Application Note:
AP-MIB-MDCOMBI01-0607

Facilitating Biofilms Assays With Thermo Scientific Multidrop® Combi Dispenser

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Abstract
This application note describes the use of the Thermo Scientific Multidrop Combi (Thermo Fisher Scientific) dispenser in biofilm assays. In this experiment, bacterial suspension of *Staphylococcus aureus* was dispensed into the 96-microtiter well plates both manually and with the Multidrop Combi. The results show that the use of Multidrop Combi to plate *S. aureus* had no effect on the bacterial ability to grow in suspensions or in biofilms, and additionally provided typical automation benefits.

Introduction
Discovery of new antimicrobial drugs has progressively been demanding the development of new reliable techniques, allowing the testing of bioactivity against biofilm infections as they represent a key problem in today's medical microbiology (Lasa, 2006). In recent years, the automation of cell (including bacteria)-based screening assays has consistently shown to provide reduced times and considerable labor saving, while maintaining or increasing assay quality during screening campaigns. These facts have progressively been favoring a shift towards the cell-based screening assays across both the environments of both the academia and pharma companies (Johnston and Johnston, 2002). This application note shows experiments conducted to evaluate the use of the Thermo Scientific Multidrop Combi dispenser during different stages (seeding, staining) of an assay intended for the bioactivity profiling of inhibitory compounds against *Staphylococcus aureus* biofilms on 96-microtiter well plates.

Methods
S. aureus (DSM 20231) was grown for 4 hours at 37°C, 200 rpm in Tryptic Soy Broth (TSB). In the first group of experiments, the effect of automated dispensing on the bacterial ability to form biofilms was assessed. For that purpose, bacterial suspensions (10^6 CFU/ml, in TSB) or TSB alone (for negative controls) were dispensed into 96-microtiter well plates (200 µl/well) either manually or using the Multidrop Combi (kept in laminar flow) at the three available speeds (low, medium or high). Bacterial cells were then allowed to form biofilms for 18 hours at 37°C, 200 rpm. At the end of this period, the bacterial cells growing on suspension were removed and their concentration assessed by absorbance measurement (at 595 nm), while bacterial biofilms left in the wells were quantitatively and manually stained using crystal violet (Kolari et al., 2001). In the second group of experiments, the Multidrop Combi was used for the bacterial dispensing and staining steps in the assessment protocol of *S. aureus* biofilms, in conjunction with a liquid handling workstation where the rest of the pipetting steps were made. In that case, medium speed was used during the Multidrop Combi steps, and the approach was compared to a totally manually performed assay. For the second group of experiments, statistical parameters and repeatability measures characterizing the performance of the assay were calculated as in Zhang et al., (1999) and Bollini et al., (2001).

Results and discussion
The use of the Multidrop Combi to plate *S. aureus* was proven to have no effect on the bacterial ability to grow in suspension or in biofilms at the medium and high dispensing speeds (Table 1). Using the Multidrop Combi, the initial process leading to biofilm formation was speeded up, as dispensing time was reduced from at least 60 seconds per plate in the manual (time always depending on the individual ability) to 24, 22, and 18 seconds, using the low, medium, and high dispensing speeds, respectively.

In addition, the introduction of the Multidrop Combi for dispensing bacterial suspension and also for staining *S. aureus* biofilms within a fully automated approach provided with a high-quality assay that has reduced variability around the maximal signal on the plate-to-plate and day-to-day basis

(Table 2 and Figure 1). A particularly advantageous result of introducing the Multidrop Combi to this assay was that no spills were generated during the staining step with crystal violet, which therefore significantly facilitated the implementation of this assay of biofilms.

Thus, the automated bacterial handling on 96-microtiter well plates using the Multidrop Combi alone or in conjunction with a liquid handling workstation provided typical automation benefits, without compromising the quality in the area of biofilms-based assays.

