WHITE PAPER

Assessment of Art Robbins Instruments' (ARI) Scorpion device for applications pertaining to nucleic acid normalization

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ABSTRACT

Numerous laboratory processes and procedures demand meticulous and/or repetitive actions, leading to bottlenecks in production workflows. While increasing laboratory personnel may alleviate some of these tasks, it doesn't necessarily address the challenge of executing them accurately, as they often involve error-prone processes that could impact data quality. While automation holds promise in resolving some of these issues, many available automation instruments require skilled programmers to integrate protocols, thus adding to the financial burden on laboratories. In our pursuit of an optimal liquid handler, Avrok Biosciences assessed the performance of the Scorpion instrument from Art Robbins Instruments for nucleic acid normalization, with the aim of integrating it into our production workflow. The findings indicate that the Scorpion instrument exhibits a CV of under 5% for volumetric transfers and less than 5% for nucleic acid normalization, accompanied by a standard deviation of 0.11. These results underscore the instrument's reproducibility and minimal variability, establishing its suitability for a diverse range of applications in laboratory settings.

INTRODUCTION

While scientific research offers gratification, it frequently involves monotonous tasks that can diminish the enjoyment of the scientific process. This holds true in research and production settings, prompting the hiring of laboratory staff to alleviate bottlenecks. While increasing personnel may appear to be a solution, the repetitive nature of tasks can hinder performance, especially over prolonged periods. Although it resolves the personnel issue, precision remains uncertain. Addressing this challenge may require extended training periods, often lasting several months. Both solutions ultimately demand time and financial resources, impacting the overall efficiency of any laboratory.

At Avrok Biosciences, a contract research organization (CRO), we engage in a diverse array of services. Our expertise spans from Next Generation Sequencing, real-time PCR, ELISA assays, and mass spectrometry, biospecimen processing, all the way to biobanking. Given the multitude of assays and methodologies we employ, there exists a significant opportunity for the incorporation of automated processes. These range from simple tasks such as reagent plate preparation and aliquot generation to more intricate procedures like nucleic acid normalization or library pooling for sequencing. With our organization's high throughput demands, managing both research and clinical projects (under our CAP/CLIA accreditation), it became apparent that we required a dependable and reliable instrument to handle the intricate sample processing for our diverse clientele, including academic partners and large pharmaceutical industry collaborators.

When evaluating automation instruments, a myriad of questions must be taken into account. Does the instrument necessitate specialized personnel, such as proficient programmers and developers, for devising protocols and procedures? Are there substantial costs involved if I need to modify the protocol? Does the instrument come with specific handling instructions, requiring frequent calibrations and maintenance? Can the instrument

seamlessly integrate with our laboratory-specific protocols and utilize the plates/consumables we currently employ, or are we compelled to adopt the expensive consumables offered by the instrument manufacturer? As we weighed these considerations, we scrutinized a broad array of instrument manufacturers. While some concerns were easily addressed, we noticed that most instruments fell short in at least one of these categories. Engaged in the performance of chemistry and molecular biology assays, Avrok Biosciences sought an instrument that could go beyond routine aspiration and dispense tasks, and perform complex automation functions, without the hefty demands that come with such automation instruments. A crucial focus of the automation was to facilitate the normalization of nucleic acids for PCR and NGS applications, a process known for its time-consuming nature, causing significant bottlenecks in high-throughput settings. We discovered that the Scorpion instrument fulfilled many of the requirements we were seeking.

The Scorpion instrument, created by ARI, serves as an automated liquid handler designed to enhance laboratory processes through efficient benchtop pipetting. True to its name, the instrument features a single channel, mimicking the movement of a scorpion, traversing across six deck positions. These positions can accommodate various types of consumables, including 96 well plates, 384 well plates, 15 and 50 mL conical tube racks, tip racks (with diverse tip sizes of 50 µL, 200 µL, and 1,000 µL), and more. Operated through a dedicated Windows PC, the instrument boasts a notably compact footprint compared to its competitors. With dimensions measuring 19 inches x 19 inches x 27.5 inches (L x W x H) and a weight of 90 lbs, it can conveniently be placed on standard laboratory benchtops.

