Technical Note

INTEGRA

Measuring Dead Volume in INTEGRA's Multichannel Reagent Reservoirs

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Abstract

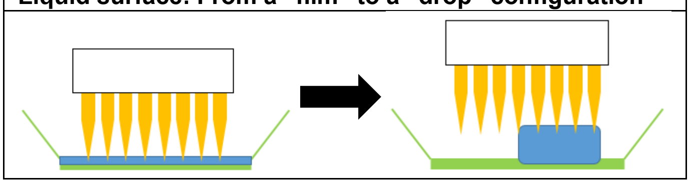
In the framework of miniaturization and high throughput processing, required volumes of reagents volumes for lab assays decrease while their value increases. This is particularly true in reference to expensive Master Mix, enzymes or self-expressed antibodies and other new compounds developed over many hours of work. Under these circumstances, any loss of reagent due to high dead volume is unacceptable. However, this is a reality that many researchers face on a daily basis in the lab when transferring a reagent from a reservoir into a multi-well plate using a multichannel pipette. Most reagent reservoirs feature a V-shaped bottom to minimize dead volumes. However, when the liquid level lowers, the liquid starts to pool unevenly and as soon as a single pipette tip aspirates air, the rest of the reagent cannot be used for the defined application: this is defined as **dead volume**.
To overcome this issue, INTEGRA developed a new 25 ml divided reservoir combining two novel innovations: the SureFlo™ anti-sealing array and its unique surface treatment. Enabling the pipette tips to sit on the bottom and preventing liquids from pooling, INTEGRA proposes a two-compartment reservoir offering the lowest dead volume on the market.
The aim of this Technical Note is to demonstrate this statement based on experimental data, but more importantly to provide a guidance to support users in setting up their experiments.

The Problem - Dead Volume

In the context of multichannel pipetting; dead volume refers to the volume of liquid that cannot be used for an application without risking aspirating air in the pipette tips. The dead volume depends on several factors: material and geometry of the container, nature and initial volume of the liquid, number of pipette channels and the tip spacing, pipetting techniques and atmospheric conditions, such as air temperature and humidity that impacts surface tension for instance. Large reagent dead volume is a serious issue in laboratories, especially when pipetting precious liquids - such as Master Mix, enzymes or antibody solutions into well plates using a multichannel pipette.
Traditional reservoirs have a V-shaped bottom to minimize dead volumes. However, as the liquid level lowers, the liquid pulls apart and starts to pool. As a result one or several tips on a multichannel pipette may aspirate air (Scheme 1). The remaining liquid in the reservoir and the pipette tips is then considered **dead volume**.

When setting up an experiment, a surplus of reagent in the reservoir is therefore needed to ensure adequate liquid to transfer. In order not to lose a large amount of precious reagents, the use of optimal reservoirs is a considerable asset for users.

Liquid surface: From a "film" to a "drop" configuration



Scheme 1: *Illustration of the tendency of a liquid to regroup and form a large drop instead of nicely spreading out in the deep trough all over its length when the liquid level lowers*.

INTEGRA's Multichannel Reagent Reservoirs

INTEGRA multichannel reagent reservoirs consist of disposable inserts that fit into reusable bases with clearly visible volume markings. Inserts are available as crystal clear polystyrene reservoirs or polypropylene reservoirs for improved chemical compatibility. For maximum fluid recovery and minimal waste, INTEGRA reagent reservoirs feature a full length, extra deep trough that is more easily accessible for pipette tips.
To further reduce dead volumes in reservoirs, INTEGRA developed new 25 ml divided multichannel reagent reservoirs with a SureFlo™ anti-sealing array on the bottom and a unique surface treatment.

The anti-sealing pattern consists of a series of small channels that allow the liquid to flow evenly across the bottom of the reservoir and prevent pipette tips from sealing. Pooling of liquid is prevented by a specially formulated, hydrophilic surface treatment. Additionally, the reservoir is divided, offering 5 and 10 ml volume compartments side by side, resulting in a reservoir with the lowest possible dead volume.
INTEGRA's divided reservoirs are designed to be compatible with all VIAFLO fixed spacing and VOYAGER adjustable tip spacing multichannel pipettes.

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Performance of INTEGRA's Reagent Reservoirs

The main purpose of this Technical Note is to support users in setting up their experiments while helping them to maximize their precious reagents. We measured the dead volumes in each INTEGRA multichannel reagent reservoir using the same test method in order to compare results in a correct manner.

