



SMALL VOLUME DRY PLATE DISPENSING WITH TOMTEC'S 60 μ L POSITIVE DISPLACEMENT PIPETTOR

There is a desire by a number of compound library dispensaries to dispense small volumes of compounds in DMSO. It enables them to distribute a common compound plate to a number of different users. Since the compound is in 100% DMSO its' shelf life can be extended by freezing. As assays move to the smaller volume of the 384 well plate, the interest is in 0.5microliters. This allows the 100 fold dilution of the compound, in 100% DMSO, to the 1% DMSO requirement of most assays, when the assay constituents are added to the well.

Dispensing 0.5 μ L aliquots with precision is a challenge. Ink jet or piezo methods can accomplish it with ease. They generate sufficient back pressure at the dispensing orifice to deliver the aliquot as a stream or jet. However, their utility relies on the ability to repeatably dispense the same liquid. This is lost when asked to aspirate a discreet sample from one location and dispense in another, without carry over. This is the requirement in compound handling. That application is best handled by piston displacement.

Piston displacement falls in two primary categories, air displacement and positive displacement. With air displacement, there is an air column between the orifice and the piston. This is usually the volume of the disposable tip. A typical tip volume may be 100 μ L. If asked to aspirate or dispense 0.5 μ L the piston moves 0.5% of its tip volume. This creates a negative pressure in the tip, which in the theoretical case will allow 0.5 μ L to flow in to equilibrate the negative pressure that was created. In practice since $P_1V_1 = P_2V_2$ the internal tip pressure only decreases 0.5% below atmospheric. This creates very little negative head pressure to overcome the capillary forces at the tip orifice. This is particularly true on the dispense stroke, since now there is only a 0.5% increase in theoretical pressure. The result is loss of precision at the small volumes.

A true positive displace system has the piston in direct contact with the liquid. Thus, any change in the piston is an equivalent change in volume. In practical terms there is a small air gap between the piston and the liquid interface. In Tomtec's small volume Quadras, in both the 384 and 96 well versions, the air gap is about 1 to 2 μ L. A change of 0.5 μ L creates a 25-50% change in the internal air pressure. The piston cannot reach the orifice, due to the taper at the bottom of the tip. It is essential to have a small orifice and surrounding sidewall. This provides the minimum surface to retain liquid by surface tension. The larger diameter is required in the piston, above the orifice, to achieve a total dispense volume capability. In the case of the Quadras it is 60 μ L.

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In working with 0.5 μ L aliquots, aspirating is not the problem. Mother Nature will fill the vacuum created by the displaced piston. Dispensing is the problem. Surface tension and its' associated capillary action, is the culprit of precision. Since sufficient back pressure cannot be exerted to eject the aliquot, it must be touched off to a surface. That plate surface should be more hydrophilic than the tip surface to attract the drop from the tip surface. The common material for microplates is polypropylene, Disposable tips are also polypropylene making two similar surfaces. Thus, dispensing from a polypropylene tip to a polypropylene plate, provides no difference in surface tension attraction. The dispensed droplet can adhere equally well to the tip, as to the desired plate surface.

The Quadra small volume pipettors use a stainless steel tip that is coated both inside and outside with Teflon[®]. Teflon[®] is far more hydrophobic than polypropylene and polystyrene, creating less surface tension attraction to the tip. However, testing has also shown that both Teflon[®] and polypropylene exhibit less hydrophobicity to DMSO than to water.

A testing program was undertaken to evaluate aspirating and dispensing 0.5 μ L aliquots of 100% DMSO. A 0.1% solution (by weight) of Methyl Orange was made in DMSO. A Quadra model 230 with a 384 well small volume head was used for the pipetting. The Quadra program sets the stage height in stepper motor steps. One step is equivalent to a 0.002" change in height.

Initially, the testing was done by aspirating 0.5 μ L of the DMSO and dispensing it on the lid of a microplate. This facilitated wiping the lid dry between tests. At a stage setting of 840, the tips were barely touching the lid. The dispense missed 20-30% of the drops. They remained on the tips. At a setting of 850, we obtained 100% touch off of all 384 wells. The lid was lightly pinned by the tips (i.e. no lid movement). At a setting of 860, steps we missed 3 wells. Further testing, and with different parameters, verified that we could obtain 100% touch off of all 384 wells with a stage setting between 850 and 855. That is a distance range of 0.010". That thickness is equivalent to two sheets of copy paper. We successfully ran 50 plates at this setting, testing other variables. On all 50 plates we had 100% touch off of all 384 wells. It should be noted these stage settings were for this particular machine with the same manufacturers' plates. A different Quadra with different plates may require a different stage number to be set. But, we would expect to find a 0.010" tolerance that would duplicate these results. The 0.010" tolerance should also compensate for molding differences between plates from the same manufacturer. Greiners' 384 well plates were used but we would expect the same results with other manufacturers, such as CoStar[®] or NUNC[®].

