Sample prep

Functionality of the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit on KingFisher instruments

Purpose

This document provides guidance for the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit on Thermo Scientific™ KingFisher™ instruments. Deviating from the indicated guidance may result in excessive reagent foaming during sample preparation and nucleic acid extraction.

Background

Foaming of the binding solution and wash reagents of the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit can occur, as both reagents contain detergent to assist in lysing the virus in the targeted sample type. If the binding and wash solutions alone are shaken or mixed vigorously, they will bubble and foam as is common with most detergent-containing reagents. If this foaming of reagents occurs prior to mixing with samples during semi-automated or fully automated extraction, there is concern the foam will build up excessively, resulting in leakage into adjacent wells of the sample plate and potential contamination between samples. Here we outline various methods used to observe the occurrences of excessive foaming of the binding solution and determine the impact when running the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit workflow on the KingFisher Flex Purification System.

Methods

Method 1—impact of binding solution volume and mixing

A bulk binding bead slurry was prepared by combining the binding solution and magnetic beads from the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit using the ratio given for the 200 µL protocol in the instructions for use (IFU) [1]. Using nuclease-free water as a sample, different volumes of the prepared binding bead slurry and sample were then added to a sample plate to create variable conditions A–C (Table 1), which represent the suggested volumes in the IFU that correlate with the 200 µL protocol option. For each variable condition, the sample plates were tested with and without premixing the binding bead slurry in the plate 3 times with a 12-channel 1,000 µL pipette set at 500 µL. After the sample plate was prepared, it was placed on the KingFisher Flex Purification System and run using the MVP_2Wash_200_Flex script.

Table 1. Method 1 fill volumes.

Condition	Fill volume of bead slurry	Fill volume of sample	Premixing
A	275 µL (53%)	0 µL	Yes (+) or no (–)
B	515 µL (100%)	0 µL	Yes (+) or no (–)
C	515 µL (100%)	200 µL	Yes (+) or no (–)

Method 2—impact of binding solution volume

Using the instrument scripts for 200 µL (MVP_2Wash_200_Flex) and 400 µL (MVP_2Wash_400_Flex) sample inputs with the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit, various volumes of binding bead slurry were tested with and without the addition of sample from pooled nasopharyngeal swabs stored in viral transport medium (NPVTM, Table 2).

Table 2. Method 2 fill volumes.

Condition	Binding bead slurry volume	NPVTM sample volume
MVP_2Wash_200_Flex script		
A	275 µL	0 µL
B	138 µL	0 µL
C	413 µL	0 µL
D	275 µL	200 µL
E	138 µL	200 µL
F	413 µL	200 µL
MVP_2Wash_400_Flex script		
A	550 µL	0 µL
B	275 µL	0 µL
C	600 µL	0 µL
D	550 µL	400 µL
E	275 µL	400 µL
F	600 µL	400 µL

Method 3—impact of plate-balancing effects

Balanced and unbalanced plate designs (Figure 1) were evaluated on the KingFisher Flex Purification System. A 96 deep-well plate was filled with the bead slurry at recommended volumes in all 96 wells. Nuclease-free water and binding bead slurry were added to wells, as denoted in Figure 1 by "+", to achieve quadrant balancing across the X and Y axes of the plate map.

Balanced plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	+	+	+	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	+	+	+	+	+	+
E	+	+	+	+	+	+	+	+	+	+	+	+
F	+	+	+	+	+	+	+	+	+	+	+	+
G	+	+	+	+	+	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	+	+	+	+	+

Unbalanced plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	+	+	+								
B	+	+	+	+								
C	+	+	+	+								
D	+	+	+	+			+					
E	+	+	+	+				+				
F	+	+	+	+								
G	+	+	+	+								
H	+	+	+	+								+

\+ **Contains 200 or 400 µL of nuclease-free water added on top of bead slurry**

Figure 1. Balanced and unbalanced plate designs. Wells without sample (water) added contained only the bead slurry.

Results

Method 1—impact of binding solution volume and mixing

Figure 2 shows the sample plate after premixing with a multichannel pipette and before being placed on a KingFisher Flex Purification System for extraction. Figure 3 shows the sample plate after completing the extraction run on the instrument. Although all premixed samples produced foam during extraction, wells containing the lowest volume of combined sample and reagents (condition A) produced foam that migrated outside of the wells. The least foaming occurred with samples under condition C with no sample premixing before extraction.

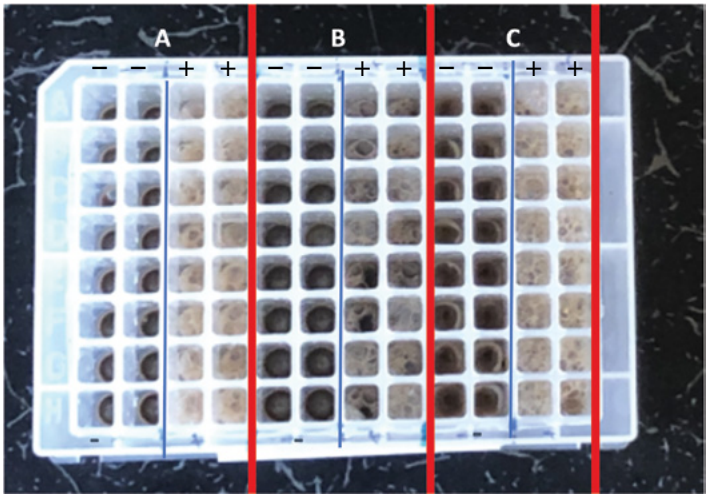

Figure 2. Sample plate before extraction. The plate contains binding bead mix and sample according to variable conditions A–C, without (–) or with (+) premixing with a multichannel pipette.