After conducting a trial of the instrument before finalizing the purchase, the installation process was straightforward and required no specialized tools. The Windows PC comes preloaded with the Scorpion Software, seamlessly connecting with the Scorpion Instrument. The software includes a comprehensive list of pre-loaded plates and racks from various consumable vendors. Art Robbins Instruments also offers the flexibility to add additional definitions upon consumer request, allowing them to send over precise product details. Utilizing precision tools, they accurately

Figure 1. Scorpion (ARI) Instrument Setup

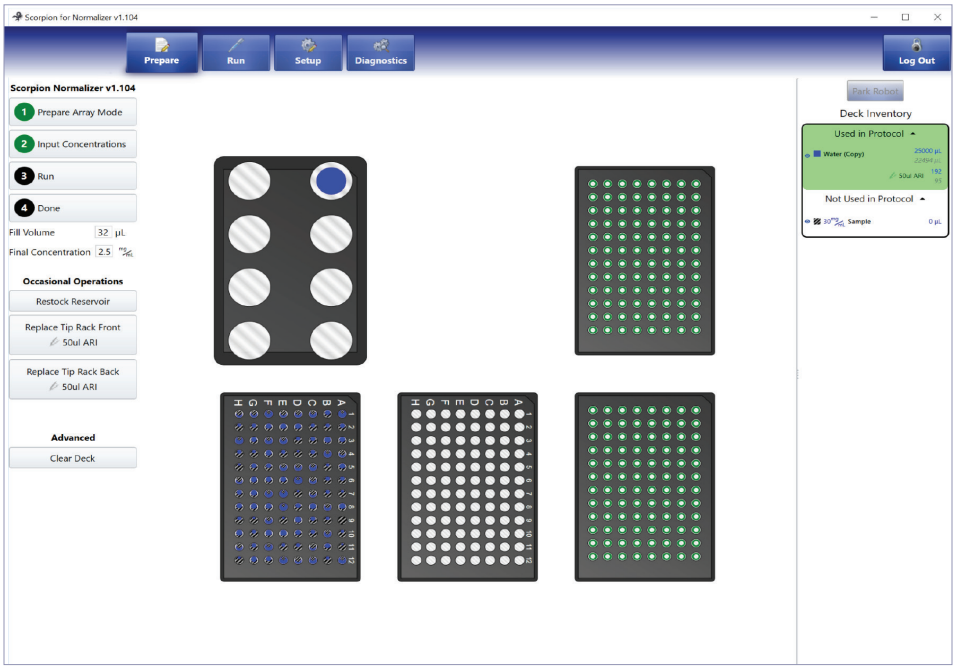

Figure 2. Scorpion (ARI) User Interface

define the consumables and provide the necessary design files for your specific requirements.

The instrument is furnished with a user interface that facilitates rapid protocol creation. Thanks to its straightforward design, it does not demand specialized individuals such as developers or programmers. Accompanied by a comprehensive user manual for the software, end users have all the necessary resources to construct protocols with ease. In cases where challenges surpass user capabilities, the instrument can be configured to enable remote access by ARI for swift issue resolution. Coupled with the fact that the instrument does not require any time consuming maintenance and frequent service, it proves to be superior compared to the competitor instruments.

The normalization feature of the instrument operates smoothly, necessitating the loading of a .CSV file onto the device. Users are required to specify the desired final volume and concentration, prompting the software to compute the necessary volumes of the sample and diluent. Once the consumables are set on the instrument and the run commences, the final output plate will generate samples at the precise working concentration needed for subsequent downstream applications.

