Technical Note:

TN-MIB-MDcombiNL-0508

Optimization of a bulk reagent liquid handler for low volume dispensing

Sini Suomalainen, Heikki Oranen, Ari Kukkonen and Merja Mehto
Thermo Fisher Scientific (Vantaa, Finland)

Introduction

This technical note describes the performance of Thermo Scientific Multidrop Combi nL in low volume dispensing and the operating principle of the valves. Multidrop Combi nL provides consistent, high-throughput dispensing into 1536-, 384- and 96-well microplates covering the volume range 50 nl – 50 µl. In this experiment, liquids with different surface tension properties were tested by dispensing multiple volumes into 1536- and 384-well plates. Descriptions of the liquid calibration and the valve correction feature with the Thermo Scientific FILLit Software are also presented.

Functional operation of the valve

Unlike other Multidrop dispensers Multidrop Combi nL uses valves and a pressurized reagent container for liquid flow. When the valve is closed, the ruby closing ball is pressed into the sapphire seat. By applying current, the ruby ball moves to open the valve (Figure 1). The dispensing volume is based on the duration of the opening pulse and depends on the loading pressure at the reagent reservoir. The pressure system can also produce vacuum, which is used for the backflush of reagents.

There are eight microsolenoid dispensing valves inserted into the dispensing valve head of the Multidrop Combi nL. The valves operate separately and it is possible to replace an individual valve instead of the whole dispensing head, if needed.

Figure 2. Eight dispensing valves inserted into the manifold.

Replacing the dispensing valve is easy and there is no need for recalibration after the replacement, because the valve performance goes through the performance inspection.

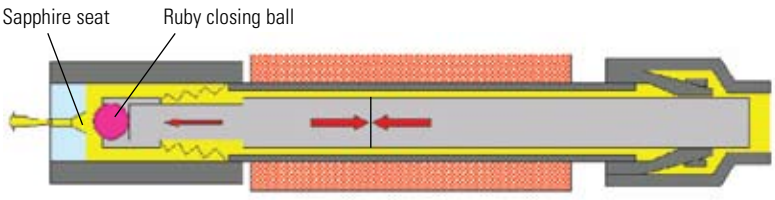
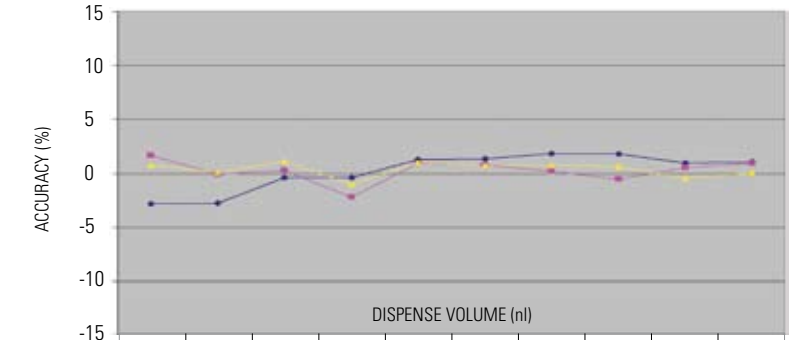


Figure 1. The microsolenoid valve open.

Dispensing and calibration of various liquids

Multidrop Combi nL is designed for dispensing of all kinds of liquids. The tubing is made of tygon, to reach the maximum resistance to the chemicals. Besides excellent durability and exceptional smooth inner surface, tygon is inert material for flexible tubing.



	50	100	200	500	1000	2000	5000	10000	20000	50000
BSA 1 %	-2.8	-2.7	-0.4	-0.4	1.3	1.3	1.8	1.8	0.9	1.0
DMSO	1.6	-0.1	0.3	-2.2	0.9	0.8	0.2	-0.5	0.5	0.9
Glycerol 30 %	0.7	0.1	1.1	-1.0	1.0	0.7	0.8	0.6	-0.4	0.1

Figure 3. The accuracy of dispensing of different liquids.