As water is strongly influenced by environmental factors in the reality of a non-controlled environment lab, i.e. air temperature and humidity, and in order to get closer to real lab conditions, we decided to use a foaming blocking buffer containing diverse salts and low surface tension liquids.

Results were obtained by differential weighing of the reagent reservoirs using an analytical balance in the controlled environment of a calibration laboratory.
Each measurement was repeated 10 times per reagent reservoir type to ensure result consistency.

For each reservoir size, we filled in the reservoir with an initial volume corresponding to 36 % of the nominal volume of the reservoir – number easily divisible by the 8- and 12-channels of a multichannel pipette.
Tips were pre-wetted three times to optimize pipetting performance and results.

Standard Multichannel Reagent Reservoirs

In a first series of tests we measured the dead volume of INTEGRA's standard polystyrene and new 25 ml polypropylene reservoirs using a blocking buffer composed of 0.1 % Triton X-100 and 0.05 % Tween 20 in a 1x TBS (0.05 M TRIS.HCl and 0.15 M NaCl in water ISO 3696:1995, grade 3).

The main part of the buffer was aspirated using a VIAFLO 8-channel 1250 µl pipette at speed 3. To determine the dead volume in the reservoir, we carefully aspirated the rest of the foaming buffer using a VOYAGER 12-channel 50 µl pipette at speed 1 to ensure that no air entered in the pipette tips. The tip spacing of the VOYAGER pipette was set up at 6.5 mm for the 10 ml reagent reservoirs and at 9 mm for the 25 and 100 ml reservoirs. Results of the experiments are presented in both Table 1 and Figure 1.

Reservoirs	Initial Volume (µl)	Dead Volume (µl)	% Dead Volume / Initial Volume
PS 10 ml	3 600	51 ±10	1.4 %
PS 25 ml	9 000	80 ±12	0.9 %
PP 25 ml	9 000	65 ±10	0.7 %
PS 100 ml	36 000	120 ±14	0.3 %

Table 1: *Measuring dead volumes with a blocking buffer in INTEGRA's polystyrene (PS) and polypropylene (PP) reservoirs: initial and dead volumes in µl and corresponding dead volume percentage of the initial volume.*

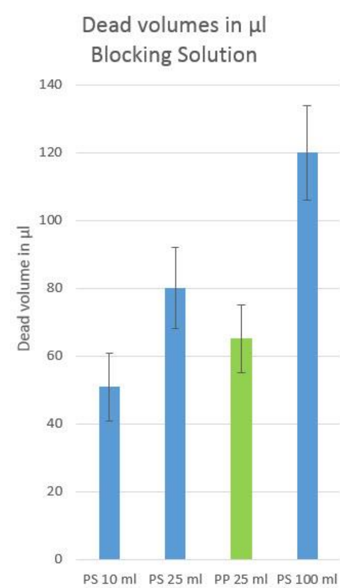

Figure 1: *Average and error bars of dead volumes (in µl) in INTEGRA's polystyrene (PS) and polypropylene (PP) reservoirs starting from an initial volume of 36 % of the nominal volume of the reservoirs.*

Reagent reservoirs of large volumes (25 and 100 ml) show excellent results in terms of low dead volume with less than 1 % of the initial volume. In case of 10 ml reservoirs, measured dead volume in experimental conditions is 51 ±10 µl starting from a volume of 3.6 ml.

To provide users with an ideal solution for small quantities of precious or rare reagents, INTEGRA designed new 25 ml divided reservoirs with a SureFlo™ anti-sealing array on the bottom, a unique surface treatment and two compartments of 5 and 10 ml.

Divided Reagent Reservoirs

In a second series of tests, we measured the dead volumes of new polystyrene and polypropylene 25 ml divided reservoirs in both 5 ml and 10 ml compartments.

Tests were performed in the same conditions that the ones previously described, using the blocking solution, but also water ISO 3696:1995, grade 3, a 10 % Tween 20 solution in grade 3 water and an 80 % isopropanol solution in grade 3 water. Aim of testing different solutions was to evaluate the influence of liquid on dead volumes and to provide some more detailed insights.

The main part of the liquid was aspirated with a VOYAGER 4-channel 300 µl at speed 5 for the 5 ml side and a VIAFLO 8-channel 1250 µl at speed 3 for the 10 ml side.
The remaining liquid was then carefully aspirated using a VOYAGER 8 channel 50 µl with tip spacing 4.5 mm for the 5 ml side and a VOYAGER 12-channel 50 µl pipette with tip spacing 6 mm for the 10 ml side, at speed 1 to avoid any air to enter in the tips.
Results of these tests are presented in Figure 2 and Table 2.