All factors considered, as scientists, our trust lies in evidence-based claims. Therefore, our objective was to conduct a comprehensive assessment of the instrument's suitability for production settings. We evaluated its performance in volumetric transfers and rigorously tested its capacity for nucleic acid normalization.

MATERIALS AND METHODS

Diluent

The dilution solutions utilized in assessing the Scorpion Instrument included Molecular Grade Nuclease Free Water and Tris EDTA buffer.

Test Sample

The nucleic acids utilized in this research originated from cultured cells. DNA extraction from the cells was carried out using the KingFisher Flex instrument (ThermoFisher Scientific) with the MagMax DNA Multi-Sample Ultra 2.0 kit. Eluate fractions were consolidated into a single specimen vial for DNA concentration measurement. Quantification was performed using the VarioSkan LUX instrument (ThermoFisher Scientific) with the Quant-iT dsDNA High Sensitivity Kit (ThermoFisher Scientific), adhering to the manufacturer's provided instructions. For the experiment, three working concentrations (40 ng/µL, 10 ng/µL, and 5 ng/µL) were prepared by diluting the DNA sample in Molecular Grade Nuclease Free Water.

Volumetric Evaluation

The volumetric test involved utilizing Tris EDTA buffer as both the sample and diluent. A 96-well plate was prepared with 50 µL in each well, representing sample volumes obtained from the respective extraction method. This plate, termed the input plate, was positioned on the deck allocated for samples. Additionally, a 50 mL conical tube containing 25 mL of Tris EDTA buffer was placed on the deck designated for diluent. The Scorpion Instrument was assessed across various transfer volumes of sample and diluent. Table 1 below outlines the different ranges of sample and diluent volumes utilized in this assessment. A representative .CSV file was loaded onto the instrument, with concentrations entered to achieve the desired sample and diluent transfer volumes. The target volume in each well, post normalization was 32 µL. Each well volume was measured on the completion of the run, using a single channel pipettor. A total of three runs were conducted to assess performance.

Test Case	Sample Volume (µL)	Diluent Volume (µL)	No. of Test Wells
Condition 1	32	0	32
Condition 2	16	16	16
Condition 3	8	24	16
Condition 4	4	28	16
Condition 5	2	30	16

Table 1. Volumetric Test Conditions

Nucleic Acid Normalization

The assessment for normalization involved utilizing three distinct DNA concentrations: 40 ng/µL, 10 ng/µL, and 5 ng/µL. Each concentration, totaling 50 µL, was dispensed into one-third of a 96-well PCR plate, covering 32 wells. This distribution ensured that the different DNA concentrations were evenly spread across the entire plate. A representative .CSV file specifying the concentration ranges on the PCR plate was uploaded onto the instrument. The target concentration was configured to be 2.5 ng/µL with a final volume of 32 µL. After inputting the parameters, the instrument was initiated. Three runs were executed to evaluate performance.

RESULTS

Volumetric Evaluation

A. Run 1: As outlined in table 2 below, transfer volumes of sample and diluent performed as expected.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Volumes (µL)	A	32	32	32	32	32	32	32	32	32	32	32	32
	B	32	32	32	32	32	32	32	32	32	32	32	32
	C	32	32	32	32	32	32	32	32	32	32	32	32
	D	32	32	32	32	32	32	32	32	32	32	32	32
	E	32	32	32	32	32	32	32	32	32	32	32	32
	F	32	32	32	32	32	32	32	32	32	32	32	32
	G	32	32	32	32	32	32	32	32	32	32	32	32
	H	32	32	32	32	32	32	32	32	32	32	32	32
Diluent Transfer Volume (µL)		0				16		24		28		30	
Sample Transfer Volume (µL)		32				16		8		4		2	
Target Volume (µL)		32.00				32.00		32.00		32.00		32.00	
Mean		32.00				32.00		32.00		32.00		32.00	
Variance		0.00				0.00		0.00		0.00		0.00	
Standard Deviation		0.00				0.00		0.00		0.00		0.00	
Coefficient of Variation (%)		0.0%				0.0%		0.0%		0.0%		0.0%	
Deviation (%)		0.0%				0.0%		0.0%		0.0%		0.0%	

