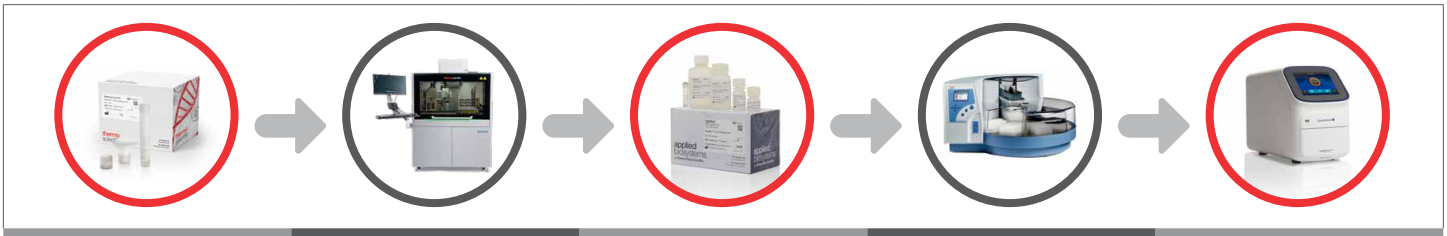
Clinical research

Full high-throughput workflow with the KingFisher SpeciTRAX Sample Transfer System

Introduction

High-throughput molecular biology research has pushed the limits of discovery in areas ranging from infectious disease investigation to genomic analysis and biobanking. Some of the most tedious and time-consuming tasks in a high-throughput laboratory workflow are related to sample preparation and processing. High quality and consistency in sample processing are two key elements for infectious disease and genomic workflows. Samples must be accessioned and stored, and collection devices must be carefully uncapped. The samples must be transferred to a sample processing workflow with accurate pipetting techniques, and sample-containing tubes must be re-capped safely to protect the operator from pathogen transmission. This portion of the sample processing workflow can be time-consuming and fatiguing for the operator, reducing accuracy and overall process quality.

In response to the need for greater sample processing capability, robotics and automation have streamlined efficiency and accuracy, providing laboratories an opportunity to reduce operator hands-on time. The use of barcode scanners can help with sample tracking, while liquid handling systems can improve pipetting consistency and accuracy. The Thermo Scientific™ KingFisher™ SpeciTRAX™ Sample Transfer System combines barcode scanning for sample accessioning, liquid handling for sample transfer, and uncapping and re-capping in one simple-to-use system that is fully enclosed. The KingFisher SpeciTRAX system also offers safety features like HEPA filtration and UV lights. When combined with the Thermo Scientific™ KingFisher™ Flex Purification System and a magnetic bead–based workflow like that of the Applied Biosystems™ MagMAX™ kit, the KingFisher SpeciTRAX system can help save time and money and reduce strain while improving accuracy and safety in sample transfer, whether you are a big lab looking to support staff shortages in a high-throughput environment or a small lab looking to improve quality and speed. Figure 1 details the proposed workflow from sample to answer with the KingFisher SpeciTRAX system.



Sample collection	Automated sample transfer	Viral nucleic acid isolation	Automated nucleic acid purification	Real-time PCR analysis
Thermo Scientific™ SpeciMAX™ saliva collection kits	KingFisher SpeciTRAX Sample Transfer System	Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	Thermo Scientific™ KingFisher™ Flex or Apex systems	Applied Biosystems™ QuantStudio™ real-time PCR systems

Figure 1. Full workflow with the KingFisher SpeciTRAX Sample Transfer System for infectious disease sample processing. The workflow includes sample collection, sample transfer on the KingFisher SpeciTRAX system, nucleic acid isolation and purification, and nucleic acid detection by real-time PCR.

In this application note, we explore the KingFisher SpeciTRAX system as part of a full workflow for infectious disease testing in a high-throughput laboratory that processes saliva and general samples in viral transport media. We analyze several aspects of this full workflow solution to determine the feasibility of using the KingFisher SpeciTRAX system in a high-throughput laboratory for infectious disease applications. Analyses included pipetting precision and accuracy, time savings, contamination risk from the sample transfer system, and the feasibility of extracting specimens obtained from humans using bead-based extraction technology in the MagMAX MVP II kit workflow with the KingFisher Flex Purification System.

Experimental overview

Full workflow

The MagMAX MVP II kit was used to prepare sample processing plates for 192 samples, which were then set aside. Previously frozen raw saliva was pooled and split into two equal portions. One portion was spiked with SARS-CoV-2 inactivated virus (from BEI Resources Repository) to a concentration of 100 copies per µL of stabilized saliva, while the other remained unspiked to represent a sample without SARS-CoV-2 virus. The spiked and unspiked saliva portions were each distributed in 1 mL aliquots into 48 unfilled tubes of the Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit, with 100,000 virus copies per tube for the spiked saliva. The samples were placed on the KingFisher SpeciTRAX system in a checkerboard pattern, alternating samples containing and not containing SARS-CoV-2 (Figure 2). A previously prepared sample processing plate with MagMAX MVP II reagents was placed on the SpeciTRAX system deck's Deep Well plate location, and the sash of the KingFisher SpeciTRAX system was closed.

