Characterization of the FlexJet® with Multi-Array Dispense for Reduction of Chemistry Waste Due to Overage

ABSTRACT

The Nexar® System is a flexible and automated high throughput laboratory system for rapid analysis in miniaturized reaction volumes. Nexar includes the FlexJet non-contact dispense jet that provides high speed liquid handling for building reactions in Array Tape®. A set of experiments was conducted with FlexJet to analyze the volume of reagent waste used with multi-array dispense and to evaluate data quality. The study described here demonstrates the efficacy and economic advantages of using FlexJet with multi-array dispense. Results show the Nexar System with FlexJet's multi-array dispense feature can significantly reduce master mix consumed as dead volume.

INTRODUCTION

The Nexar System from Douglas Scientific® is an automated, inline solution that includes the Nexar liquid handling and assay processing system, Soellex® high-capacity thermal cycler, and Araya® fluorescence detector. The Nexar features FlexJet, a non-contact dispense jet that provides high speed liquid handling for applications in Array Tape. One challenge facing many laboratories is the total reagent cost, including the cost of waste incurred due to overage volume requirements. Amortizing the overage across multiple arrays versus a single array reduces total reagent costs. To address this issue, Douglas Scientific designed the FlexJet to provide multi-array aspiration and dispense of up to eight arrays of assay per tip.

A series of experiments was completed to analyze the volume of reagent waste that was used with the multi-array dispense and to evaluate data quality. Assay-specific reagents were prepared to assess dispensing performance of the FlexJet with multi-array dispense using the Artel MVS® Multichannel Verification System.

MATERIALS AND INSTRUMENTATION

Supplies and Sample Preparation: Analysis was completed using an Artel MVS, which included 384-well corning plates, Range C solution, diluent and baseline solution.

Corn Crude DNA Preparation: Post-harvest corn seeds were donated by local farmers and grain elevators in Central Minnesota and were provided without any genetic information. A sodium hydroxide method was used to prepare crude corn DNA samples. Individual corn seeds were pulverized using a mini bead beater and a dilute solution of sodium hydroxide was added to lyse the cells at 50 ^{0}C for 10 minutes. The samples were cooled and neutralized with Tris-HCl buffer, pH 7.8. After centrifugation, the supernatant was collected and diluted 1:50 in water before use.

Master Mix and Assays: Genotyping ToughMix®, Low ROX™ (Quanta Biosciences) with assays targeting the Lipid Protein Transfer (LTP) gene and the 35S promoter sequence was used to genotype all samples. BHQplus® SNP assays for the LTP and 35S assays were described previously (R. Alary, 2002) and were purchased from LGC Biosearch Technologies. Oligos were added at 2X concentration to the 2X master

mix to achieve a final concentration in the PCR reaction of 200 nM probes, 900 nM primers, and 1X master mix. The ROX™ labeled oligo calibration dye was obtained from LGC Biosearch Technologies.

The FlexJet multi-array dispensing process requires 60 µL of dead volume per aspiration, including the volume of the dispense nozzle and valve. The dead volume was maintained for both the eight-array and single-array dispense processes.

Instrumentation: The Nexar System, which includes the Nexar, Soellex and Araya as described in Figure 1, was used for this experiment. The PCR reactions contained 800 nL of sample dispensed with the multi-channel, 384-tip dispense pipette head from the CyBi® product line and 800 nL of 2X master mix containing 2X assay dispensed with the non-contact FlexJet to create 1.6 µL total volume reactions.

The FlexJet (8-tip) includes eight dispense channels each with its own pump, allowing aspiration and dispense of liquids with different viscosities. Parallel aspiration capability is also available for the FlexJet (4-tip) with four pumps and channels.

PCR amplification and thermal cycling were performed in the Soellex using the reagent manufacturer's instructions. An initial activation step of three minutes at 95 ^{0}C was followed by 45 cycles of 95 ^{0}C for 15 seconds and 60 ^{0}C for 60 seconds.

End-point fluorescence values were determined by scanning the Array Tape in the Araya. Cluster plot analysis was completed using the Intellics Software Suite.

MATERIALS

Acceptance Criteria: Acceptance criteria for Artel testing using Artel plates required FlexJet to meet or exceed 5% CV and inaccuracy for all Artel tests. In addition, the average volume shift from array to array had to be 5% or less. Throughout the testing, any dispensing variability within a single array or across multiple arrays were characterized. Any dispense variability whether the volumes and CVs meet the other acceptance criteria was documented.