For convenience, the above testing was initiated by dispensing on the lid. Comparable heights were then reset to dispense on the bottom of the plate. The same 0.010" tolerance was verified. Having established the ability to achieve 100% dispensing, other parameters were evaluated. The Quadra can dispense at three speed settings. Testing showed no difference in dispense speed using 100% touch off as the criteria of success.

The next parameter was blow out air. We were able to obtain 100% touch off using no blow out air. 0.5 μ L was aspirated and 0.5 μ L was dispensed. 2 μ L of blow out air was then evaluated. The test sequence was to aspirate 2 μ L of air then 0.5 μ L of DMSO. At touch off in the plate, a total of 2.5 μ L was dispensed blowing out the 0.5 μ L of DMSO. Using the criteria of 100% spots, both with and without blow out air, gave the same results - 100% transfer of DMSO.

The spots were larger with the blow out, which was attributed to an air bubble. To test the actual volume that was dispensed, 25 μ L of DI water was added to the wells as a secondary operation. The test procedure was to aspirate 0.5 μ L of DMSO dye solution with and without blow out air as the only variable. This was dispensed into a dry plate. The tips were washed in an ultrasonic tip washing station. 25 μ L of deionized water was aspirated, from a reservoir, and dispensed into the plate. It was mixed by three aspirate/dispense cycles. The plate was read bichromatically (492/620nm) on an SLT Image Reader. The results were as follows:

<u>0.5µL Dye + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
1	14.90	921
2	14.30	449
3	17.50	597

<u>0.5µL Dye with 2.0µL blow out air + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
1	7.01	1159
2	8.24	957
3	8.22	947

The 2.0µL of blow out air gave better CV and also a more consistent average optical density. Based on these positive results the blow out air was increased to 5.0µL.

<u>0.5µL Dye with 5.0µL blow out air + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
1	7.66	1197

One well was obviously lighter in density by eye, in addition to the OD reading. There appeared to be a tiny drop of dye on the sidewall of that well above the liquid level. The test was repeated.

<u>0.5µL Dye with 2.0µL blow out air + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
2	8.40	999

There was one well with no dye. Eliminating that from the data gave a CV = 6.7 on the rest of the plate. Based on these results it was concluded that an excessive amount of blow out air is not recommended. It may cause splashing of the drop and subsequent loss of precision. The use of 2.0µL of blow out air gave the best results.

Using the test parameters established with the above testing (i.e. stage height and blow out air), the next test looked at 1.0µL and 2.0µL dispenses instead of 0.5µL. The same CV test protocol was used.

<u>1.0µL Dye with 2.0µL blow out air + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
1	4.31	497
2	5.22	491
3	5.78	553

<u>2.0µL Dye with 2.0µL blow out air + 25µL H2O</u>	<u>CV</u>	<u>Mean O.D.</u>
1	2.84	1168
2	2.67	1171
3	2.60	1007

Conclusions

Careful setting of the relationship of the tips to the plate surface can provide 100% touch off of small volume samples from 0.5µL to 2.0µL and larger, in a dry plate. The use of 2µL of blow out air will improve the CV. The speed of dispense was not a contributing factor. There was no observed difference between speed 1 (slow) and speed 3 (fast).

Summary of Results

Using Tomtec's small volume Quadras in either the 96 or 384 well format can provide the following expected results.

- Dispensing 0.5µL of DMSO and washing it out with 25µL of buffer will provide CV of 2-3%.
- Dispensing 0.5µL of DMSO dry into a well with no blow out air will provide CV of 15-17%.
- Dispensing 0.5µL of DMSO dry into a well with 2.0µL blow out air will provide CV of 7-8%.
- Dispensing 1.0µL of DMSO dry into a well with 2.0µL blow out air will provide CV of 4-6%.
- Dispensing 2.0µL of DMSO dry into a well with 2.0µL blow out air will provide CV of 2-3%.