The KingFisher SpeciTRAX system software was initiated to uncap the tubes containing the 96 contrived saliva specimens, transfer 200 µL from each specimen to the sample processing plate, and re-cap each tube. After the KingFisher SpeciTRAX system had transferred all 96 samples and the software program was complete, the 96 samples were processed as per instructions for a 200 µL saliva workflow with the MagMAX MVP II kit on the KingFisher Flex Purification System. The extracted eluates from the samples were sealed and stored on ice until further processing. The collection tubes containing the unspiked saliva specimens were retrieved from the KingFisher SpeciTRAX instrument, and an additional 200 µL sample was taken from each for manual processing using a 200 µL saliva workflow for the MagMAX MVP II kit on the KingFisher Flex Purification System. These saliva specimens were processed manually after the transfers on the KingFisher SpeciTRAX system, to determine if any cross-contamination between tubes occurred during the automated uncapping, sample transfer, and re-capping. Time was recorded for manual specimen processing and compared to the time recorded for processing on the KingFisher SpeciTRAX system. Extracted nucleic acids were analyzed using Applied Biosystems™ TaqMan™ chemistry and reagents to detect SARS-CoV-2 after the 200 µL workflows for automated and manual processing. The results were analyzed for consistent detection of the RNase P gene across the wells and amplification of SARS-CoV-2 targets.

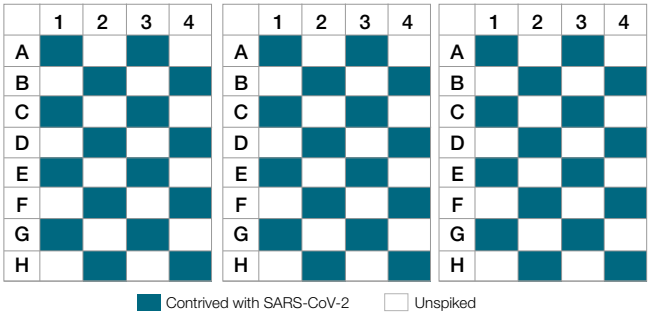


Figure 2. Layout of samples on the KingFisher SpeciTRAX system. The 96 saliva samples were alternated within the sample holder attachments on the SpeciTRAX system deck between those spiked with SARS-CoV-2 and unspiked.

Accuracy and precision of specimen transfer

Nuclease-free water was used to fill 33 empty tubes from the Thermo Scientific™ SpeciMAX™ Saliva Collection Kit, at 2.5 mL per tube. Weights were recorded for all 33 filled tubes. The KingFisher SpeciTRAX system was programmed to transfer 1,000 µL, 100 µL, and 10 µL volumes, with weights checked between each sample transfer event to assess pipetting accuracy.

Results and discussion

Full workflow

All 96 samples were successfully transferred by the KingFisher SpeciTRAX system to the MagMAX MVP II extraction sample plate in 200 µL volumes. Table 1 indicates the results of the post-extraction RT-qPCR using TaqMan chemistry on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System using a 384-well block. All 96 SpeciMAX saliva samples spiked with SARS-CoV-2 were detected with qPCR for all three targets. The unspiked saliva specimens did not result in amplification of the N gene or *orf1ab*, but they did result in amplification of the RNase P gene. Amplification of the RNase P target in each well indicated successful transfer of the stabilized saliva specimens from SpeciMAX Stabilized Saliva Collection Kit tubes to the MagMAX MVP II sample plate in 200 µL volumes on the KingFisher SpeciTRAX system. As none of the unspiked samples had amplification of the SARS-CoV-2 targets, it is concluded that there is minimal risk of cross-contamination from SARS-CoV-2 at 714 copies per tube on the KingFisher SpeciTRAX system when transferring samples from the tubes to the sample plate.

Table 1. Amplification of SARS-CoV-2 targets and the endogenous RNase P gene with TaqMan chemistry and workflow. Extracted contrived saliva specimens were transferred from tubes of the SpeciMAX Stabilized Saliva Collection Kit to a MagMAX MVP II kit sample plate on the KingFisher SpeciTRAX system.

