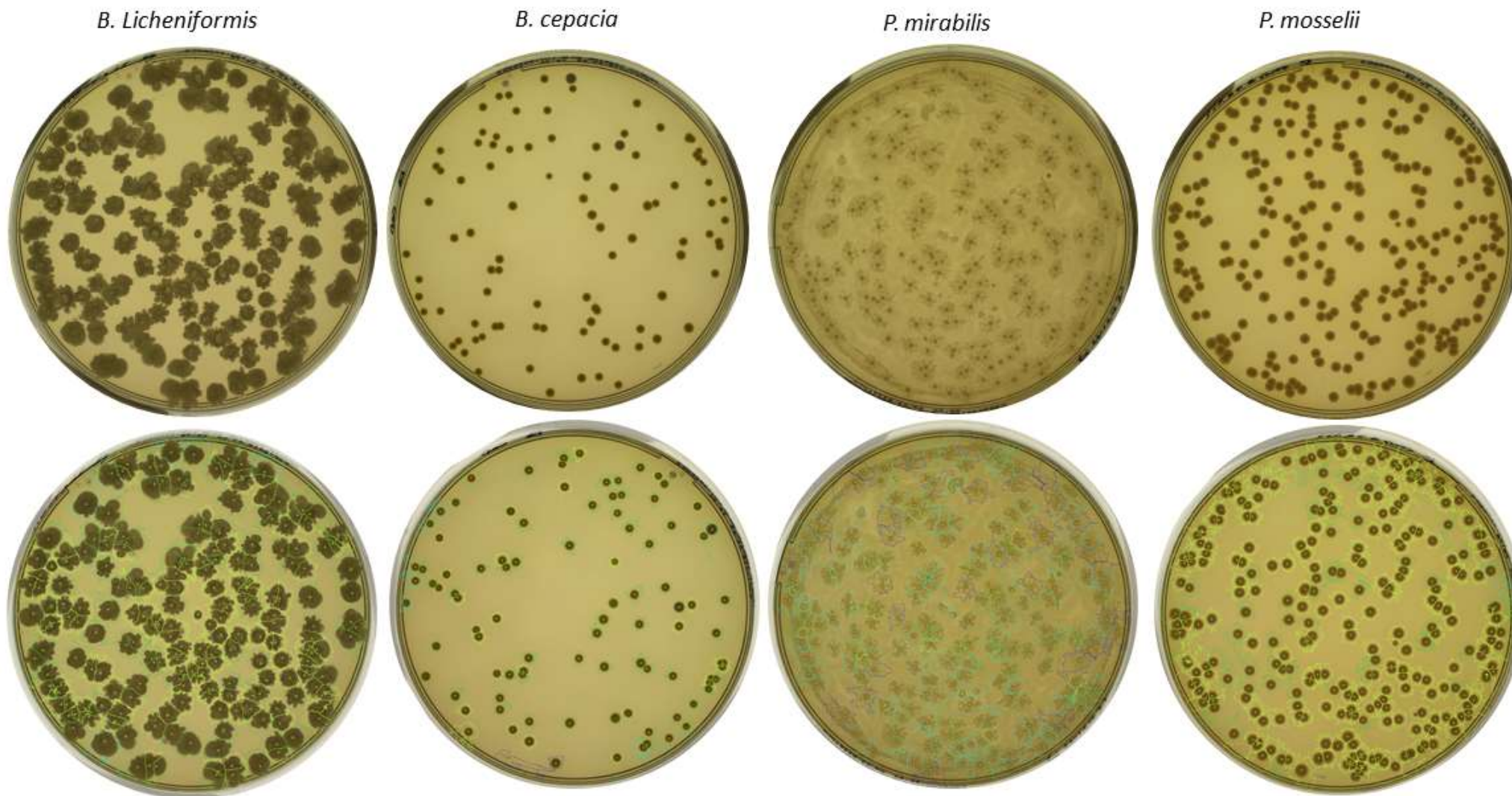


Figure 7. Top pictures show images of the agar plates taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is white background.

