interscience

APPLICATION NOTE

HOW CAN REAL-TIME COLONY COUNTING PROVIDE ANTICIPATED RESULTS ON SELECTIVE MEDIA?

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*Unit: **ScanStation**® - Software version:1.30*

Introduction

The microbiological quality control in food industrial production is a critical step to prevent sanitary crisis. Two types of analyses are carried out for this quality control: quality indicator research, which involves enumerating more or less selective flora (such as total flora, lactic flora or coliforms etc. …), and, pathogen detection, which consists of testing for presence or absence of pathogens such as *Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp..

To detect these microorganisms, reference methods are based on their growth on Petri dish containing agar culture media. Indeed, after incubation, microorganisms form colonies (CFU for Colony Forming Unit) on the surface of the agar that can be counted. Selective media are commonly used to highlight these diverse microbiological criteria.

A selective medium allows only a specific type of microorganisms to grow on it. All the other microorganisms present in the sample will be inhibited by this medium. Several characteristics of the medium provide its selectivity: incubation temperature, medium pH, specific nutritive source (e.g.: nitrogen, carbon), resistance to an antiseptic or an antibiotic and enzymatic activity highlighted by a chromogenic complex.

The **ScanStation** is a smart incubator that allows to automate the colony detection in real time. Indeed, for each Petri dish loaded in the incubator chamber, a picture is taken every 30 or 60 min during the whole duration of the incubation period. Those images, once analyzed by the monitoring software, are used to display a curve of the bacterial growth kinetics, available to the user in real-time. An example of this reporting is presented in **figure 0**.

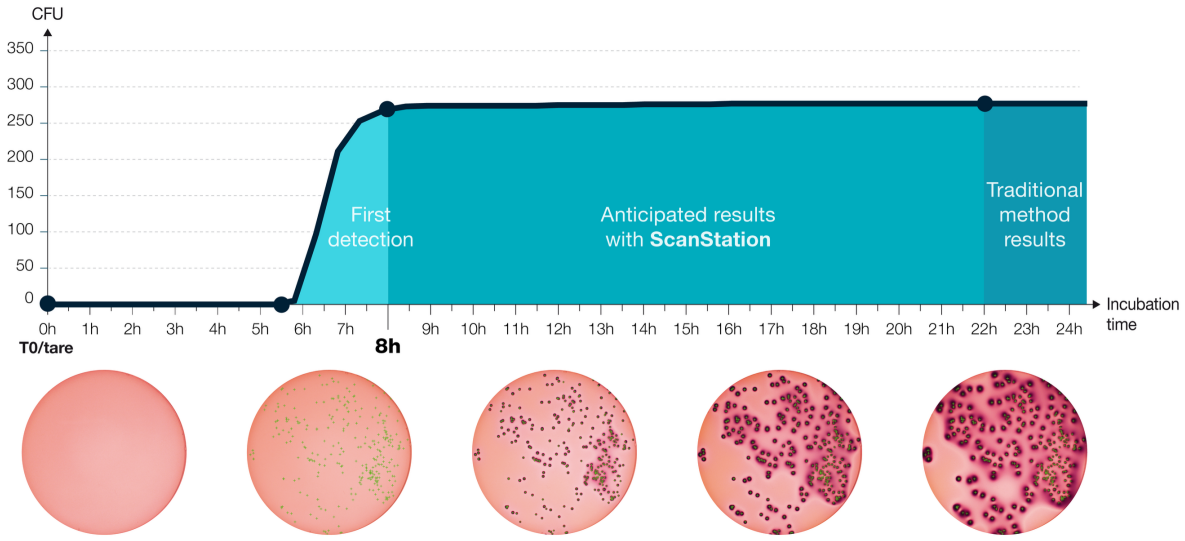

Figure 0: Example of report for coliforms on VRBL medium by the ScanStation. Above - growth curve established by CFU count in real time. Below - six pictures taken at different intervals of the 24-hour incubation period.

Real-time monitoring of bacterial and fungal growth affords a number of advantages beyond the advanced detection of colonies: switching from end-point to real time counting also increases the accuracy of the enumeration. Firstly, the use of a "T0" image captured at the beginning of the incubation and effectively used as a "tare" for all subsequent counts reduces the number of false positives in case of debris/particles in the matrix, which are often falsely counted as CFUs during end-point enumeration (manual or automatic). Secondly, the detection and marking of colonies as soon as they appear on the Petri dish means that subsequent overgrowth or confluent growth are less likely to lead to false negatives.
The **ScanStation** has the possibility to detect colonies by their color. Therefore, it is adapted for the chromogenic media analysis in which different microorganism species could be enumerated in real time and give a specific CFU number for each of them.