Table 2. Volumes transferred and calculations for the first run of Volumetric Evaluation

B. Run 2: As outlined in table 3 below, transfer volumes of sample and diluent performed as expected.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Volumes (µL)	A	32	32	32	32	32	32	32	32	32	32	32	32
	B	32	32	32	32	32	32	32	32	32	32	32	32
	C	32	32	32	32	32	32	32	32	32	32	32	32
	D	32	32	32	32	32	32	32	32	32	32	32	32
	E	32	32	32	32	32	32	32	32	32	32	32	32
	F	32	32	32	32	32	32	32	32	32	32	32	32
	G	32	32	32	32	32	32	32	32	32	32	32	32
	H	32	32	32	32	32	32	32	32	32	32	32	32
Diluent Transfer Volume (µL)		0				16		24		28		30	
Sample Transfer Volume (µL)		32				16		8		4		2	
Target Volume (µL)		32.00				32.00		32.00		32.00		32.00	
Mean		32.00				32.00		32.00		32.00		32.00	
Variance		0.00				0.00		0.00		0.00		0.00	
Standard Deviation		0.00				0.00		0.00		0.00		0.00	
Coefficient of Variation (%)		0.0%				0.0%		0.0%		0.0%		0.0%	
Deviation (%)		0.0%				0.0%		0.0%		0.0%		0.0%	

Table 3. Volumes transferred and calculations for the second run of Volumetric Evaluation

C. Run 3: The results are outlined in table 4 below. A total of 3 wells underperformed, resulting in final transfer volumes of 26 µL, 29 µL, and 30 µL.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Volumes (µL)	**A**	32	32	32	32	32	32	32	32	29	32	32	32
	B	32	32	32	32	32	32	32	32	30	32	32	32
	C	32	32	32	32	32	32	32	32	32	32	32	32
	D	32	32	32	32	32	32	32	32	32	32	32	32
	E	32	32	32	32	32	32	32	32	32	32	32	32
	F	32	32	32	32	32	32	32	32	32	32	32	32
	G	32	32	32	32	32	32	32	32	32	32	32	32
	H	26	32	32	32	32	32	32	32	32	32	32	32
Diluent Transfer Volume (µL)		0				16		24		28		30	
Sample Transfer Volume (µL)		32				16		8		4		2	
Target Volume (µL)		32.00				32.00		32.00		32.00		32.00	
Mean		31.81				32.00		32.00		31.69		32.00	
Variance		1.09				0.00		0.00		0.71		0.00	
Standard Deviation		1.04				0.00		0.00		0.85		0.00	
Coefficient of Variation (%)		3.3%				0.0%		0.0%		2.7%		0.0%	
Deviation (%)		-0.6%				0.0%		0.0%		-1.0%		0.0%	

Table 4. Volumes transferred and calculations for the third run of Volumetric Evaluation