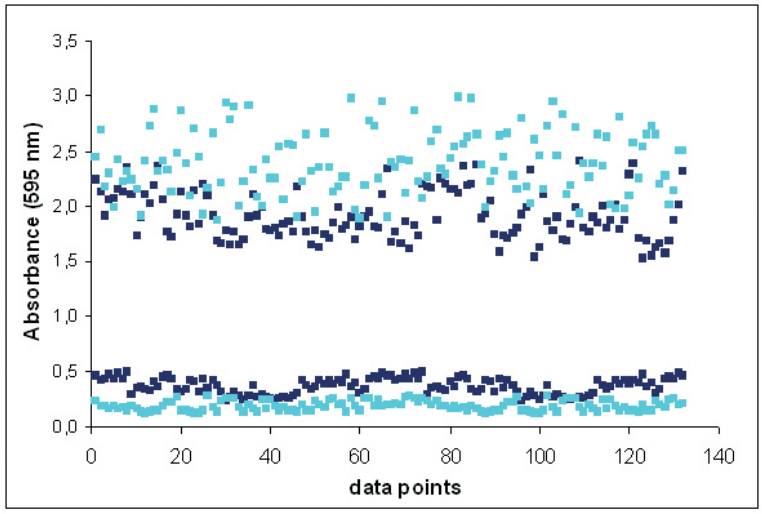

Figure 1. Signal window obtained for the automated and manual assays. The variability from plate-to-plate associated to the maximal signal is reduced by using the automated approach with Multidrop Combi.

Table 1. Bacterial growing (in suspension and in biofilms) after automated or manual plating

Dispensing mode	Planktonic growth	Biofilm growth
	Absorbance (595nm)	Absorbance (595nm)
Manual	0.20 ± 0.07	2.28 ± 0.36
Multidrop Combi LOW	0.24 ± 0.07 ns	1.55 ± 0.24***
Multidrop Combi MED	0.22 ± 0.06 ns	2.34 ± 0.45 ns
Multidrop Combi HIGH	0.17 ± 0.02 ns	2.32 ± 0.43 ns
***- statistically different when compared to the manual assay (p< 0.001) ns- no statistically different when compared to the manual assay		

Table 2. Comparison of assays performed manually and using Multidrop Combi in conjunction with another liquid handling workstation

Parameters	MANUAL	AUTOMATED
Z'	0.49 ± 0.06	0.45 ± 0.03
S/N	6.9 ± 0.47	6.9 ± 0.46
S/B	11.9 ± 1.8	5.3 ± 0.27
Separation band	1.26	1.08
Well-to-well repeatability (CV %) §	10.2	19.4
Plate-to-plate repeatability (CV %) §	3.5	0.6
Day-to-day repeatability (CV %) §	35.2	8.9
§- calculated for the maximal signal (main source of variability in the assay)		

References

- *Bollini S, Herbst JJ, Gaughan GT, Verdoorn TA, Ditta J, Dubowchik GM, Vinitsky A. (2001). High throughput fluorescence polarization method for identification of FKBP12 ligands. Journal of Biomolecular Screening 7(6): 526- 530.*
- *Johnston PA, Johnston PA. (2002). Cellular platforms for HTS: three case studies. Drug Discovery Today 7(6): 353- 363.*
- *Kolari M, Nuutinen J, Salkinoja-Salonen MS. (2001). Mechanisms of biofilm formation in paper machine by Bacillus species: the role of Deinococcus geothermalis. J. Industrial Microbiology 27: 343- 351.*
- *Lasa I. (2006). Towards the identification of the common features of bacterial biofilm development. International Microbiology 9:21-28.*
- *Zhang JH, Chung TDY, Oldenburg KR. (1999). A simple statistical parameter for use in evaluation and validation of High Throughput Screening assays. Journal of Biomolecular Screening 4(2): 67- 73.*

KR. (1999). A simple statistical parameter for use in evaluation and validation of High Throughput Screening assays. Journal of Biomolecular Screening 4(2): 67- 73.

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