The aim of this study is two-fold. First, the performance of the **ScanStation** is assessed by comparing the real manual with the automatic enumeration of real samples from food quality control laboratories. For this purpose, four selective media were selected for their common use in food industry: Baird Parker for *Staphylococcus* spp. detection with black colonies surrounded by a halo specifically for *S. aureus*, VRBL for coliforms detection with purple colonies, Symphony for yeasts and molds detection and TBX for *Escherichia coli* beta-glucuronidase-positive detection with blue colonies. Pure culture analyses were performed only with the Symphony medium to increase the chance of yeast and mold colony detection. It is important to note that, in order to avoid any inter- and intra-operator variation, which is very common with end-point counting, the **ScanStation** performance was assessed by comparing the automatic **ScanStation** counts to a manual count based not on the traditional reading of the plates at the end of the incubation, but rather on the time-lapse created by all the images collected during incubation. In a similar fashion as with replay footage in sports, the operator has access to a "back in time" function that should allow them to avoid any false positives and negatives. We will call this measure "true count".

Second, we also sought to model the "Time to Result" for the same four selective media, as defined as the duration of incubation at which the **ScanStation** can provide a stable and reliable Colony Forming Unit (CFU) count for 85% of the samples of each strain. This model for each selective media provides information about the saved time the **ScanStation** is able to afford to the user in routine analysis.

Material and methods

Inoculation of samples

100 µL for surface plating or 1000 µL for pour plating of liquid samples were inoculated on each selected media according to the recommended standards of food industry quality control laboratories and loaded them in the **ScanStation** to start incubation.

Incubation

All the samples for each strain were incubated at the temperature and for the time listed in the **table 1** below:

Strain	Medium	Temp. (°C)	Time (h)
Staphylococcus spp.	Baird Parker	37	24
Coliforms	VRBL	30	24
Yeast and molds	Symphony	25	72
Escherichia coli β-glucuronidase +	TBX	44	24

Table 1: incubation conditions.

Correlation assessment method

The manual readings were performed by manually counting CFUs on the unmarked time-lapse of the growth as they appeared, therefore improving upon traditional end-point manual counting. Indeed, possible colonies hidden by an overlap in growth could be missed during classical readings on the Petri dish. This is the reason why the manual readings are qualified as "true count" in this study. All reading values are reported in the counted Colony Forming Unit (CFU) log. The difference between the automatic and real manual readings has been calculated and the average and the standard deviation per strain are given.

Time to result

The **ScanStation** captured an image of each sample every 30 minutes. These images were

then processed by the **ScanStation** counting software version v1.30, which detected the apparition time of each colony and yielded a kinetics curve for each sample. We aggregated those growth curves for each strain using Excel. The curves were normalized as a percentage of the total count for each sample for comparison purposes and the first colony detection time and the 85% of final result were calculated for each selective medium.

Results

1. Baird Parker medium

1.a. Manual vs. ScanStation enumeration comparison

The **supplemental table 1** (see appendices) shows the manual and automatic reading of 36 samples from milk industry inoculated on Baird Parker medium for *Staphylococcus* spp. detection. The value of this reading is reported in counted CFU log. The difference between manual and automatic has been calculated and the difference of 0.3 log (absolute value) has been selected as a threshold for statistical significance.

The calculated difference for all of the samples is close to 0 with a maximum of 0.12 and with a total average of all calculated differences equal to 0.01 ± 0.02 CFU log. These results do not show a significant difference between the two enumeration modes. Furthermore, the following graph (**figure 1**) shows the correlation summarizing all manual and **ScanStation** enumerations performed with these 36 samples.

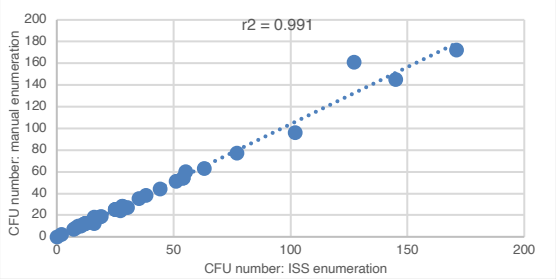

Figure 1: correlation graph of *Staphylococcus* spp. manual and automatic enumeration by Scanstation (ISS) on Baird Parker.

The correlation coefficient R^2 shows a value close to 1, meaning there is close to no difference between manual and **ScanStation** enumerations. The **ScanStation** is therefore performant for *Staphylococcus* spp. analysis on Baird Parker medium.

1.b. Microbial load graph in real-time

The graph of real time growth has also been drawn for the same 36 samples (**figure 2**). A time to result (TTR) has been implemented when the CFU value reached 85% of the final result.

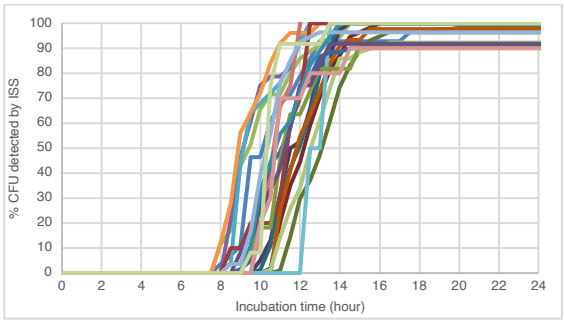

Figure 2: aggregated growth curves of the 36 samples of *Staphylococcus* spp. grown on Baird Parker.

