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# Optimizing Low Volume Transfers Using Automated Liquid Handling Instruments

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**Key Words:**

- Low Volume Dispensing
- Dispense Optimization
- Liquid Transfer Technique
- PlateMate
- % CV Testing
- % Error Testing
- Buffer
- DMSO
- Glycerin

## Abstract

Liquid handling operations, regardless of throughput, demand precision and accuracy to minimize experimental variation. Broadly, most applications include handling chemical buffers with varying properties and/or biological macromolecules. Modifying several minor parameters in automated liquid handling procedures can result in better performance and improved downstream results. In this report we describe several liquid handling parameters that can be improved to enhance performance. We present the difference between non-optimized and optimized conditions. Our testing with four commonly used reagents indicates the recommended optimized conditions. Users can choose suitable parameters for appropriate reagents and introduce features in the software to achieve better performance for their respective liquid handling procedures.

## Introduction

Automating and miniaturizing biological experiments is the primary approach to economical nanoscale experimentation. In general, two independent variables govern the success of automated experiments:

1. Performance of the automation platform.
2. Inherent experimental variables.

The first is easier to universally control and optimized parameters can be used for desired applications. The second is more specific and can vary with every experimental process. The success of an experiment depends on achieving consistency and reproducibility by minimizing variation that can occur. In this report we describe various parameters which can be modified with relevance to Thermo Scientific PlateMate automated liquid handling platforms. The PlateMate instruments are controlled via the Thermo Scientific ControlMate software interface which offers several features to efficiently control the instrument. Optimization of these features will help achieve better liquid handling results.


**Figure 1A:** **384- Channel, 0.1-50 µl Positive Displacement Pipetting Head**

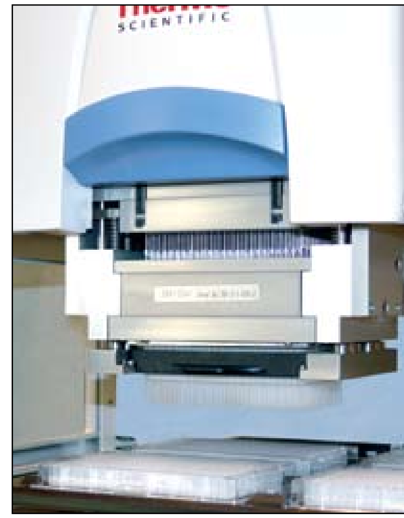


**Figure 1B:** **384- Channel, 0.5-30 µl Air Displacement Pipetting Head**

In addition to optimizing the operational parameters, the type of dispense mechanism also plays an important role in liquid handling. Two pipetting mechanisms are generally used on automated liquid handling platforms; positive displacement (syringe based) and air displacement (tip based). The compatibility of these liquid handling mechanisms varies with experimental requirements. Positive displacement pipetting can result in greater accuracy when used with non-aqueous liquids and solutions at volumes below 0.5µl. In contrast air displacement pipetting is compatible with most aqueous solutions at volumes above 0.5µl and offers disposable tips which eliminate risk of liquid carryover and cross contamination.

The choice to utilize either pipetting mechanism should be considered in conjunction with the desired transfer volume range, throughput, and other application requirements. Two pipetting heads with positive displacement and air displacement dispensing mechanisms are shown in Fig 1. The parameters that can be optimized as presented in this study however hold true for either mechanism.

## Methods

All dispenses were performed on a PlateMate 2x3 or PlateMate Plus using 384-channel air and positive displacement pipetting heads. Dry microplate dispensing was performed in all testing. 200 nl (0.2µl) was dispensed with the positive displacement head and 500 nl (0.5µl) was dispensed with the air displacement pipetting head. The dispense reagents were fortified with 10% dye to obtain a colorimetric reading. 50 µl of water was added to the dry dispense microplates and the absorbance was recorded at 412 nm after 1 minute orbital shaking and 1 minute 1750 rpm centrifugation.

## Materials

1. Yellow Dye
2. Acros Glycerin, 99% (Cat. No. 15892-0010)
3. Fisher DMSO (Cat. No. BP231-4)
4. Mallinckrodt Potassium Chloride (Cat. No. 6858)
5. Thermo Scientific Matrix PlateMate 2x3(Cat. No. 801-10005)
6. Thermo Scientific Matrix PlateMatePlus (Cat. No. 501-10001)
7. Thermo Scientific Matrix D.A.R.T.s (Cat. No. 5316)
8. Thermo Scientific Matrix 384- well plates (Cat. No. 4310)
9. Thermo Scientific Matrix WellMate (Cat. No. 201-10001)
10. Microplate Reader
11. Titer Plate shaker

## Results

### I. Optimization is needed to achieve the best performance:

In order to understand the effect of optimization, we compared data obtained before and after optimization. Non-optimized data was obtained using randomly set parameters for all ControlMate software commands. Optimized data was obtained by introducing the optimum parameters based on empirical knowledge with automated liquid handling. Furthermore, the parameters were adjusted according to the reagent under study. Significant improvement was observed after optimization (Table I).

