Nucleic acid isolation

Evaluation of potential contamination risk in the sequential workflow for large RNA volumes with the MagMAX FFPE DNA/RNA Ultra Kit

Purpose

Thermo Scientific™ KingFisher™ plastics for 96 deep-well formats support working volumes of up to 1,000 µL for processing on Thermo Scientific™ KingFisher™ instruments. The Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit uses an optimized script and chemistry suitable for large-volume sequential isolation workflows from formalin-fixed, paraffin-embedded (FFPE) samples on KingFisher magnetic processing instruments.

For large-volume sequential isolation of DNA and RNA from one FFPE curl, workflows were developed for a total working volume of >1,000 µL in the RNA isolation. Table 1 shows the working volume contained in the sample plate for DNA isolation, the first extraction within the sequential DNA/RNA FFPE isolation workflow. After DNA processing, the same sample plate can then be used for RNA isolation without cutting more curls or using additional sample. Table 2 shows the working volume contained in the sample plate for RNA isolation using the sequential isolation workflow. In this case, with the total working volume greater than 1,000 µL, it is necessary to evaluate if there is a contamination risk in the large-volume, sequential isolation protocol, stemming from reagent movement on the KingFisher processing instrument.

Table 1. Working volume in the sample plate for DNA isolation using a large volume with sections >40 µm in the sequential isolation workflow.

Description	Volume
DNA binding solution (binding buffer and beads)	270 µL
Protease-digested FFPE sample	200 µL
Total volume	470 µL

Table 2. Working volume in the sample plate for RNA isolation using a large volume with sections >40 µm in the sequential isolation workflow.

Description	Volume
DNA binding solution (leftover binding buffer)	250 µL
Protease-digested FFPE sample	200 µL
Nucleic acid binding beads	20 µL
RNA binding buffer	650 µL
Total volume	1,120 µL

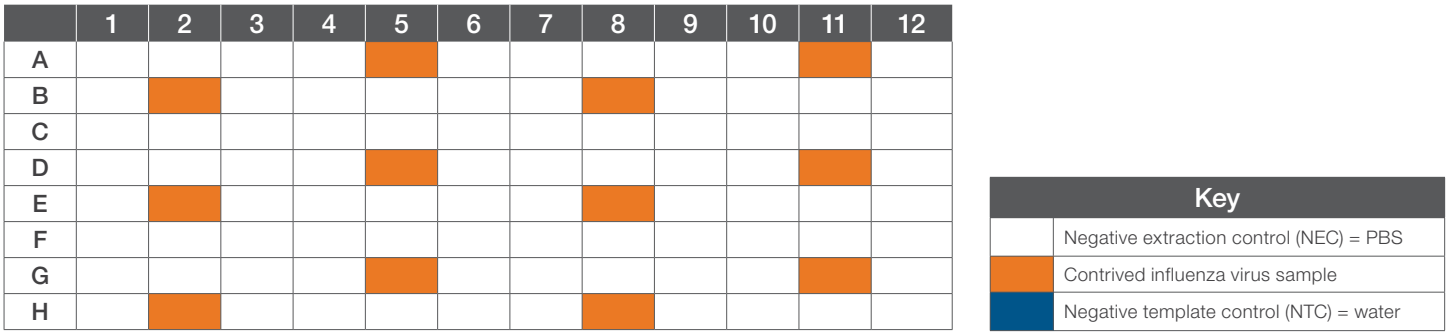
Here we evaluate the risk of cross-contamination using the Thermo Scientific™ KingFisher™ Flex Purification System with the working volumes and reagents shown in Table 2 for large-volume sequential isolation of RNA from FFPE curls. Taking a careful experimental approach to the evaluation of cross-contamination, the data show that using the MagMAX FFPE DNA/RNA Ultra Kit with the KingFisher Flex Purification System, with its current optimal validated scripts and workflows, has a low risk of cross-contamination.

Methods

A checkerboard layout was used to identify trends of sample and/or reagent movement within a 96 deep-well plate by surrounding a positive sample entirely with negative extraction controls (NECs; Figure 1A). To ensure that reagent movement from one well to another could be identified, a high titer of combined influenza virus targets was used as the sample. A viral concentration of approximately 8 x 10^{11} copies/mL was used for the large-volume sequential isolation protocol. Post-extraction, qPCR was performed with reagents of the Applied Biosystems™ VetMAX™-Plus Multiplex One Step RT-PCR Kit on the Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System. Using a 384-well plate for PCR, a double checkerboard method was used to distribute extracted samples across the plate layout.