Test Method:

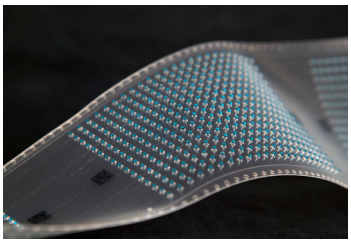
Dispense Performance - Using the Artel MVS and the aspiration formula listed in the equation below, a protocol was established to characterize the number of arrays that could be dispensed with a single aspiration.

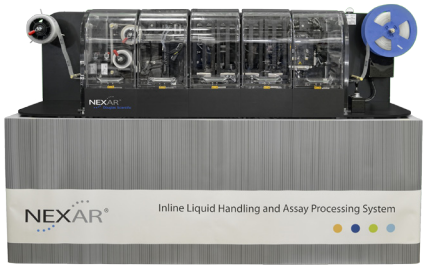
Equation 1: Maximum Arrays Dispensed (MAD) Value

MAD = target volume (nL) wells dispensed per array* arrays to dispense) +60 µL*
*= (800 * 384 * 8)+60 µL*
= 2,467,660 nL

First, dispense volumes of 800 nL were aspirated and dispensed across four Artel plates. The test was increased by one plate (up to eight plates total) as long as data passed acceptance criteria described above, while also staying under the 2.7 mL maximum aspiration volume.

Using the MAD value of 2,457,660 nL, the Artel test series was repeated three times to ensure repeatability. Then, using this same MAD value, testing was repeated with ROX calibration dye and also PCR end-point chemistry to verify and validate the Artel results.





ARRAY TAPE	NEXAR	SOELLEX	ARAYA
• Flexible microplate replacement	• Liquid handler optimized for Array Tape	• High capacity water bath PCR	• End-point fluorescence scanner
• Reduced reaction volumes	• 800 nL DNA, 384-channel dispense	• Optimized for Array Tape	• Optimized for Array Tape
• Total well volume of 2 µL	• 800 nL master mix, 384-well dispense in 48 seconds	• Three tanks for PCR optimization	• Scan 384-wells in 28 seconds
• Optically clear cover seal	• Seal Array Tape for thermal cycling	• Touchdown or traditional PCR	• Data ready for analysis in Intellics

Figure 1: Nexar System Overview

Next, a single channel 192-array run with calibration dye was performed to characterize the aspiration-to-aspiration variability and wash performance across a 384-well protocol using Array Tape. The no-dye test master mix and test master mix with ROX-labelled oligos were alternately dispensed into 96 arrays each. The alternating liquids were grouped into sets of arrays. Grouping began with a set of eight no-dye test master mix arrays, then a set of eight oligo-ROX arrays, with continuing alternation until 192 arrays was reached. Results were checked for cross contamination after washing as well as the pre-determined acceptance criteria. Finally, equal Artel, calibration dye, and chemistry dispense performance were verified through repeat testing with two additional channels on the FlexJet.

End-point PCR Chemistry Testing – End-point testing consisted of 40 arrays with dispense of a 50:1 dilution crude prep corn DNA using Array Tape. Test master mix and Genotyping ToughMix®, Low ROX™ with assays targeting the Lipid Protein Transfer (LTP) gene and the 35S promoter sequence were dispensed into alternating sets of eight arrays. Using one tip, the last set of PCR arrays included a 20-minute wait after aspiration to characterize any effects tip dehydration might have on dispensing. The chemistry run order was: eight live arrays, eight test master mix, eight live arrays, eight test master mix, and finally eight live arrays with a 20-minute wait.

RESULTS

Dispense Performance - Analysis compared the eight individual dispenses for each channel against each other, characterizing dispense performance from plate to plate for each channel. As shown in Figure 2 Artel summary chart, inaccuracies consistently were less than 2% from plate to plate, ranging from 0.98% to 1.02%. Confidence values also were consistently low, ranging from 0.7% to 2.5%.

End-point PCR Chemistry Testing - Using one tip, chemistry was run sequentially with eight arrays each of the following: PCR arrays, test master mix, PCR arrays, test master mix, and PCR arrays with a 20-minute wait. All PCR reactions in this experiment produced clusters that were easily scored in Intellics. Cluster plot images for the first array and the fortieth array are shown in Figure 3 and Figure 4 respectively.

The dispensed PCR chemistry worked well with no unexpected patterns observed. All data remained scoreable and cluster plots were consistent throughout the 40-array run.