The time of first detection varies from 8 to 12.5 hours and 85% of the final result is reach with an average of 15.6 ± 5.0 hours. By comparison with the 24-hour traditional method, TTR reading allows *Staphylococcus* spp. result anticipation on Baird Parker medium and therefore it gives the possibility to the user to define in advance a corrective action, if necessary.

1.c. Representative photos of a real-time *Staphylococcus* spp. growth on Baird Parker

The **figure 3** shows the example of sample 9259 at t = 0, t = 8.5 h and t = 15.5 h:

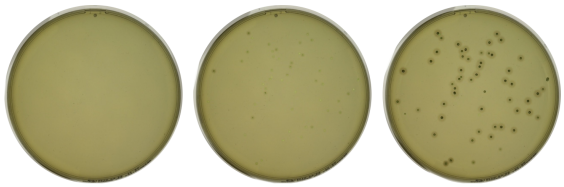

Figure 3: representative photos of *Staphylococcus* spp. real time growth of the sample #9259 on Baird Parker.

2. VRBL medium

2.a. Manual vs. ScanStation enumeration comparison

The **supplemental table 2** shows the manual and automatic reading of 48 samples from milk industry inoculated on VRBL medium for coliforms detection. The value of this reading is reported in counted CFU log. The difference between manual and automatic has been calculated and the difference of 0.3 log

(absolute value) has been selected as a threshold for statistical significance.
The calculated difference for all of the samples is close to 0 with a maximum of 0.25 and with a total average of all calculated differences equal to 0.04 ± 0.04 CFU log. These results do not show a significant difference between the two enumeration modes. Furthermore, the following graph (**figure 4**) shows the correlation summarizing all manual and **ScanStation** enumerations performed with these 48 samples.

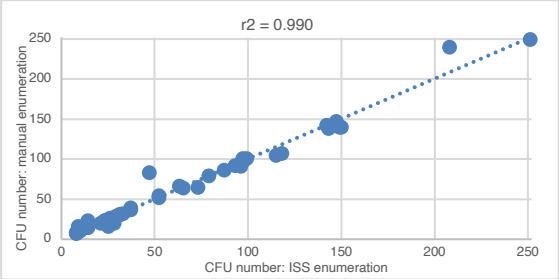

Figure 4: correlation graph of coliforms manual and automatic enumeration by Scanstation (ISS) on VRBL.

The correlation coefficient R^2 shows a value close to 1, meaning there is close to no difference between manual and **ScanStation** enumeration. The **ScanStation** is therefore performant for coliforms analysis on VRBL medium.

2.b. Microbial load graph in real-time

The graph of real time growth has also been drawn for same 48 samples (**figure 5**). A time to result (TTR) has been implemented when the CFU value reached 85% of the final result.

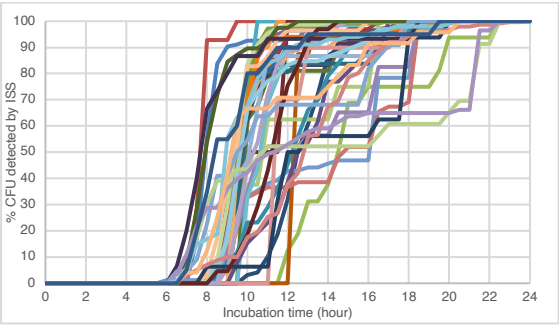

Figure 5: aggregated growth curves of the 48 samples of coliforms grown on VRBL.

The time of first detection varies from 6 to 12.5 hours and 85% of the final result is reach with an average of 15.6 ± 5.0 hours. By comparison with the 24-hour traditional method, TTR reading allows coliforms result anticipation on VRBL medium and therefore it gives the possibility to the user to define in advance a corrective action, if necessary.

2.c. Representative photos of a real-time coliform growth on VRBL medium

The **figure 6** shows the example of sample 3957 at t = 0, t = 6.5 h and t = 17 h:

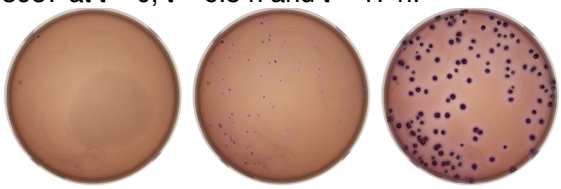

Figure 6: representative photos of coliforms real time growth of the sample #3957 on VRBL.

3. Symphony medium

3.a. Manual vs. ScanStation enumeration comparison

For each microorganism, the **supplemental tables 3 to 9** show the manual and automatic readings of colonies after pure culture growth on Symphony medium. The value of these readings is reported in counted CFU log. The difference between manual and automatic has been calculated and the difference of 0.3 log (absolute value) has been selected as a threshold for statistical significance.

The majority of the results does not exceed the CFU 0.3 log threshold with a total average of all calculated differences equal to 0.13 ± 0.10 CFU log. These results do not show significant difference between the two enumeration modes. Furthermore, the following graph (**figure 7**) shows the correlation summarizing of all manual and **ScanStation** enumerations performed on Symphony medium.