In this layout, each reaction containing extracted nucleic acid is surrounded by a negative template control (NTC) containing only nuclease-free water plus the VetMAX-Plus PCR reagents (Figure 1B). This method allows for determination of potential contamination within the workflow by means of amplification observed in an NTC or NEC. All amplified NTCs were due to human or workflow setup at the qPCR level. All NECs that were amplified were retested in triplicate to confirm their putative negative status at the extraction level. In total, 3 runs were performed across 3 different KingFisher Flex instruments. There were 252 potential contamination events that could result from reagent movement on the KingFisher instrument.

A



B

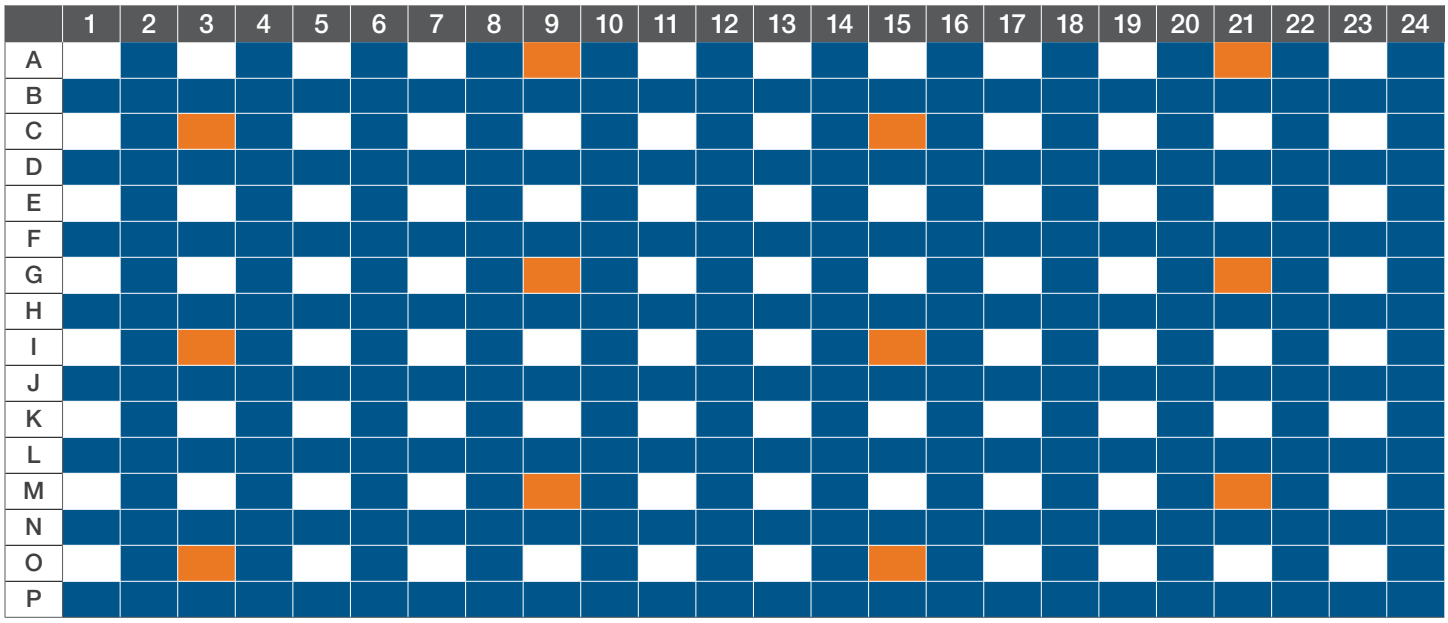


Figure 1. Sequential isolation using the MagMAX FFPE DNA/RNA Ultra Kit with influenza virus targets and the VetMAX-Plus Multiplex One Step RT-PCR master mix. (A) Extraction plate layout. The low-density checkerboard layout incorporated 12 high-titer samples (C_t ≈10) of influenza virus at 8 x 10^{11} copies/mL (orange). **(B)** PCR plate layout. A double checkerboard layout was used to distinguish between contamination from extraction and contamination from the subsequent qPCR. The wells surrounding all extracted samples (white and orange) were filled with NTCs (blue). NECs (white) contained only PBS.