8 Plate Artel Dispensing				
Channel	**AVG (nL)**	**AVG Inacc. %**	**STDEV**	**%CV**
1	808	1.01	5.9	0.7%
3	818	1.02	11.7	1.4%
7	781	0.98	19.5	2.5%

Figure 2: Dispensing Performance for FlexJet with multi-array dispense using Artel Verification System

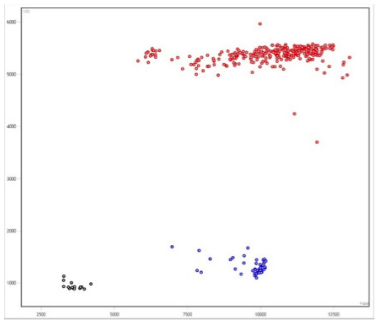


Figure 3: Cluster plot results for the first array of the 40-array run.

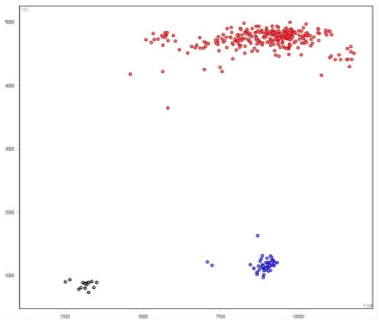


Figure 4. Cluster plot results for the last array of the 40-array run. The last eight array set also had a 20-minute wait time from aspiration to dispense, which had little effect on the end data.

Overage Savings - A single array dispense with the standard Dispense Jet requires a 60 µL dead volume usage to aspirate and dispense. With FlexJet multi-array dispense, one aspiration of 60 µL dead volume is amortized across as many as eight arrays. Therefore, the cost of unused reagent is significantly reduced. For example, running eight arrays using Dispense Jet single array dispense uses 480 µL in overage, whereas eight arrays with FlexJet multi-array dispense is only 60 µL in overage. This results in a reagent savings of 87.5% (or 52.5 µL per array) for every eight arrays analyzed as shown in Table 1.

	Dispense Jet single array aspiration	**FlexJet (8-Channel) eight array aspiration**
384-Well Arrays Dispensed Using Array Tape	8 Arrays (3,072 wells)	8 Arrays (3,072 wells)
Per Channel Dead Volume Per Aspiration	60 µL	60 µL
Total Volume of Reagent Used Per 8 Arrays	2917.6 µL	2517.6 µL
Total Volume of Overage Reagent Consumed/8 Arrays	480 µL	60 µL
Average Overage/Array	60 µL	7.5 µL
% Savings of Master Mix Required for Overage/8 Arrays	0%	87.5%

Table 1. Total Overage Volume

At 400 arrays per day, 260 days per year, this equals 5.46 L of master mix saved annually. See Figure 5 for FlexJet master mix savings with multiple-array dispense of eight arrays versus Dispense Jet single-array dispense.

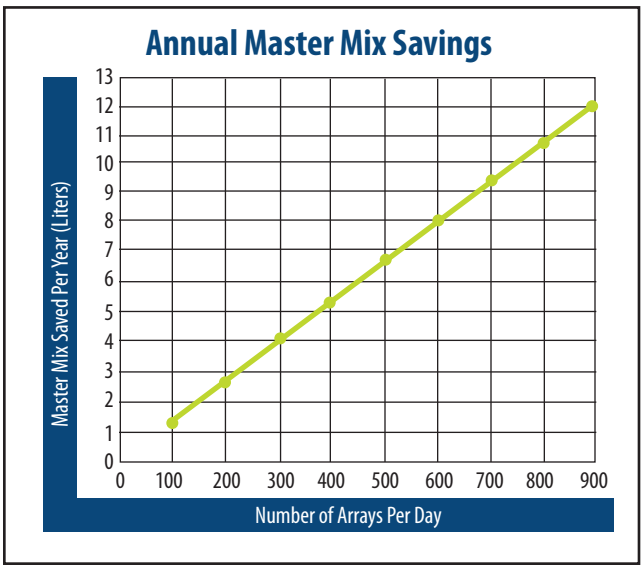


Figure 5. FlexJet master mix savings with multi-array dispense of eight arrays versus Dispense Jet single-array dispense

CONCLUSIONS

Test data supports multiple array dispensing of eight arrays with each aspiration, using FlexJet with multi-array dispense. There were no observable patterns or phenomena that negatively affected dispense performance.

This study demonstrates that the Nexar with the FlexJet multi-array dispense feature can reduce master mix consumed as dead volume by 87.5% compared to Dispense Jet single-array dispense. The capability to perform multi-array dispense with one aspiration also produces repeatable and accurate results and is an ideal solution for genotyping applications.

REFERENCES

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Alary R., Serin, A., Maury D., Jouira H.B., Sirven, J.P., Gautier M.F., Joudrier, P. Comparison of simplex and duplex real-time PCR for the quantification of GMO in maize and soybean. Food Control 2002; 13:235-244.

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