interscience

APPLICATION NOTE

SCANSTATION COUNTING PERFORMANCE ASSESSMENT ON FILTRATION MEMBRANE

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Unit: ScanStation® - Software version: 9

Introduction

Environmental monitoring is an important part of the microbiological quality control in diverse industrial production fields (pharmaceutical, cosmetic or food). This quality control includes surfaces, air and water testing. While surface testing is mainly carried out using contact agar, air and water testing requires the use of filtration membranes to filter out microorganisms and reveal their presence.

The aim of this applicative note is to assess the performance of colony counting on different types of filtration membranes by the real time colony counter ScanStation.

Methods

Automate and software version

ScanStation #439300S00156 equipped with the telecentric UI-337X camera was used for this study. Detail configurations for image acquisition white membrane are presented in the following table. The colonies on white membranes were enumerated with the lighting condition so-called HFBR (for ''Haut Fond Blanc rasant'', top low-angle white background in French).

	White membrane
Client	8.23, 8.25, 8.26
Server	8.28.99, 8.31
Camera	UI-3371 uEye
Driver	4.93.13.14
Counting dll	1.39.00

Strains

Escherichia coli ATCC 8739
Pseudomonas aeruginosa ATCC 9027
Ralstonia pickettii ATCC 27511
Stenotrophomonas maltophilia ATCC 13637
Sphingomonas paucimobilis ATCC 29837
Candida albicans ATCC 10231

Filtration membranes

- PVDF (polyvinylidene fluoride) membrane 0.45 µm Merck Millipore #HVWP047S6,
- Cellulose MCE (mixed cellulose ester) membrane 0.45 µm Merck Millipore #HAW04700 (noted MCE_1 in the report),
- Cellulose MCE membrane 0.45 µm with grid Merck Millipore #HAW047S6 (noted MCE_2 in the report),
- Cellulose CA (cellulose acetate) membrane 0.45 µm Sartorius #11106--47------N,
- PES (polyethersulfone) membrane 0.45 µm Sartorius 15406Z-47---SC.

Protocol

After filtration, membrane deposited on TSA plates, incubated at 32.5°C for 48 hours.

Results

Counting performance on white membranes

The following tables 1 to 6 show the synthetic results of ScanStation counting compared with the reference manual counting, expressed in CFU and percentage for the six strains and four white membranes listed in the Methods paragraph.

Escherichia coli				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
2358	MCE_1	19	22	86
2359	MCE_1	36	37	97
867	MCE_1	20	20	100
868	MCE_1	36	36	100
869	MCE_1	31	29	107
2361	MCE_1	32	33	97
2362	MCE_1	44	51	86
2615	MCE_1	8	8	100
2617	CA	25	26	96
2619	CA	38	40	95
864	PES	29	29	100
865	PES	23	23	100
866	PES	22	22	100
2364	PES	18	22	82
2365	PES	31	47	66
2616	PES	19	17	112
861	PVDF	32	32	100
862	PVDF	24	24	100
863	PVDF	22	23	96
2619	PVDF	23	22	105
2620	PVDF	35	35	100

Table 1: *E. coli* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

Pseudomonas aeruginosa				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
849	MCE_2	22	23	96
850	MCE_2	20	20	100
851	MCE_2	23	23	100
2597	MCE_2	28	23	122
2682	MCE_2	14	17	82
3336	MCE_2	16	16	100
3337	MCE_2	24	24	100
2600	CA	32	31	103
2601	CA	35	36	97
2684	CA	12	11	109
3340	CA	10	8	125
3341	CA	10	10	100
846	PES	20	20	100
847	PES	19	19	100
2488	PES	22	45	49
2598	PES	21	20	105
2599	PES	29	38	76
2683	PES	11	11	100
3338	PES	19	19	100
3339	PES	10	11	91
841	PVDF	27	27	100
842	PVDF	34	34	100
843	PVDF	28	29	97
2685	PVDF	17	17	100
2686	PVDF	21	22	95
3342	PVDF	20	20	100
3343	PVDF	28	28	100

Table 2: *P. aeruginosa* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

Ralstonia pickettii				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
2417	MCE_1	4	4	100
2418	MCE_1	3	3	100
2419	MCE_2	12	12	100
2420	MCE_2	5	5	100
2577	MCE_2	29	32	91
2578	MCE_2	54	56	96
3312	MCE_2	4	4	100
2581	CA	23	31	74
2582	CA	72	77	94
3314	CA	2	2	100
3315	CA	2	4	50
2421	PES	3	5	60
2422	PES	5	5	100
2579	PES	28	30	93
2580	PES	70	79	89
3313	PES	1	3	33
3316	PVDF	3	3	100
3317	PVDF	2	2	100

Table 3: *R. pickettii* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

Shingomonas paucimobilis				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
2501	MCE_1	4	4	100
2502	MCE_1	8	8	100
2503	MCE_2	8	8	100
2504	MCE_2	8	8	100
2641	MCE_2	6	6	100
2642	MCE_2	9	7	129
2643	MCE_2	18	18	100
3267	MCE_2	21	22	95
3268	MCE_2	23	24	96
2647	CA	6	6	100
2648	CA	11	11	100
2649	CA	22	22	100
3270	CA	15	15	100
3271	CA	19	19	100
2505	PES	10	10	100
2506	PES	7	7	100
2644	PES	7	7	100
2645	PES	10	10	100
2646	PES	14	15	93
3269	PES	10	10	100
2650	PVDF	11	12	92
2651	PVDF	7	7	100
3272	PVDF	11	11	100
3273	PVDF	22	22	100