Results

Isolating contamination stemming from extraction with the sequential isolation for RNA, 2 contaminations out of 252 potential contamination events were identified. Figure 2 shows the amplification plots from the three EDS files of the double checkerboard evaluation approach. A confirmation qPCR run was performed with the suspected contaminated eluates in a triplicate reaction. The confirmation runs indicated one suspected contamination (from KingFisher Flex instrument #1) to have resulted from qPCR, not extraction, as all three replicates were negative. One contamination event (from KingFisher Flex instrument #3) had potential contamination stemming from extraction, as 2 of the 3 replicates indicated no amplification while 1 of the 3 replicates showed some low-level amplification.

Because only 1 of the 3 replicates amplified in the confirmation run, this could indicate user or pipette contamination when the qPCR 384-well plate was set up. Even though user error was suspected, the extracted sample was considered a true positive caused by extraction. The final results indicated that there was 1 out of 252 potential contamination events (equivalent to <0.4% contamination). This contamination event was thought to have originated during the RNA extraction. Given these results, there is a low risk of potential contamination stemming from movement of well contents on the KingFisher Flex instrument when using the optimized extraction script (A31881_FLEX_large_vol_RNA_script) with the current manufacturer-recommended volumes.

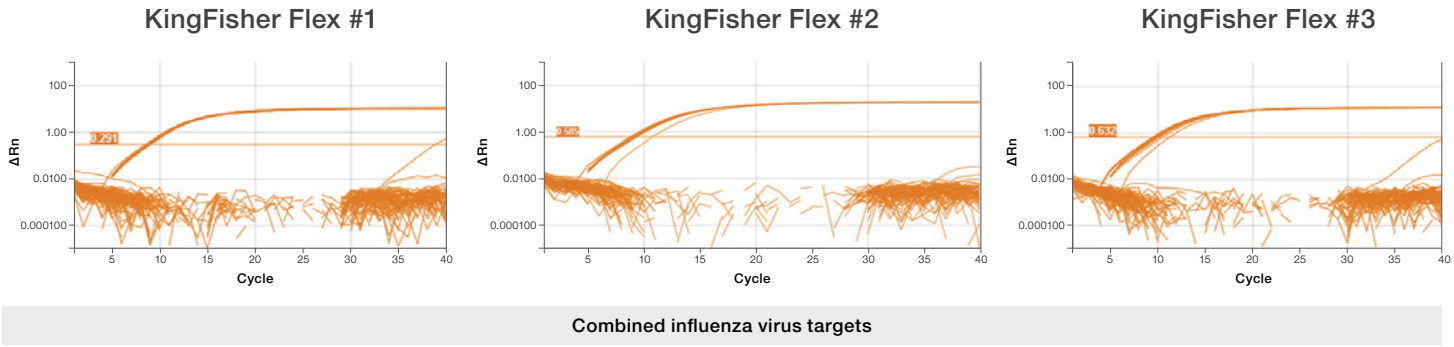


Figure 2. Evaluation of potential cross-contamination in plate setup on the KingFisher Flex Purification System. Among 252 samples processed across three different KingFisher Flex instruments, we observed a contamination rate of <0.4. The RNA extraction method (sequential DNA/RNA isolation) described in the MagMAX FFPE DNA/RNA Ultra Kit User Guide (Pub. No. MAN0017541) was used.

Conclusions

This study provides data indicating a low risk of contamination with the current workflow for the MagMAX FFPE DNA/RNA Ultra Kit with high volumes. The workflow and reagents are used in the sequential isolation of DNA and RNA from FFPE samples, and the risk of cross-contamination was low even when the total working volume within the sample plate was >1,000 µL. The workflow, working volumes, and scripts were developed and optimized to ensure that sequential isolation of high-quality DNA and RNA is reproducible using the MagMAX FFPE DNA/RNA Ultra Kit on KingFisher instruments. Caution should be exercised if exceeding recommended volumes with unvalidated workflows and reagents, which is not recommended.

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Ordering information

Product	Cat. No.
MagMAX FFPE DNA/RNA Ultra Kit	A31881
KingFisher Flex Purification System with 96 Deep-Well Head	A32681

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