Nucleic Acid Normalization

A. Run 1: Table 5 below details the concentrations attained for each well position. Among the 96 wells, findings from four wells exceeded a 10% variability from the intended target of 2.5 ng/µL.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Concentrations (ng/µL)	**A**	2.45	2.39	2.50	2.55	2.68	2.63	2.65	2.47	2.60	2.52	2.49	2.64
	B	2.50	2.52	2.42	2.43	2.65	2.65	2.74	2.54	2.78	2.45	2.42	2.41
	C	2.41	2.40	2.49	2.51	2.79	2.63	2.62	2.53	2.53	2.59	2.56	2.41
	D	2.47	2.44	2.56	2.43	2.62	2.67	2.67	2.53	2.62	2.59	2.59	2.49
	E	2.41	2.49	2.45	2.42	2.60	2.51	2.65	2.63	2.47	2.60	2.62	2.46
	F	1.94	2.36	2.31	2.53	2.57	2.55	3.05	2.60	2.54	2.68	2.59	2.59
	G	2.37	2.56	2.46	2.45	2.61	2.58	2.61	2.57	2.53	2.58	2.60	2.64
	H	2.40	2.43	2.41	2.43	2.60	2.51	2.48	2.58	2.46	2.54	2.60	2.64
Approx. Initial Concentration (ng/µL)		40				10				5			
Desired Final Concentration (ng/µL)		2.5				2.5				2.5			
Approx. Sample Transfer Volume (µL)		2				8				16			
Approx. Diluent Transfer Volume (µL)		30				24				16			
Mean Concentration (ng/µL)		2.43				2.62				2.56			
Standard Deviation		0.11				0.10				0.08			
Coefficient of Variation (%)		4.3%				4.0%				3.3%			

Table 5. Concentrations and calculations for the first run of Nucleic Acid Normalization

B. Run 2: Table 6 below details the concentrations attained for each well position. Among the 96 wells, findings from two wells exceeded a 10% variability from the intended target of 2.5 ng/µL.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Concentrations (ng/µL)	**A**	2.27	2.49	2.44	2.52	2.62	2.62	2.60	2.61	2.59	2.56	2.57	2.57
	B	2.42	2.40	2.41	2.45	2.63	2.61	2.59	2.54	2.61	2.56	2.60	2.57
	C	2.44	2.44	2.40	2.39	2.62	2.54	2.57	2.58	2.57	2.57	2.52	2.53
	D	2.44	2.36	2.40	2.40	2.57	2.56	2.60	2.57	2.53	2.57	2.53	2.55
	E	2.41	2.78	2.43	2.68	2.44	2.56	2.52	2.59	2.55	2.59	2.57	2.43
	F	2.41	2.40	2.29	2.70	2.56	2.57	2.61	2.52	2.47	2.57	2.48	2.43
	G	2.50	2.40	2.41	2.36	2.58	2.53	2.52	2.49	2.54	2.54	2.56	2.52
	H	2.29	2.41	2.40	2.46	2.53	2.55	2.66	2.56	2.44	2.21	2.46	2.47
Approx. Initial Concentration (ng/µL)		40				10				5			
Desired Final Concentration (ng/µL)		2.5				2.5				2.5			
Approx. Sample Transfer Volume (µL)		2				8				16			
Approx. Diluent Transfer Volume (µL)		30				24				16			
Mean Concentration (ng/µL)		2.44				2.57				2.53			
Standard Deviation		0.11				0.05				0.07			
Coefficient of Variation (%)		4.4%				1.8%				2.9%			

Table 6. Concentrations and calculations for the second run of Nucleic Acid Normalization

C. Run 3: Table 7 below details the concentrations attained for each well position. All of the 96 wells reported results that were within 10% variability from the intended target of 2.5 ng/µL.

		1	2	3	4	5	6	7	8	9	10	11	12
Final Well Concentrations (ng/µL)	**A**	2.32	2.36	2.29	2.49	2.63	2.69	2.66	2.60	2.56	2.54	2.51	2.53
	B	2.38	2.25	2.40	2.41	2.53	2.61	2.45	2.58	2.53	2.60	2.54	2.53
	C	2.30	2.42	2.35	2.37	2.55	2.66	2.57	2.58	2.46	2.51	2.56	2.43
	D	2.25	2.42	2.32	2.40	2.52	2.57	2.63	2.58	2.49	2.58	2.55	2.47
	E	2.30	2.35	2.35	2.49	2.53	2.54	2.55	2.58	2.61	2.46	2.56	2.44
	F	2.33	2.48	2.29	2.39	2.52	2.55	2.54	2.54	2.49	2.45	2.51	2.48
	G	2.31	2.38	2.28	2.38	2.44	2.55	2.50	2.53	2.47	2.48	2.49	2.45
	H	2.29	2.70	2.47	2.40	2.52	2.55	2.52	2.59	2.53	2.57	2.50	2.40
Approx. Initial Concentration (ng/µL)		40				10				5			
Desired Final Concentration (ng/µL)		2.5				2.5				2.5			
Approx. Sample Transfer Volume (µL)		2				8				16			
Approx. Diluent Transfer Volume (µL)		30				24				16			
Mean Concentration (ng/µL)		2.37				2.56				2.51			
Standard Deviation		0.09				0.05				0.05			
Coefficient of Variation (%)		3.7%				2.1%				2.0%			