Divided Reagent Reservoirs	Initial Volume (µl)	Water (µl) Ratio (%)	Blocking solution (µl) Ratio (%)	10 % Tween 20 (µl) Ratio (%)	80 % iPrOH (µl) Ratio (%)
Div. PS 5 ml	1 800	6 ±3 0.3 %	9 ±2 0.5 %	12 ±1 0.7 %	14 ±1 0.8 %
Div. PP 5 ml	1 800	6 ±3 0.3 %	10 ±4 0.6 %	10 ±2 0.6 %	17 ±3 0.9 %
Div. PS 10 ml	3 600	27 ±6 0.8 %	27 ±5 0.8 %	27 ±5 0.8 %	38 ±6 1.1 %
Div. PP 10 ml	3 600	29 ±10 0.8 %	30 ±10 0.8 %	43 ±6 1.2 %	40 ±8 1.1 %

Table 2: *Dead volumes in µl and dead volume percentage of the initial volume in divided reservoirs for the 4 tested solutions.*

Results of this series of tests demonstrate the performance of the new 25 ml divided reagent reservoirs from INTEGRA. The new design of the 10 ml compartment allows reducing the dead volume of a foaming blocking solution from 51 ±10 µl to 27 ±5 µl while keeping the same working procedure.

For smaller quantities of the precious reagents, the 5 ml side of the divided reservoirs allows reaching dead volumes to around 10 µl, i.e. 0.5 % of the initial volume in the reservoir.

Polypropylene divided reagent reservoirs present similar performance while offering an improved chemical compatibility in comparison with polystyrene.

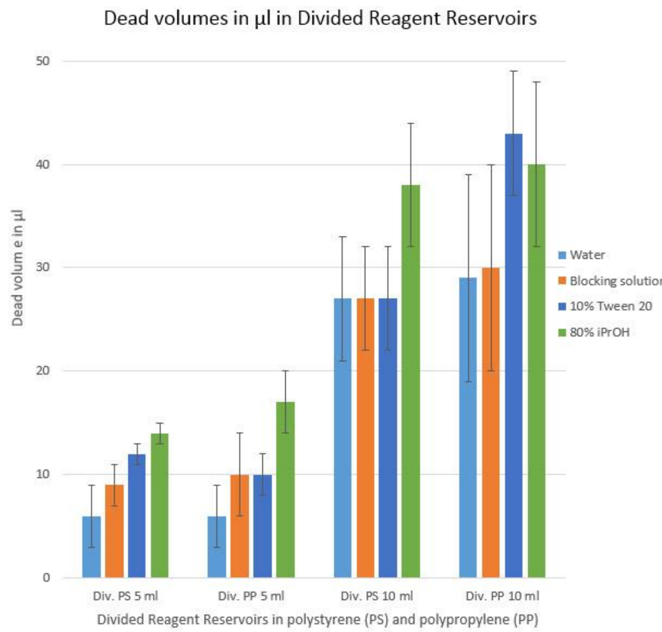

Figure 2: *Average and error bars of dead volumes (in µl) in INTEGRA's polystyrene (PS) and polypropylene (PP) divided reservoirs (Div.). Tested solutions: Water, foaming Blocking solution, 10 % Tween 20 and 80 % isopropanol (iPrOH).*

Conclusion

With multichannel pipetting and transfer of reagents into multi-well plates, dead volume is a real problem especially when handling expensive or rare solutions.
In this study, we measured dead volumes of INTEGRA's multichannel reagent reservoirs using as a reference a foaming blocking solution to reflect realistic laboratory experimental conditions while trying to provide a guide for scientists when setting up their protocols.
INTEGRA's 25 and 100 ml multichannel reagent reservoirs showed excellent results in terms of low dead volume with less than 1 % of the starting volume when pipetting the reference buffer.

To offer users an appropriate solution for small quantities of precious or rare reagents, INTEGRA rethought the concept of reagent reservoirs by including two innovations in its newly released 25 ml divided reservoirs. This led to highly reduced dead volumes when pipetting the foaming reference buffer, with an average of 0.5 % of the starting volume in the 5 ml compartment and 0.8 % of the initial volume in the 10 ml side.
Thus, by combining INTEGRA's multichannel reagent reservoirs to best pipetting practices and appropriate pipetting system in which tips and adjustable tip spacing pipettes perfectly fit together, scientists have at their disposal advanced tools to minimize their dead volume and save precious reagents.

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