| Positive Displacement (PD) | Randomized %CV | % Error | Optimized %CV | % Error |
|---|---|---|---|---|
| Buffer A | 105.8% | -110.0% | 5.4% | 5.6% |
| DMSO | 24.1% | 136.8% | 7.6% | 37.6% |
| Dye | 1.0% | 188.2% | 9.1% | 5.3% |
| Glycerin | 8.9% | -115.4% | 5.3% | 12.4% |

| Air Displacement (AD) | Randomized %CV | % Error | Optimized %CV | % Error |
|---|---|---|---|---|
| Buffer A | 27.4% | 190.5% | 9.2% | 6.9% |
| DMSO | 5.0% | 127.9% | 7.2% | 10.0% |
| Dye | 3.4% | 182.9% | 4.3% | -0.1% |
| Glycerin | 18.6% | -18.0% | 13.9% | 1.9% |

**Table I: Randomized vs. Optimized Dispense Conditions.**
Demonstrates the effect on precision, as measured by %CVs and accuracy as measured by % Error rates of randomized processes and optimized processes for the Positive Displacement (PD) and Air Displacement (AD) pipetting heads. The PD tests were obtained by pipetting 200 nl, while the AD tests used 500 nl.

### II. Parameters that Can be Optimized on the Automated Liquid Handling Platform:

The following are the different parameters that can be optimized on automated liquid handling platforms. The following parameters are discussed with relevance to PlateMate automated liquid handling platforms and ControlMate software interface.

## Terms and Definitions

1. **Air Gap:** Volume of air aspirated before any reagents. An air gap combined with a blow out will allow complete dispense of the liquid into the destination plate. Compare this to dispensing to a purge point using a manual pipetting technique.
2. **Blow out:** Blow out is the command used to move the pipetting head pistons past the “zero volume” dispense point, pushing as small amount of air after the liquid. This command in conjunction with the air gap will aid in pushing any remaining liquid in or on the outer orifice of the tip or needle into the destination labware to completely dispense of the liquid.
3. **Aspiration and Dispense Speeds:** The speed of aspiration and dispense will affect liquid handling results. In general, thick, viscous liquids require slower aspiration and dispense. The common occurrence of wicking (liquid adhering to the side of the tip or needle after dispense), hanging droplets (liquid not fully dispensing from the tip or needle), or full dispense of viscous liquids can be achieved by slowing the aspirate and/ or dispense speeds.
4. **Dwell Times:** Dwell time is the amount of time the tips or needles remain in the aspirate or dispense location after moving liquid. This command allows time forpressure to equalize in all 384 pistons and allows viscous liquids to completely aspirate or dispense.
5. **Neat vs. Incremental Dispense:** Neat dispense is the process of aspirating from a source and dispensing directly to the destination labware. The incremental pre-dispense cycle involves aspiration with a dispense prior to dispensing the desired volume in the destination labware. In this experiment a neat dispense was completed as listed here, while an incremental dispense included aspirating 3 µl additional reagent followed by three 1 µl pre-dispenses of reagent back into the reservoir.
6. **Overstroke:** An overstroke includes the aspiration of the reagent and dispense back to souce labware. This is routinely used in incremental dispensing technique.
7. **Tip Heights:** Tip height and placement in the labware well is an important factor in achieving optimal automated liquid handling performance.
8. **Tip Touch:** Tip touch is the “touch off” on the side wall or bottom of a microplate well that removes droplets adhering to the pipetting head tip or needle after an aspirate or dispense. This command allows droplets to fall into the well rather than be carried away with the tips or needles.
9. **Offset Move Command:** This ‘Move’ command allows X and Y stage alignment and positioning within labware wells for aspirate and dispense procedures.
10. **Volume Correction:** The ability to adjust pipetting head piston movements and timing for viscosity and specific gravity of solutions used in liquid handling aspirate and dispense procedures.

| | Buffer | Air Gap | Asp/Disp Speed | Blowout | Dwell Time | Neat/Incremental | Overstroke | Pos/Air displace | Tip Heights | Tip Touch | Offset | Volume Correction | Volume Desired | CV | Error |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Positive Displacement | DMSO | 0.1 µl | Fast | Air gap | -- | Inc | 5 µl | PD | Dispense | Bottom | Bottom Left | 0.694 | 200 nl | 4.2% | 6.9% |
| | Dye | -- | Fast | -- | 5 sec | Inc | 5 µl | PD | Dispense | Bottom | -- | 0.901 | 200 nl | 9.1% | 5.3% |
| | Buffer A | -- | Fast | -- | 3 sec | Inc | 5 µl | PD | Dispense | Bottom | -- | 1.205 | 200 nl | 5.4% | 5.6% |
| | Glycerin | -- | Slow | -- | 5 sec | Neat | 5 µl | PD | Aspirate | Bottom | -- | 1.736 | 200 nl | 8.3% | 6.1% |
| Air Displacement | DMSO | 2 µl | Fast | Air gap | 3 sec | Neat | -- | Air | Bottom | -- | -- | 0.73 | 500 nl | 7.2% | 10.0% |
| | Dye | -- | Fast | -- | 3 sec | neat | -- | Air | Aspirate | Bottom | Top Right | 0.833 | 500 nl | 4.3% | -0.1% |
| | Buffer A | 1 µl | Fast | Air Gap | 3 sec | Neat | 5 µl | Air | Aspirate | Bottom | -- | 0.17 | 500 nl | 9.2% | 6.9% |
| | Glycerin | -- | Fast | -- | 3 sec | Neat | 5 µl | Air | Aspirate | Bottom | Top Right | 1.205 | 500 nl | 13.9% | 1.9% |