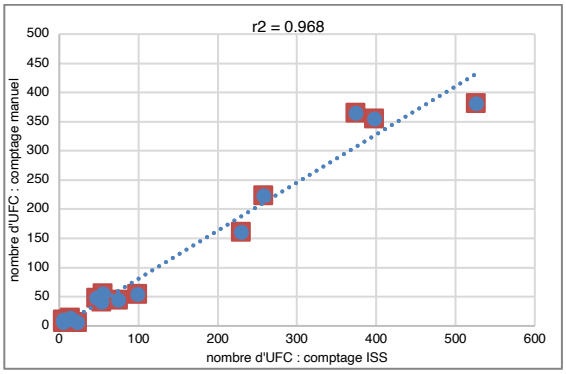

Figure 7: correlation graph of yeasts and molds manual and automatic enumeration by Scanstation (ISS) on Symphony.

The coefficient correlation R^2 shows a value close to 1, meaning there is close to no difference between manual and **ScanStation**

enumeration. The **ScanStation** is therefore performant for yeasts and molds analysis on Symphony medium.

3.b. Microbial load graph in real-time

Graphs of real-time growth have also been drawn for each microorganism (**figure 8 to 14**). A time to result (TTR) has been implemented when the CFU value reached 85% of the final result.

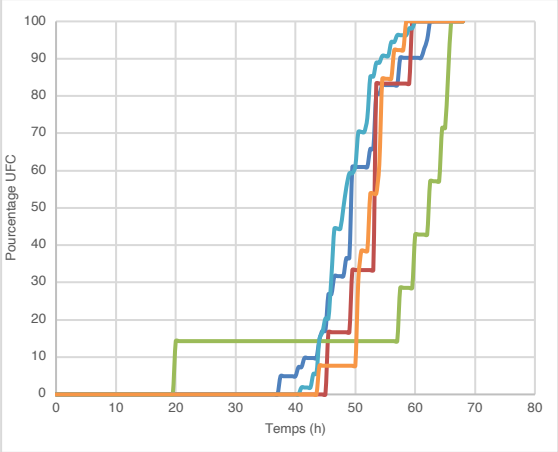

Figure 8: aggregated growth curves of *Penicillium* grown on Symphony with a value of average 85% TTR = 57 h.

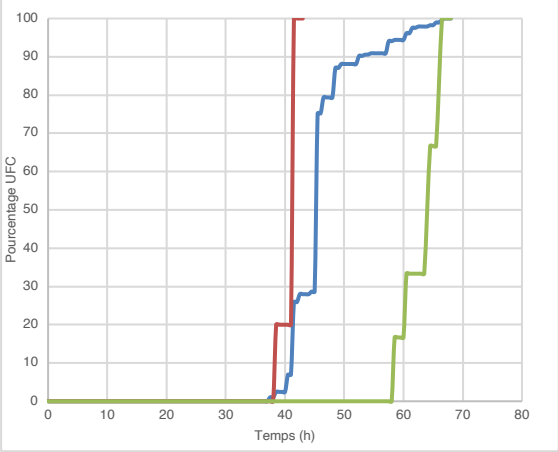

Figure 9: aggregated growth curves of *Zygosaccharomyces* grown on Symphony with a value of average 85% TTR = 48.5 h.

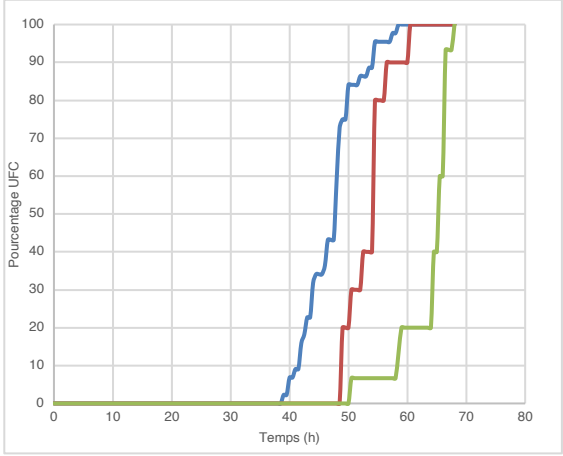

Figure 10: aggregated growth curves of *Aspergillus* grown on Symphony with a value of average 85% TTR = 56 h.

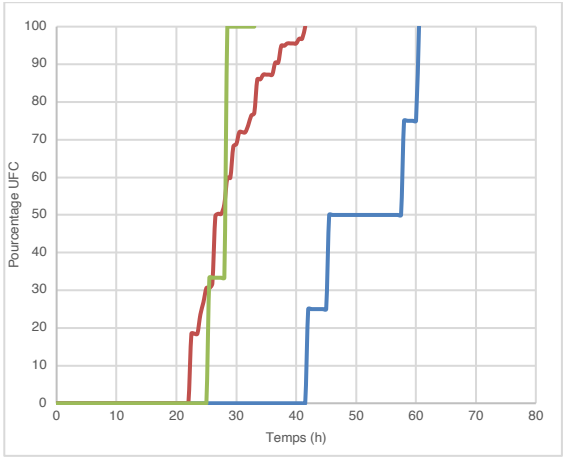

Figure 11: aggregated growth curves of *Mucor* grown on Symphony with a value of average 85% TTR = 33.5 h.

