interscience

APPLICATION NOTE

EASYSPIRAL® / EASYSPIRAL PRO® VALIDATION STUDIES

*Units: **easySpiral® / easySpiral Pro®***

1. **Validation of easySpiral® / easySpiral Pro® Spiral plating**

Purpose:

To study the feasibility of easy**Spiral®** / easy**Spiral Pro®** **Spiral** plating in comparison with the reference technique (classical surface plating).

Equipments and techniques:

Five bacterial strains (three gram+ and two gram-) are tested: *Lactobacillus casei var. rhamnosus*, *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATTC 9027).

Bacterial cultures are diluted in buffered peptonized water before being plated on Petri dishes with agar (90 mm) using classical technique and **Spiral®** technique (50 µL in exponential mode).

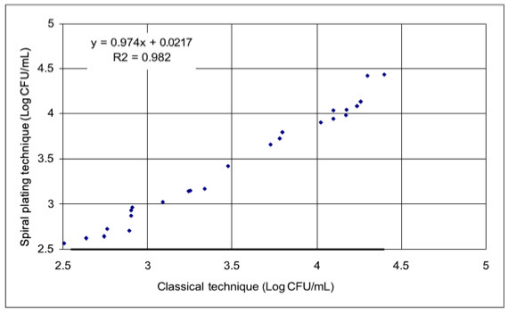
After 48 hours of incubation at 37°C (±1) on MRS (De Man, Rogosa, Sharpe) for *Lactobacillus casei* and 18 hours (±2) at 37°C (±1) on PCA (Plate Count Agar) for *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and on Macconkey for *Escherichia coli*, the counting is performed by an automatic colony counter (**Scan® 1200**).

Results analysis:

Counting results are given in Log CFU/mL (Colony Forming Unit) and a regression analysis is made to evaluate the correlation between the **Spiral®** plating technique on easy**Spiral Pro®** and the classical plating techniques.

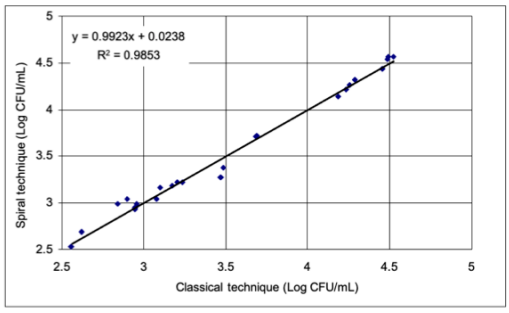
Results:

- *Bacillus Cereus*:



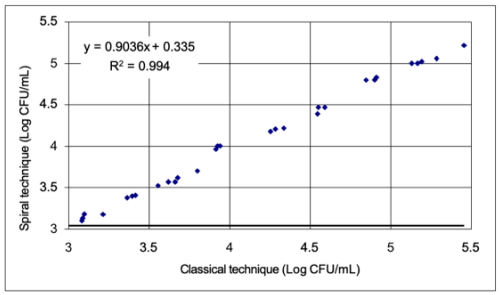
Picture 1: Correlation between Bacillus cereus counting (in Log CFU/mL) obtained by plating with Spiral® and classical techniques.

- *Bacillus subtilis*:



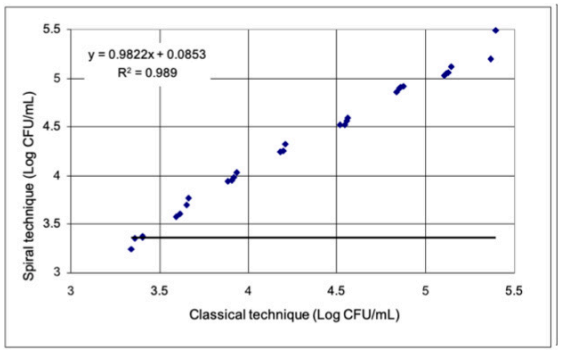
Picture 2: Correlation between the counting of Bacillus subtilis counting (in Log CFU/mL) obtained by plating with Spiral® and classical techniques.