Performance of Multidrop Combi nL

Dispensing liquids with different viscosities and surface tension properties were tested to estimate the performance of Multidrop Combi nL. Multiple volumes of various liquids were dispensed into 1536- and 384-round well plates to evaluate the accuracy and precision of the dispensing. Factory settings for different liquids were used for dispensing speed. Reproducibility was tested by dispensing 50-50000 nl of 0.04 mM fluorescein in 1% BSA, 30% glycerol and DMSO to 384-well plates. The filling was done for the whole plate and the test was repeated twice with every volume used. After dispensing of different liquids with fluorescein, 10-50 µl Tris-Saline (pH 8.0) buffer was added to the plates. The volume of Tris-Saline depended on the volume of dispensed liquid, 50 µl for 50-5000 nl, 40 µl for 10000 nl, 30 µl for 20000 nl and 10 µl for 50000 nl. After centrifugation and shaking, the plates were measured in Thermo Scientific Varioskan ex/em 485/518 nm. The internal software Liquid Factor -function was used to measure the accuracy of dispensing all liquids with every volume. The liquid factor was calculated by weighing the liquid dispensed in to the 1x8 strip and compared this to the expected volume to be dispensed (Figure3).

Reliability of dispensing

The reproducibility test was done by dispensing 0.04 mM fluorescein in water to 384-well plates. The dispensing of 100 nl to 4300 plates was done within two days to test the affect on the accuracy. The tubing was emptied for the night and filled and primed to continue dispensing with the same parameters in the morning. There were no peaks or empty wells detected during dispensing 4300 plate run with 100 nl of water. The repeatability test was also done with 500 nl of 0.04 mM fluorescein in DMSO (1000 plates), 1% BSA (1320 plates) (Figure 4a).

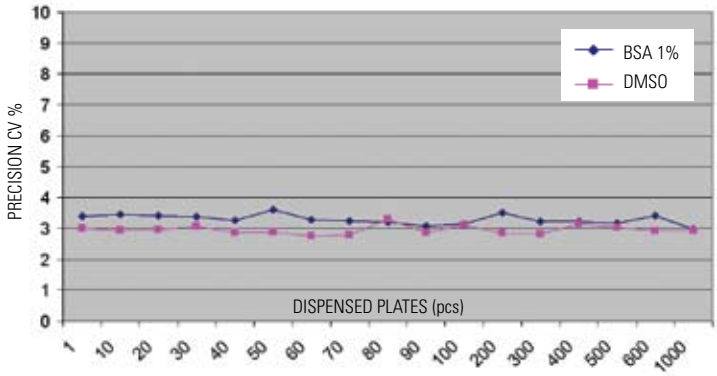


Figure 4a. The reproducibility of dispensing 500 nl of BSA 1% and DMSO to 384 plates (100 pcs.).

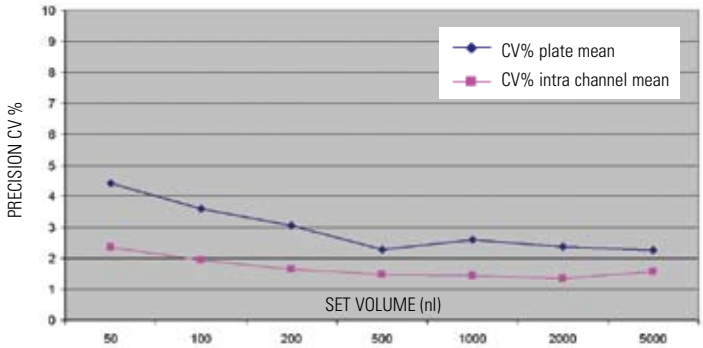


Figure 4b. Precision of dispensing Tris-Saline (pH 8.0) buffer to 1536 plates.

Calibration

Because of the different densities that affect the movement of the liquid, the calibrations are needed to ensure that the instrument dispenses the set volume accurately. Multidrop Combi nL has five calibrated liquids in store; water, DMSO, 1% BSA, methanol and 30% glycerol and with the Multidrop FILLit Software it is straight forward to calibrate a new liquid to the instrument. Calibration creates a calibration curve for the combination of liquid and speed. The curve contains a series of five calibration points, each of which consists of a volume/time pair (Figure 5).