Target detection	Samples transferred	N gene	*orf1ab*	RNase P gene
Detected	48	48	48	48
Not detected	48	0	0	48

After all 96 stabilized saliva specimens were transferred to the sample plate by the KingFisher SpeciTRAX system for extraction with the MagMAX MVP II kit workflow, the 48 unspiked specimens were removed from the KingFisher SpeciTRAX system. These specimens were then manually extracted with the MagMAX MVP II kit workflow at 200 µL input volumes on the same KingFisher Flex Purification System used previously. In these 48 saliva specimens, there was no amplification of the SARS-CoV-2 N gene or *orf1ab* targets. Because this workflow was performed after sample handling on the KingFisher SpeciTRAX system, the confirmation of unamplified SARS-CoV-2 genes indicates that there was no tube-to-tube contamination in sample transfer with the SpeciTRAX system workflow. The samples derived from the unspiked saliva and processed after automated transfer on the KingFisher SpeciTRAX system were compared to the samples derived from the same saliva but transferred manually, for detection of the RNase P gene (Table 2). Amplification of the RNase P gene in samples transferred with the KingFisher SpeciTRAX system had a higher mean C_q; also, the standard deviation (SD) and the coefficient of variation (CV) across 48 samples were significantly lower for the samples transferred on the automated system. C_q values of the manually transferred samples and those transferred on the KingFisher SpeciTRAX system were within 1 cycle of each other, an acceptable range for an endogenous control target. The lower standard deviation and CV seen with samples transferred on the KingFisher SpeciTRAX system indicate that the automated system provides increased consistency compared to the manual transfer method.

Calculations to estimate the theoretical time needed to transfer 192 samples included accessioning time, liquid transfer time, processing time, and hands-on time. The calculations were based on the 96 samples transferred on the KingFisher SpeciTRAX system and the 48 samples transferred manually. Table 3 shows theoretical estimates of the time needed to complete sample transfer with the KingFisher SpeciTRAX system and manual transfer by a human operator. The total processing time is estimated to be approximately 42 minutes for the KingFisher SpeciTRAX system, compared to 271 minutes for a human operator, to manually process all 192 samples. Time for completion may vary depending on extraction workflows and operator.

Table 3. Theoretical times for different time components of sample transfer from data processed with the KingFisher SpeciTRAX system and manual transfer methods. The estimates for the KingFisher SpeciTRAX system are based on an actual time record of transferring 96 samples. The estimates for the manual workflow are based on an actual time record of transferring 48 samples.

	Time for 192 samples (minutes)	
Time component	**KingFisher SpeciTRAX system**	**Manual**
Total setup	2	7
Accessioning	1	126
Liquid transfer	40	60
Total sample processing time	42	271
Direct hands-on time	1	132

Table 2. Variation between the KingFisher SpeciTRAX system and manual sample transfer. Stabilized unspiked saliva specimens were transferred to a sample plate prepared with MagMAX MVP II kit reagents and were extracted on the KingFisher Flex Purification System. The mean, standard deviation (SD), and coefficient of variation (CV) of the C_q values for amplification of the endogenous RNase P gene target across 48 samples are shown for each sample transfer method.

Target	Transfer method	C_q mean	C_q SD	C_q CV
RNase P gene	Manual	24.81	1.59	6.39
	KingFisher SpeciTRAX system	25.51	0.26	1.04

Accuracy and precision of specimen transfer

Process capability was measured for transfer of nuclease-free water to a sample processing plate at 1,000 µL, 100 µL, and 10 µL volumes. Figure 3A is a summary table of precision (%CV) and accuracy (%DEV) measured at each transfer volume across 33 samples. The upper and lower specification limits with 95% confidence were established to be ±20 µL for the 1,000 µL transfer, ±5 µL for the 100 µL transfer, and ±1 µL for the 10 µL transfer. Figure 3B shows the process capability reports for transfer of nuclease-free water by the KingFisher SpeciTRAX system at 1,000 µL, 100 µL, and 10 µL volumes. For the 1,000 µL and 100 µL transfers, all 33 samples had transfer volumes that were between the target and upper specification limits. The upper specification limit for 10 µL transfers was exceeded for this 33-sample set.

A

Precision (%CV)			Accuracy (%DEV)		
1,000 µL	**100 µL**	**10 µL**	**1,000 µL**	**100 µL**	**10 µL**
0.1%	0.2%	2.5%	1.7%	2.9%	9.9%