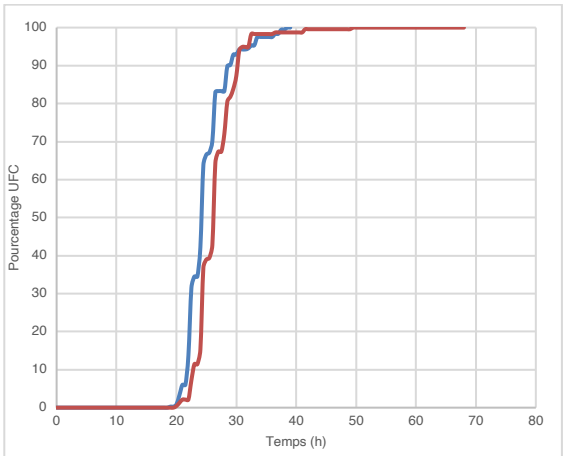

Figure 12: aggregated growth curves of *Torulopsis* grown on Symphony with a value of average 85% TTR = 29.25 h.

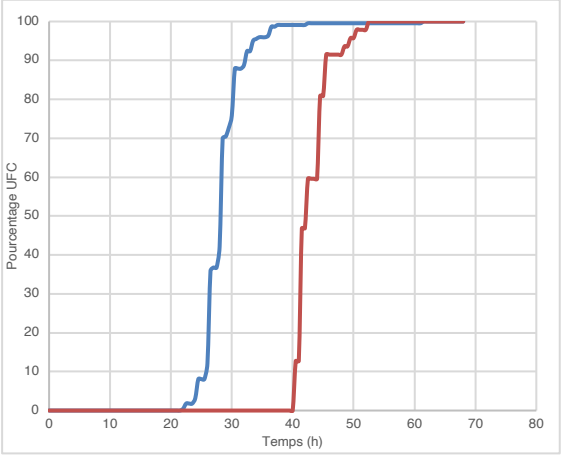

Figure 13: aggregated growth curves of *Saccharomyces* grown on Symphony with a value of average 85% TTR = 38 h.

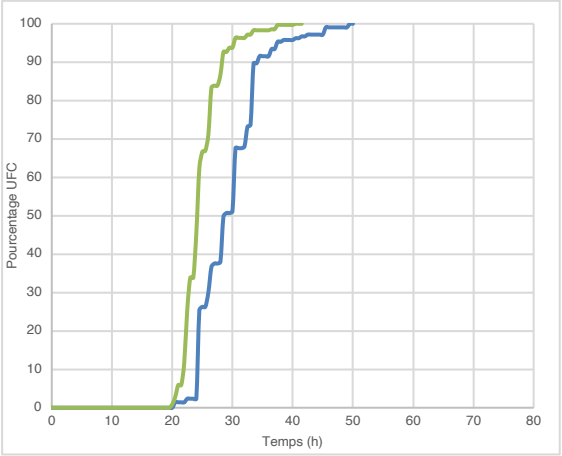

Figure 14: aggregated growth curves of *Candida* grown on Symphony with a value of average 85% TTR = 30.74 h.

TTR reading allows enumeration result anticipation on Symphony medium and therefore it gives the possibility to the user to define in advance a corrective action, if necessary. For example, knowing that the 85% TTR of *Candida* is 30.75 h, a user reading 170 colonies at t = 30.75 h could estimate the total number of CFU for that sample to be 200 CFU. The exact total will be confirmed at the end of the incubation. The precision of the average 85% TTR can be increased by the user by running a significant number of samples in the same conditions.

3.c. Representative photos of a real-time microorganism growth on Symphony medium

The **figure 15** shows the example of *Aspergillus* (sample 2965) at t = 0, t = 49.5 h and t = 65 h:

Figure 15: representative photos of *Aspergillus* real time growth of the sample #2965 on Symphony.

4. TBX medium

4.a. Manual vs. ScanStation enumeration comparison

These analyses were performed with naturally contaminated samples from different quality control food industries. The growth of beta-Glucuronidase-positive *Escherichia coli* on TBX medium was therefore consistent with typical stress condition frequently encountered during daily bacterial analysis.

The **supplemental table 10** shows the manual and automatic reading of *E. coli* colonies after growth on TBX medium. The value of this reading is reported in counted CFU log. The difference between manual and automatic has been calculated and the difference of 0.3 log (absolute value) has been selected as a threshold for statistical significance.

The majority of the results does not exceed the CFU 0.3 log threshold with a total average of all calculated differences equal to 0.12 ± 0.09 CFU log. These results do not show significant difference. Furthermore, the following graph (**figure 16**) shows the correlation summarizing of all manual and **ScanStation** enumerations of *E. coli* growth on TBX medium:

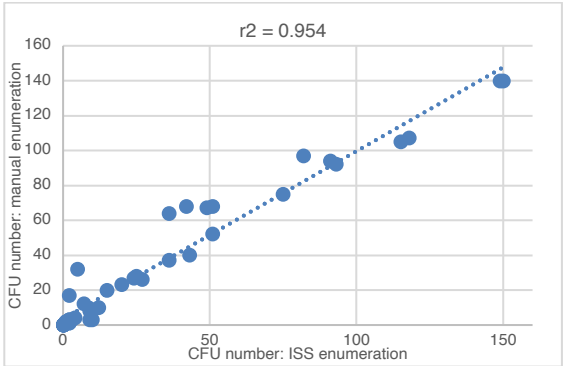

Figure 16: correlation graph of *E. coli* manual and automatic enumeration by Scanstation (ISS) on TBX.