**Table II: Optimization Parameters for the Four Reagent Types.**

The conditions that can be optimized using ControlMate for a simple dispense have been adjusted to the parameters shown here. The desired volume and the %CV for the conditions listed are also reported.

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| | | Randomized % CV | Randomized % Error | Minimally Optimized %CV | Minimally Optimized % Error | Optimized %CV | Optimized % Error |
|---|---|---|---|---|---|---|---|
| Positive Displacement | DMSO | 22.3% | -57.6% | 17.7% | 23.2% | 4.2% | 6.9% |
| | Dye | 4.3% | 24.0% | 9.2% | 12.4% | 9.1% | 5.3% |
| | Buffer A | 11.9% | -66.1% | 13.2% | -24.3% | 5.4% | 5.6% |
| | Glycerin | 7.0% | 466.6% | 9.2% | -24.2% | 8.3% | 6.1% |
| Air Displacement | DMSO | 15.2% | 13.7% | 12.8% | 18.3% | 7.2% | 10.0% |
| | Dye | 7.1% | 101.2% | 11.3% | 21.9% | 4.3% | -0.1% |
| | Buffer A | 5.1% | 124.8% | 8.3% | 25.6% | 9.2% | 6.9% |
| | Glycerin | 12.0% | 82.2% | 16.7% | -14.5% | 13.9% | 1.9% |

**Table III: Mid Error**

Additional measurements were taken comparing randomized %CV and % Error to minimally optimized and optimized procedures.

### III. Performance with Four Reagent Types:

Experiments were performed using four reagents with various liquid properties. The four reagents used exhibit different physical and chemical properties.

1. **90% Dimethyl sulfoxide (DMSO) with dye:** DMSO is commonly used in compound screening experiments.
2. **10mM Tris·HCl, 1.5mM MgCl2 (Buffer A) with dye:** This buffer will serve as a model as it is commonly used in PCR reactions and has similar physical properties of viscosity and pH.
3. **20% Glycerin with dye:** Glycerin is a common viscous liquid that stands as an example for this class of solutions. The glycerin solution was prepared with 70% deionized water.
4. **Water with dye:** A standard yellow dye is used to measure the pipetting performance in each case.

The optimized performance of the above reagents using the air displacement and positive displacement pipetting heads is shown in Table II. Table II compares non-optimized, minimally optimized, and optimized conditions to show the improvement in precision and accuracy performance that can be achieved with minimal adjustments to the above listed parameters included in the above "Terms and Conditions".

### IV. Consistency in performance:

One major advantage of using automated liquid handling platforms is the consistency with which results can be obtained after several cycles. In order to address experimental variation, resulting from either the automated liquid handling system or in downstream processes, we performed the experiment with fifty repetitions using dye solutions. Periodic recordings were obtained and comparable results were observed for dispenses 1 through 3 and 48 through 50, indicating consistent performance that can be achieved using automated liquid handling equipment.

### Conclusion:

Optimization of aspirate and dispense parameters in automated liquid handling can enhance performance significantly. We discuss several parameters in the instrument programming interface that can be optimized to achieve best results. Optimized programs for four liquid types with diverse physical and chemical properties have been generated to serve as a guideline for other similar liquids.

| | CVs | Avg for cycles 1-3 | Avg for cycles 48-50 | Difference |
|---|---|---|---|---|
| 0.5 µl | Wet | 7.6% | 8.2% | 0.7% |
| 0.5 µl | Dry | 9.5% | 12.3% | 2.8% |
| 1 µl | Wet | 1.7% | 1.9% | 0.1% |
| 1 µl | Dry | 1.9% | 2.0% | 0.1% |
| 5 µl | Wet | 1.5% | 1.0% | 0.5% |
| 5 µl | Dry | 0.9% | 0.8% | 0.1% |
| 10 µl | Wet | 0.9% | 0.8% | 0.1% |
| 10 µl | Dry | 0.7% | 0.7% | 0.1% |

**Table IV: CVs on PlateMate Plus automated platform.**

Showing the % CVs of 384-well microplates using yellow dye at 0.5 µl, 1.0 µl, 5.0 µl, and 10.0 µl. The conditions refer to the mode of dispensing to a wet or dry microplate well. "Wet" indicates 50 µl of water before in microplate well prior to dispense and "Dry" indicates no volume in microplate well prior to dispense.

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