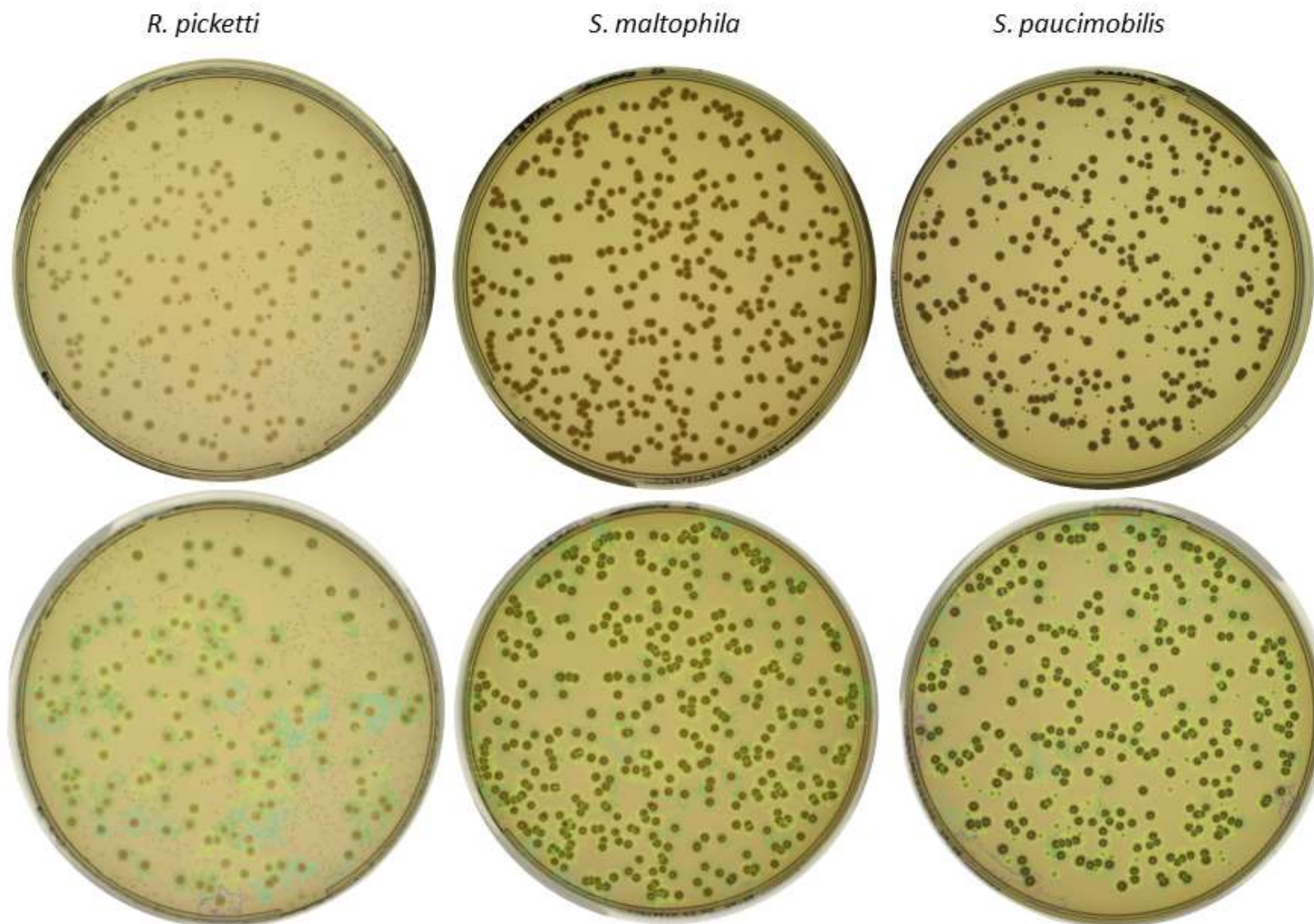


Figure 8. Top pictures show images of the agar plates taken by the ScanStation®. Bottom pictures show the images after being processed by the software for enumeration of CFU (visualization of crosses and detection area around colonies). The camera configuration is white background.