- *Lactobacillus casei var. rhamnosus*:



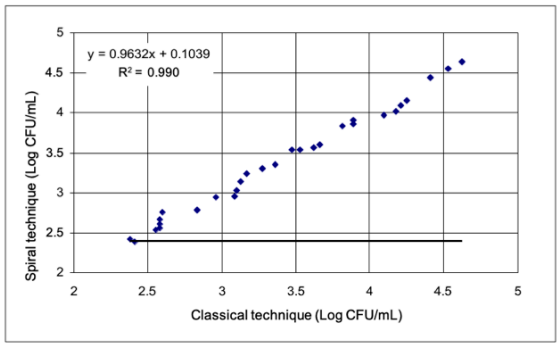
Picture 3: Correlation between Lactobacillus casei var. rhamnosus counting (in Log CFU/mL) obtained by plating with Spiral® and classical techniques.

- *Escherichia coli*:



Picture 4: Correlation between Escherichia coli counting (in Log CFU/mL) obtained by plating with Spiral® and classical techniques

- *Pseudomonas aeruginosa*:



Picture 5: Correlation between *Escherichia coli* counting (in Log CFU/mL) obtained by plating with Spiral® and classical techniques.

Conclusion:

Results show a strong correlation ($R^2 \geq 0.982$) between **Spiral®** mode on **easySpiral®** and the classical technique. Hence the average difference of CFU/mL Logs between the two techniques is 12 times inferior to the maximum deviation (0.5 log) authorized by AFNOR NF V 08/100 standard, thus not significant.

2. easySpiral® / easySpiral Pro® cleaning effectiveness

Objective:

Ensure that the cleaning of the stylus is thorough in between each series of samples. Check the disinfection effectiveness of the stylus by spreading on plates sterile diluent.

Principle:

- Contamination of the stylus of the **easySpiral®** / **easySpiral Pro®** by a highly concentrated bacterial suspension ($C=10^5$ -10^7 CFU/mL).

- Cleaning the stylus with a disinfectant solution by using either the "normal" or "long" (only for easy**Spiral Pro®**) cleaning mode of the plater.

- Verification of disinfection effectiveness by plating sterile diluent on non-selective agar plate (e.g. TSA, Tryptone Soya Agar) and incubate it at 37°C (± 1°C) during 48h (±2).

Bacterial strains:

Escherichia coli ATCC 25922,
Staphylococcus aureus ATCC 9144,
Listeria monocytogenes ATCC 13932,
Salmonella typhimurium ATCC 14028,
Pseudomonas aeruginosa WDCM 00027,
Bacillus subtilis ATCC 6633.

Disinfectants:

Ethanol 70%

Bleach 1%

H_2O_2 3% + PAA 0.08% (solution of hydrogen peroxide 3% + peracetic acid 0.08%).

Results:

The concentration of bacterial suspension used to contaminate the stylus vary from 10^5 to 6.6 x10^7 CFU/mL.

Plating sterile diluent, after a bacterial suspension sampling + cleaning step, shows that no colony is observed on agar plate (Table 1), whatever the cleaning mode used.

Bacterial strain	[bacteria] in suspension (CFU/mL)	Disinfectant	Number of CFU after cleaning	
			Normal mode	Long mode
Escherichia coli	5.5 x10^{7}	Ethanol 70%	0	0
		Bleach1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0
Listeria monocytogenes	3.3 x10^{6}	Ethanol 70%	0	0
		Bleach 1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0
Salmonella typhimurium	5.0 x10^{6}	Ethanol 70%	0	0
		Bleach 1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0
Pseudomonas aeruginosa	6.6 x10^{7}	Ethanol 70%	0	0
		Bleach 1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0
Staphylococcus aureus	2.3 x10^{6}	Ethanol 70%	0	0
		Bleach 1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0
Bacillus subtilis	1.0 x10^{5}	Ethanol 70%	0	0
		Bleach 1%	0	0
		H_2O_2 3% + PAA 0.08%	0	0

* Only for easy**Spiral Pro**®

Effectiveness of the stylus disinfection according the 'normal' or 'long' cleaning mode and the disinfectant solution tested (3 repetitions per test).

Conclusions:

The study shows that, for the six bacterial strains, the 'normal' or 'long' cleaning mode is effective to disinfect the stylus and to avoid the cross contamination.

Three disinfectants were tested: ethanol 70%, bleach 1% and a solution of H_2O_2 3% + PAA 0.08%.

Other products with different concentrations f H_2O_2 and PAA exist that can be effective to disinfect the stylus. In this case, customer should check the cleaning effectiveness of the stylus with the product and the absence of contamination.

It is recommended to plate sterile diluent on agar plate before and after each series of samples to verify the sterility of the stylus.