B

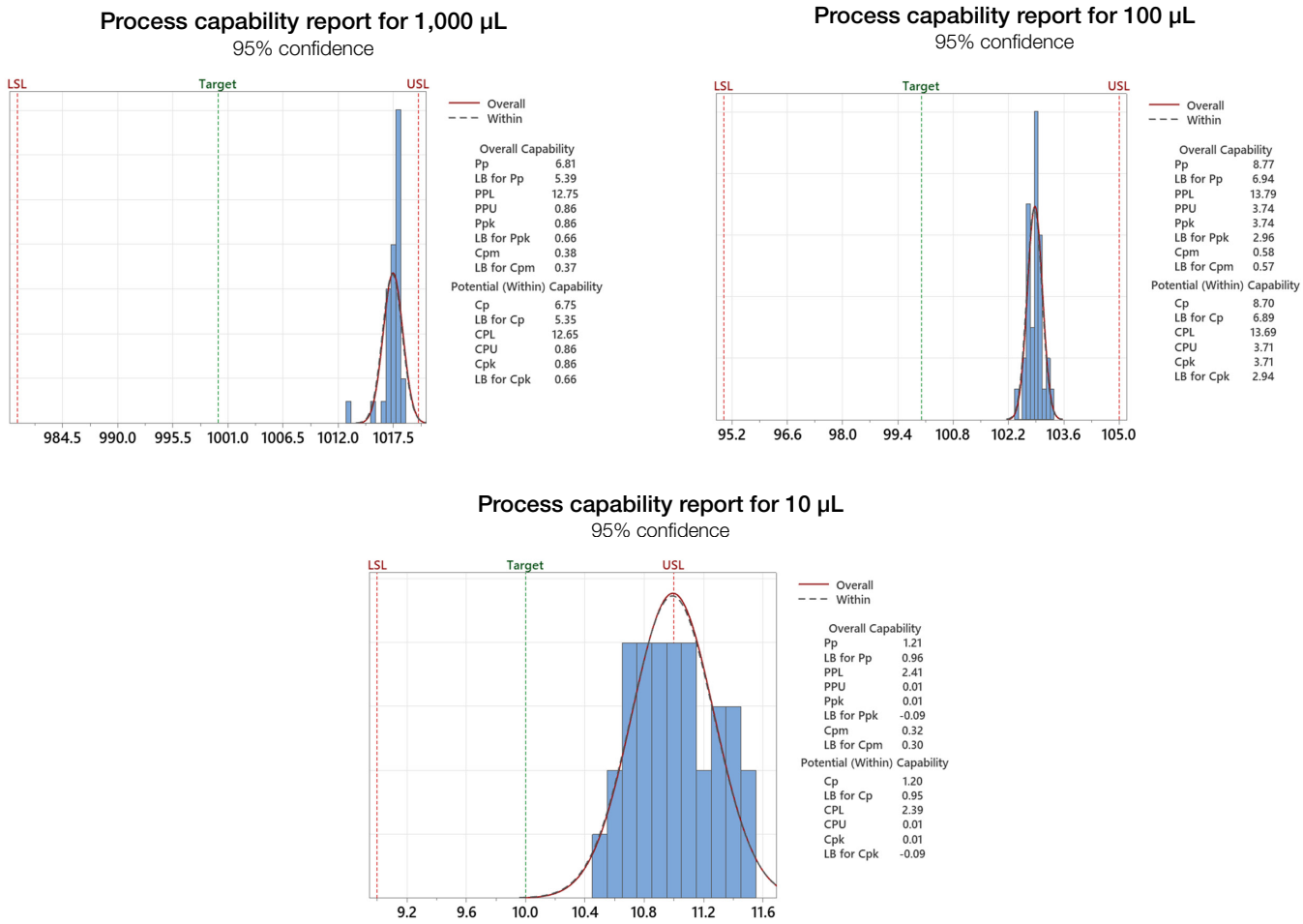


Figure 3. Process capability of the KingFisher SpeciTRAX system with 95% confidence for transfer of nuclease-free water at 1,000 µL, 100 µL, and 10 µL volumes. (A) Precision (%CV) and accuracy (%DEV) of 1,000 µL, 100 µL, and 10 µL volume transfers of nuclease-free water on the KingFisher SpeciTRAX system. **(B)** Process capability reports showing the lower specificity limit (LSL) and upper specificity limit (USL) for 1,000 µL, 100 µL, and 10 µL transfers of nuclease-free water on the KingFisher SpeciTRAX system. There were 33 samples per volume transfer.

Conclusion

The KingFisher SpeciTRAX Sample Transfer System is the time management solution needed for high-throughput laboratories that are processing specimens. When combined with the MagMAX MVP II Kit chemistry on KingFisher instruments, the full KingFisher SpeciTRAX Sample Transfer System workflow saves time and energy while increasing efficiency and reducing safety risks for the operator. The walk-away system allows for processing up to 192 samples in approximately 40 minutes and is compatible with various viral transport media and saliva specimen collection devices.

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Ordering information

Description	Cat. No.
KingFisher SpeciTRAX Sample Transfer System	5400700
SpeciMAX Saliva Collection Kit	A50696
SpeciMAX Stabilized Saliva Collection Kit	A50697
MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383R
TaqMan Gene Expression Assay (FAM)	4331182
KingFisher Flex Purification System, KingFisher with 96 Deep-Well Head (for volumes less than 1 mL)	5400630
KingFisher Flex Purification System, KingFisher with 24 Deep-Well Head (for volumes less than 5 mL)	5400640
QuantStudio 5 Real-Time PCR System, 384-well	A28140

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