Table 7. Concentrations and calculations for the third run of Nucleic Acid Normalization

DISCUSSION

We conducted a comprehensive evaluation of the Scorpion Instrument to assess its performance and suitability for use in production environments. Our volumetric evaluations revealed that two out of the three runs performed exceptionally well, accurately transferring a total volume of 32 µL, comprising both sample and diluent. However, in run three, we observed volumes of 26 µL, 29 µL, and 30 µL in three specific wells. The occurrence percentage, considering all three runs, was found to be 1.04%. This indicates that such deviations are infrequent. When examining these wells within the subset of samples corresponding to the same sample and diluent transfers, the overall population exhibited coefficients of variation (CV) of 3.3% and 2.7%, respectively.

Furthermore, while testing the nucleic acid normalization capabilities of the instrument, we observed that four specific wells in run one and two specific wells in run two resulted in values exceeding a variability of 10%. The occurrence percentage, encompassing all three runs, was found to be 2.08%. When evaluating concentrations of wells within a given population, we found the coefficient of variation to be less than 4.5%, with a standard deviation of less than or equal to 0.11.

While the obtained results fell within acceptable ranges, we endeavored to enhance our understanding and pinpoint the reasons for underperformance in three specific wells for volumetric transfers and six specific wells for nucleic acid normalization. Subsequent tests were conducted, involving adjustments such as reducing the aspiration and dispense rates of the instrument and modifying the reagent class to accommodate varying viscosities.

A significant improvement in instrument performance was identified through the implementation of an initialization run before actual usage. This initialization run involves using molecular-grade nuclease-free water as a diluent and molecular-grade nuclease-free water as samples. A 96-well plate, loaded with 50 µL of molecular-grade nuclease-free water in each well, is placed in the sample's position. The diluent tube is positioned accordingly. The instrument is initiated using a generic program, simulating the instrument's operation as it would with live samples. Afterward, the consumables and transfers are discarded, allowing the resumption of testing with actual samples. Following this test, the occurrence percentage was found to have decreased to well below 1%.

Assessing an automation instrument to meet specific laboratory requirements is often more complex than anticipated. Various factors necessitate consideration even prior to conducting evaluations, and it's crucial to acknowledge potential tradeoffs. These tradeoffs can be alleviated by incorporating quality controls within the assay to identify irregularities arising from nucleic acid normalization.

It's important to note that similar variabilities can arise in manual transfers, potentially leading to sample carryover. What may initially appear as a tradeoff may not actually be one. On average, nucleic acid normalization with the Scorpion Instrument requires approximately 20-25 minutes for a full 96-well plate. In contrast, manual execution demands the involvement of at least two laboratory technicians and about 45 minutes for the same plate.

The time and resources saved with automation readily offset the need for potential sample reruns due to quality control failures. Our experience over the past six months with the Scorpion Instrument has been free of quality control issues, indicating a low variability in the data obtained.

Considering that the instrument sustains a CV of under 4%, the volumetric transfers fall comfortably within acceptable thresholds. The nucleic acid normalization results reveal a coefficient of variation at or below 4.4% and a standard deviation under 0.11 across all sample populations. This signifies the Scorpion Instrument's high precision and low variability. Our findings strongly suggest that the instrument is well-suited for a wide array of applications in laboratory settings.