The coefficient correlation R^2 shows a value close to 1, meaning there is close to no difference between manual and **ScanStation** enumeration. The **ScanStation** is therefore performant for *E. coli* analysis on TBX medium.

4.b. Bacterial load graph in real-time

The graph of real time growth has also been performed for each sample (**figure 17**). A time to result (TTR) has been implemented when the CFU value reached 85% of the final result.

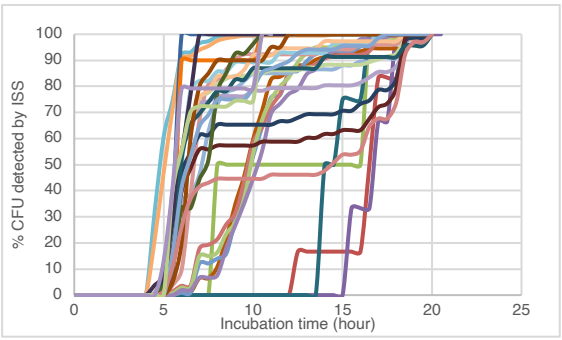

Figure 17: aggregated growth curves of *E. coli* grown on TBX.

The time of first detection varies from 5 h to 15.5 h and 85% of the final result is reach with an average of 10.5 h ± 3.5 hours. The large range of first-time detection is explained by the intrinsic conditions of the plated samples. Indeed, these samples were analyzed following real laboratory conditions and therefore suffered from different stress conditions with a different impact on bacterial growth. However, TTR reading still allows *E. coli* result anticipation on TBX medium and therefore it gives the possibility to the user to define in advance a corrective action, if necessary.

4.c. Representative photos of real-time *E. coli* growth on TBX medium

The **figure 18** shows the example of the sample 5371 at t = 0, t = 9 h and t = 17 h:

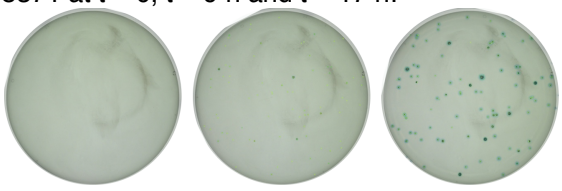

Figure 18: representative photos of *E. coli* real time growth of the sample #5371 on TBX.

Discussion

The correlation between the classical manual enumeration with the **ScanStation** automatic enumeration proved that this device is highly performant to detect wide variety of colony types on different media. Indeed, in this study, enumeration analysis of *Staphylococcus* spp. on Baird Parker, coliforms on VRBL, yeasts and molds on Symphony and *E. coli* on TBX shown more than 95 % of correlation between the two methods. These results demonstrate that the **ScanStation** enumeration method is accurate and reliable. Therefore, the **ScanStation** is a relevant equipment which can be integrated in a R&D or a quality control laboratory of a food industry.

The reliability of the **ScanStation** enumeration is enable thanks to the real time monitoring of the colony growth. Thus, the **ScanStation** performs the enumeration of a sample by detecting the dynamic formation of each colony during the complete incubation period. This method yields counts that are as close as possible to the real number of colonies on the plate. Indeed, the possibility to monitor the colony growth in real time prevents the missing of overlapped colonies that would have happened with a classical automatic end-point colony counter, or manually (false negative). Furthermore, the T0 picture of the plate taken as it is loaded into the **ScanStation** and before any growth starts is used as a tare reference for the analysis. Therefore, this first picture allows the possibility to remove all initial particles (bubbles in agar, solid fragments from sample…) that could be present on the plate, and to take into account only the dynamic apparition of colonies, thus reducing false positives.

The real time reading gives also the opportunity to follow the three microbial growth phases. These three phases are represented on the example graph of the **figure 0**. The first phase, called the lag phase, takes place from t = 0 to t = 6 h and no colony is visible on the Petri dish. The second phase, called the log phase, follows up from t = 6 to t = 8 h. This is during this shortest phase that the majority of the colonies appear over the Petri dish. The third phase between t = 8 and t = 24 h is the stationary phase during which the already appeared colony size continues to grow but no newer colony appears. At the time t = 8h corresponding to the beginning of this third phase, the final result is already completed and available by contrast with the classical method that the 24-hour of incubation period is requested. During this anticipated period, a corrective action earlier on the production could be raised in a case of a CFU threshold reached or the production lot could be released quicker than with the classical method. Subsequently,

anticipated result could reduce significantly the production cost for a company that works with ultra-fresh products like dairy products or fresh meats. Furthermore, for a pathogen detection in which the present/absence criterium is mandatory, the first colony detection is even quicker than the final result. Indeed, in this study, the real time colony monitoring by the **ScanStation** highlighted that 60 to 74% of the incubation time could be saved for bacterial detection and 52% for the yeasts and molds detection to compare with the classical method (**figure 19**).