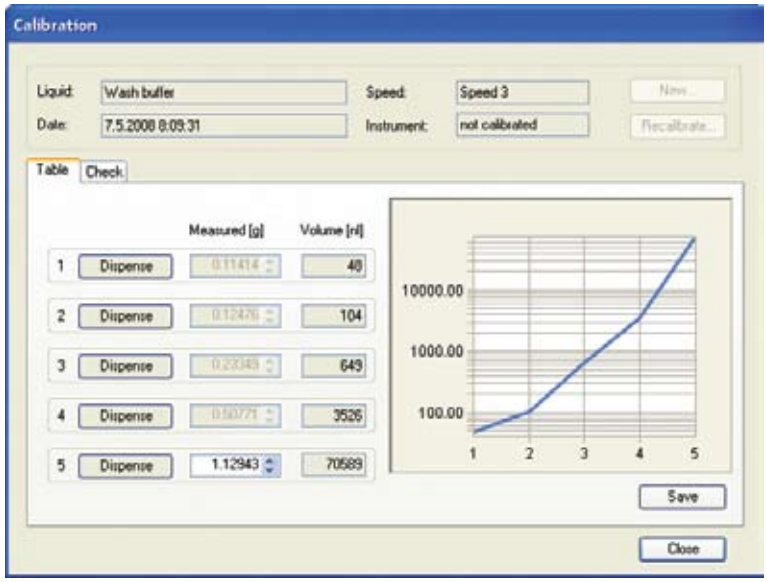


Figure 5. Five point calibration

It is possible to compensate the dispensing volume of a single valve with the valve correction feature of the FILLit Software (Figure 6). All eight valves are set to function similarly, but they are individual and function separately. If some of the valves are consistently dispensing other than the set volume, there is a possibility to enter a correction factor in percentage for each valve.

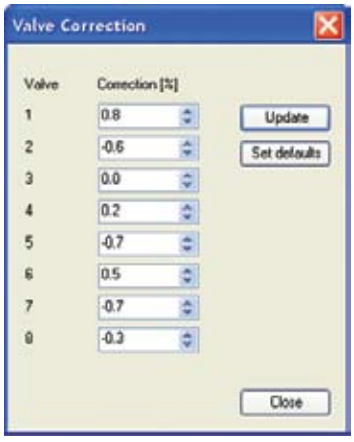


Figure 6. Valve corection feature

Dispensing of cells and cell culture media

Because of the characteristics of the instrument, Multidrop Combi nL can be used to dispense cells, culture media and beads. The dispensing of cell culture medium was tested with DMEM and 10% FBS. There was a comparison of two different dispensing heights, 11 and 16 mm, and all 5 different dispensing speeds to 384-well plates. Some frothing was seen in some of the wells with dispensing height 16 mm, but that was eliminated with the lower dispensing height and the highest dispensing speed. Different cell lines, e.g. CHO and HEK 293, have been succesfully dispensed with Multidrop Combi nL and the monolayer cells remain undisturbed after dispensing liquids on top of them (figure 7).

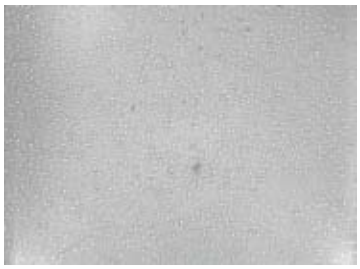


Figure 7. Reagents dispensed successfully on top of monolayer CHO cells.

Conclusion

The precision and accuracy test results of Multidrop Combi nL show reliability, reproducibility and flexibility of dispensing small volumes of liquids with different viscosities and surface tension properties. It has also been succesfully used for dispensing of living cells and cell culture media. The pressurized reagent container with valves provides accurately controlled liquid flow at all volumes and the dispensing reliability of Multidrop Combi nL has proven to be very high in dispensing tests. With the Multidrop FILLit software, the instrument can calibrate the valve open times for different liquids and the possibility of single valve correction supplements the five point calibration to achieve optimal dispensing results.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America:
USA / Canada
+1 866 984 3766

Europe:
Austria
+43 1 801 40 0
Belgium
+32 2 482 30 30
France
+33 2 2803 2000
Germany national toll free
08001-536 376
Germany international
+49 6184 90 6940
Italy
+39 02 02 95059 434-254
Netherlands
+31 76 571 4440
Nordic countries
+358 9 329 100
Russia/CIS
+7 (812) 703 42 15
Spain/Portugal
+34 93 223 09 18
Switzerland
+41 44 454 12 12
UK/Ireland
+44 870 609 9203

Asia:
China
+86 21 6865 4588 or
+86 10 8419 3588
India
+91 22 6716 2200
Japan
+81 45 453 9220
Other Asian countries
+852 2885 4613
Countries not listed:
+49 6184 90 6940 or
+33 2 2803 2000

www.thermo.com

© 2008 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.