Table 4: *S. paucimobilis* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

Stenotrophomonas maltophilia				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
2401	MCE_1	26	27	96
2402	MCE_1	20	20	100
858	MCE_2	4	4	100
859	MCE_2	6	6	100
860	MCE_2	5	5	100
2403	MCE_2	19	19	100
2404	MCE_2	21	22	95
3291	MCE_2	26	29	90
3293	CA	17	17	100
3294	CA	34	35	97
3295	CA	22	23	96
855	PES	8	8	100
856	PES	7	7	100
857	PES	8	8	100
2405	PES	27	28	96
2406	PES	23	25	92
3292	PES	22	25	88
852	PVDF	7	7	100
853	PVDF	10	10	100
854	PVDF	6	6	100
3296	PVDF	16	17	94
3297	PVDF	30	30	100

Table 5: *S. maltophilia* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

Staphylococcus epidermidis				
Plate number	Membrane	CFU_HFBR	CFU_ref	%
2349	MCE_1	21	21	100
2380	MCE_1	12	12	100
2381	MCE_2	27	27	100
2382	MCE_2	16	18	89
2383	PES	25	27	93
2384	PES	20	25	80

Table 6: *S. epidermidis* ScanStation count (CFU_HFBR) and manual count (CFU_ref) comparison and percentage difference. The green cells show a counting performance equal to 100% ± 5.

The comparison of the ScanStation counting with the manual reference counting on the four white filtration membranes are correct. Indeed, about 60% of the cases, the ScanStation counting performance is equal to 100% of the

manual reference counting, including all type of membranes and all tested strains. Furthermore, 100% ± 5 of performance is reach for 73% of the cases.

A deeper analysis detailed in the table 7 highlights that the counting on PES membranes is less performant than the counting of the three of tested white membranes.

		Performance 100% ± 5	
Membrane	Total number of plates	Number of cases	Percentage of cases
MCE_1	10	9	90
MCE_2	33	24	73
CA	19	14	74
PES	33	18	55
PVDF	23	21	91

Table 7: Number of cases (number of plates) and percentage of total analyses for which the ScanStation's counting performance is equal to 100% ± 5.

Furthermore, the following graph (figure 1) shows the correlation summarizing all manual and ScanStation counting performed on the four membrane types and the six strains. The correlation coefficient R^2 is equal to 0.95, meaning there is not significant variation between manual reference and ScanStation counting.

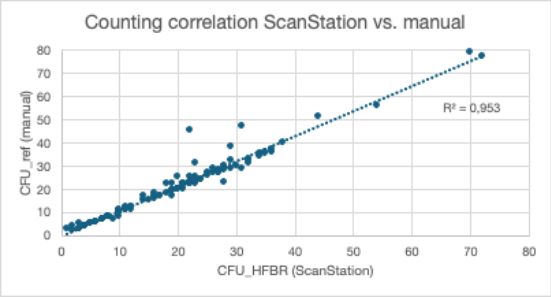

Figure 1: Correlation graph between manual reference and ScanStation of the 113 white filtration membranes analyzed for this study.

The table 8 shows examples of missing colonies or default in the plate analysis by the ScanStation. The lowest performance observed (more than 5% error compared with the reference counting) is due to the presence of colonies too close together in the majority of cases (e.g.: plate number 2383). In rarer cases, colonies are counted several times. Another default in the analysis with less impact on the counting shows a shift on the colony labelling or on the membrane alignment (e.g.: plate number 2383 and 2384).

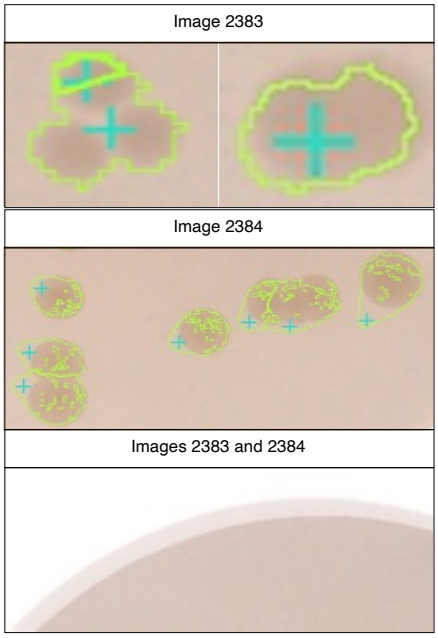

Table 8: Illustrations of some cases of counting with ''errors'' or ''defects''.

The figure 2 shows an example of a real time monitoring of *P. aeruginosa* growth on filtration membrane.

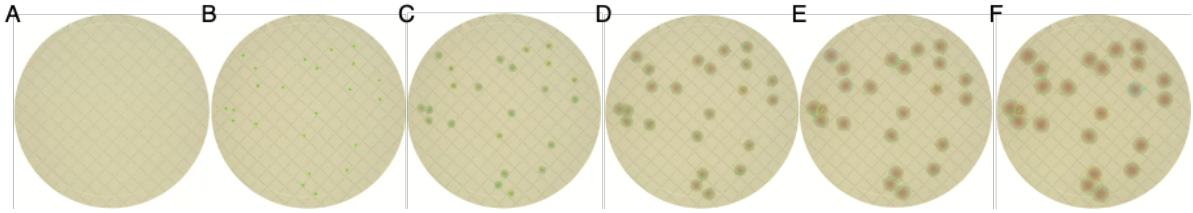

Figure 2: Representative photos of *P. aeruginosa* growth (#851) on an MCE_2 membrane after (A) 0, (B) 9, (C) 18, (D) 27, (E) 36, and (F) 48 hours of incubation.

Conclusion

The ScanStation counting assessment results highlight that colonies are automatically detected on white membranes with correct accuracy.

The ScanStation enumerates on filtration membrane with an accuracy as follow:

- PVDF = 99%.
- MCE = 97%;
- CA = 91%;
- PES = 90%.