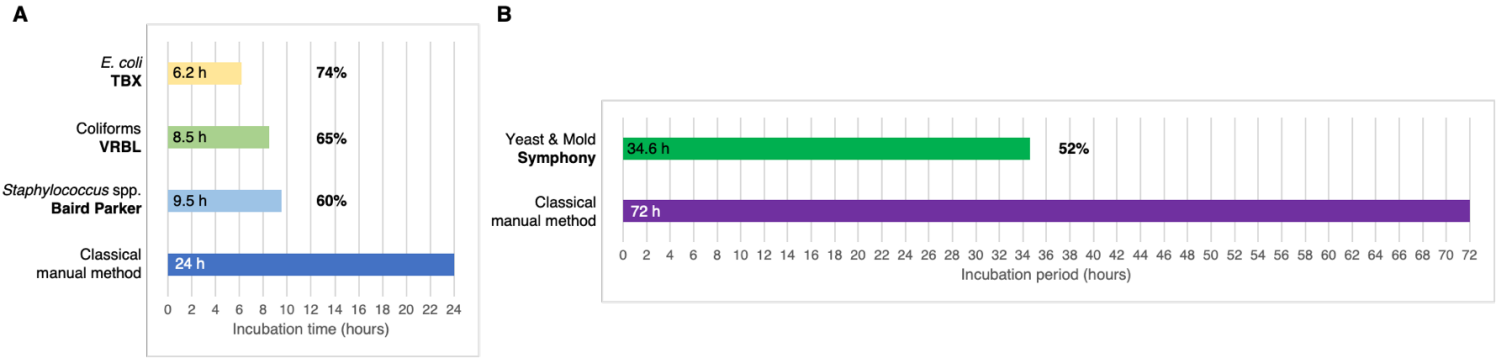

Figure 19: first detection compared to end point at 24 hours for *Staphylococcus* spp. on Baird Parker, coliforms on VRBL and *E. coli* on TBX and at 72 hours for yeasts and mold on Symphony.

According to the **table 1**, the four different media were incubated at different temperatures. However, the **ScanStation** contains only one incubator chamber with only one set temperature. Therefore, only a multi batch analysis is possible with these media at those temperatures. A new study is ongoing to determine the optimized temperature to performs all the analysis on mono batch to synchronize all the anticipated results of a routine R&D or quality control laboratory in food industry.

Conclusion

In light of the results discussed in this study, which show a significantly high correlation between the **ScanStation** automatic CFU count and the "true count" as verified by a human operator with all the pictures taken during the incubation cycle, we confidently recommend the use of the **ScanStation** for production quality control. Furthermore, the Time to Result study gives insights into the growth kinetics of each strain which can be used, if replicated and validated in the lab where it would be intended to be installed, to shorten the detection time to reduce production costs in the food industry. The **ScanStation** is particularly fit for regulated laboratories, as the 21 CFR part 11-compliant software offers full data integrity and traceability to the user.

On our end, further research is planned to assess monotemperature in the **ScanStation** in such as the Food, Pharmaceutical or Cosmetics industries.

Acknowledgements

The authors wish to express their gratitude to all of the personnel involved in the drafting, reviewing, and editing of this application note. In particular, we extend our thanks to the customers who have worked with us through many iterations of the software and shared their data to help us make the most efficient version to date. In house, we thank our microbiology experts Sylvie Viboud, Emilie Tran and Manon Laborie, for their guidance, advice, and data processing.

Appendices

Sample number	Enumeration (CFU log)		Difference (absolute value)
	TC	ISS	
5319	0.90	0.90	0.00
5320	1.43	1.38	0.05
5321	1.48	1.43	0.05
5322	0.85	0.85	0.00
5323	1.43	1.41	0.02
5324	0.00	0.00	0.00
5325	1.80	1.80	0.00
5326	2.01	1.98	0.03
5328	1.20	1.08	0.12
5329	1.20	1.26	0.05
5330	1.28	1.28	0.00
9231	1.45	1.45	0.00
9232	1.71	1.71	0.00
9233	1.54	1.54	0.00
9234	1.45	1.45	0.00
9235	1.40	1.40	0.00
9236	1.40	1.40	0.00
9238	1.40	1.40	0.00
9239	1.73	1.73	0.00
9240	2.23	2.24	0.00
9241	1.58	1.58	0.00
9242	2.16	2.16	0.00
9243	1.64	1.64	0.00
9245	1.08	1.08	0.00
9246	1.89	1.89	0.00
9247	2.10	2.21	0.10
9248	0.30	0.30	0.00
9250	1.74	1.78	0.04
9251	1.00	1,00	0.00
9252	1.04	1.04	0.00
9253	1.08	1.08	0.00
9254	1.26	1.26	0.00
9255	1.08	1.08	0.00
9256	1.73	1.73	0.00
9257	0.95	1,00	0.05
9258	1.08	1.08	0.00
Average calculated difference			**0.01**
Standard deviation			**0.02**

Supplemental table 1: True count (TC) and automatic (ISS) comparison of *Staphylococcus* spp. on Baird Parker.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	TC	ISS	
3959	0.90	0.85	0.06
3965	0.90	0.90	0.00
3972	0.90	0.90	0.00
3977	0.90	0.90	0.00
5348	0.95	1.00	0.05
4027	0.95	1.20	0.25
3961	1.00	1.00	0.00
2504	1.15	1.15	0.00
4007	1.15	1.18	0.03
4057	1.15	1.18	0.03
5579	1.15	1.36	0.22
4010	1.32	1.30	0.02
3978	1.36	1.36	0.00
5364	1.38	1.34	0.04
3967	1.38	1.38	0.00
3147	1.40	1.20	0.19
5584	1.41	1.38	0.03
3996	1.41	1.41	0.00
5585	1.45	1.30	0.15
4017	1.46	1.46	0.00
3984	1.49	1.48	0.01
3970	1.49	1.49	0.00
3986	1.52	1.51	0.01
3966	1.57	1.57	0.00
4052	1.57	1.59	0.02
5581	1.67	1.92	0.25
3975	1.72	1.72	0.00
4015	1.72	1.73	0.02
4016	1.80	1.82	0.02
3983	1.81	1.81	0.01
4047	1.86	1.81	0.05
3974	1.90	1.90	0.00
3998	1.94	1.93	0.01
4071	1.97	1.96	0.00
3985	1.98	1.96	0.02
3962	1.99	1.99	0.00
5582	1.99	2.00	0.02
4006	2.00	2.00	0.01
4075	2.06	2.02	0.04
4072	2.07	2.03	0.04
3957	2.15	2.15	0.00

3993	2.16	2.14	0.02
2500	2.17	2.17	0.00
4074	2.17	2.15	0.03
4073	2.18	2.15	0.03
5576	2.32	2.38	0.06
4011	2.40	2.40	0.00
		Average calculated difference	**0.04**
		Standard deviation	**0.04**

Supplemental table 2: True count (TC) and automatic (ISS) comparison of coliforms on VRBL.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
2962	1.73	1.61	0.12
2963	0.70	0.78	0.08
2994	2.00	1.73	0.27
2995	1.15	1.11	0.04

Supplemental table 3: True count (TC) and automatic (ISS) comparison of *Penicillium* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
3006	1.36	0.78	0.58
2974	2.72	2.58	0.14
2975	1.74	1.74	0,00

Supplemental table 4: True count (TC) and automatic (ISS) comparison of *Zygosaccharomyces* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
2964	1.88	1.64	0.23
2965	1.08	1.00	0.079

Supplemental table 5: True count (TC) and automatic (ISS) comparison of *Aspergillus* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
2998	2.36	2.20	0.16
2966	0.70	1.00	0.30

Supplemental table 6: True count (TC) and automatic (ISS) comparison of *Mucor* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
2971	2.57	2.56	0.01
3003	2.30	2.37	0.07

Supplemental table 7: True count (TC) and automatic (ISS) comparison of *Torulopsis* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
2973	2.41	2.35	0.06
3005	1.67	1.67	0.00

Supplemental table 8: True count (TC) and automatic (ISS) comparison of *Saccharomyces* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
3001	2.60	2.55	0.05
2968	2.41	2.33	0.08

Supplemental table 9: True count (TC) and automatic (ISS) comparison of *Candida* on Symphony.

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
4042	0.95	0.90	0.05
4044	0.00	0.00	0.00
4058	0.48	0.48	0.00
4059	0.00	0.00	0.00
4061	0.30	0.48	0.18
4064	0.85	1.08	0.23
4066	0.30	0.00	0.30
5370	0.60	0.60	0.00
5371	1.97	1.96	0.01
5372	2.07	2.03	0.04
5373	2.18	2.15	0.03
5374	2.17	2.15	0.02
5375	2.06	2.02	0.04
5411	1.63	1.60	0.03
5436	0.95	0.48	0.47
5437	1.00	0.95	0.05
5438	1.00	0.48	0.52
5440	0.30	1.23	0.93
5441	0.70	1.51	0.81

Sample number	Enumeration (CFU log)		Difference (absolute value)
	Manual	ISS	
5442	1.08	1.00	0.08
5456	1.69	1.83	0.14
5457	1.71	1.72	0.01
5458	1.96	1.97	0.01
5459	1.91	1.99	0.08
5460	1.40	1.45	0.05
5461	1.56	1.57	0.01
5468	1.88	1.88	0.00
5469	1.62	1.83	0.21
5470	1.38	1.43	0.05
5471	1.43	1.41	0.02
5472	1.30	1.36	0.06
5473	1.18	1.30	0.12
5474	1.71	1.83	0.12
5497	1.56	1.81	0.25
4046	0.30	0.48	0.18
4030	0.00	0.30	0.30
4031	0.00	0.30	0.30
4033	0.30	0.00	0.30

Supplemental table 10: True count (TC) and automatic (ISS) comparison of *E. coli* on TBX.