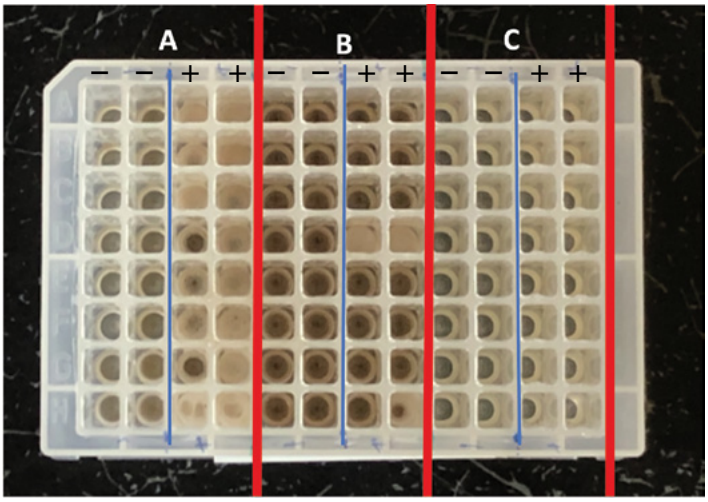

Figure 3. Sample plate after extraction. The plate contains binding bead mix and sample according to variable conditions A–C, without (–) or with (+) premixing with a multichannel pipette.

Method 2—impact of binding solution volume

Table 3 shows the overall results of method 2, which utilized plates containing under- and overfilled volumes using two different sample input instrument scripts.

Table 3. Foaming results with volume variation.

Condition	Binding bead slurry volume	NPVTM sample volume	Foaming post-extraction?
MVP_2Wash_200_Flex script			
A	275 µL	0 µL	Yes
B	138 µL	0 µL	No
C	413 µL	0 µL	Yes
D	275 µL	200 µL	No
E	138 µL	200 µL	No
F	413 µL	200 µL	No
MVP_2Wash_400_Flex script			
A	550 µL	0 µL	Yes
B	275 µL	0 µL	Yes
C	600 µL	0 µL	Yes
D	550 µL	400 µL	No
E	275 µL	400 µL	No
F	600 µL	400 µL	No

Method 3—impact of plate-balancing effects

Visual results of unbalanced plates (Figure 4) indicate that wells with binding bead mix but no sample added are most prone to foaming on the sample plate during extraction. Less foaming occurred with samples in the balanced plates than with those in the unbalanced plates, indicating the importance of quadrant balance across the sample plate on the KingFisher instrument.

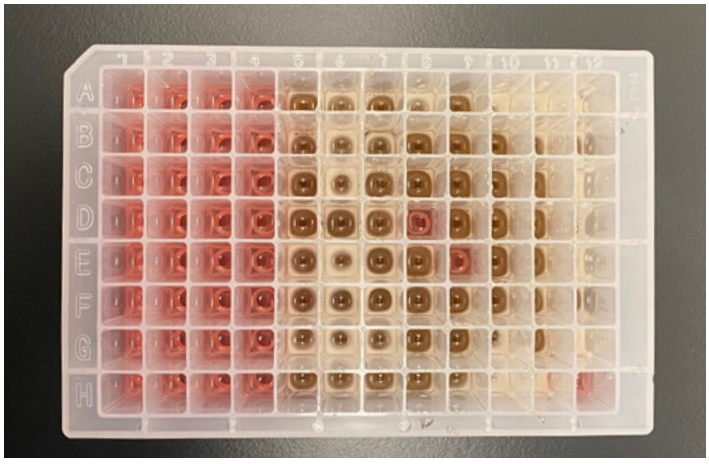

Figure 4. Unbalanced plate post-extraction.

Discussion

Method 1 confirms that the volume of reagents and sample within a plate must correspond to the indicated script volume. Z-axis movement on the KingFisher Flex Purification System is driven by the volume specified by the script, and mixing speed and volume are optimized to sample input based on the MagMAX reagents. As such, foaming is more likely to occur in wells of the sample plate containing lower than recommended volumes. Method 1 also indicates that premixing of reagents may increase the risk of excessive foaming. The manual for the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit states to not vigorously mix or overmix the binding bead mix, to minimize foaming. This recommendation should be carried over when preparing the sample plate containing the binding bead mix with sample. Mixing of reagents and sample is not necessary before extraction on the KingFisher instruments. However, if mixing is necessary in a specific workflow, foaming can be minimized by slowly pipetting up and down and ensuring that the pipette is set to a volume that will not cause bubbles while mixing.

Method 2 confirms the results of method 1 and demonstrates that the correct volume of binding bead mix plus sample is crucial for efficient extraction. The results of method 3 suggest that a balanced sample plate layout on the KingFisher Flex Purification System could reduce the risk of foaming during extraction on the instrument.

Guidance

To reduce the risk of foaming of the sample plate on the KingFisher Flex Purification System, please follow the guidance listed below. Deviation from this guidance may result in foaming of the sample undergoing extraction, which could lead to contamination and other issues.

- Ensure that the IFUs and manuals for the MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit and KingFisher Flex Purification System are followed.
- Ensure that unused wells are not filled with reagent. If using a pre-filled reagent format, use an equal volume of water or PBS in place of the sample volume.
- Ensure that the design of the sample plate map is balanced by sample and reagent volume across all quadrants.
- Avoid generating bubbles in the binding buffer or binding bead slurry before adding it to the sample plate or putting it onto the KingFisher instrument. Bubbles and foaming may occur via pipetting up and down, vigorously shaking, or a combination of both.

Ordering information

Description	Cat. No.
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (RUO)	A48383R

Reference

1. MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit: Instructions for Use. Pub. No. MAN0019746. Revision B.0.

Learn more at **thermofisher.com/magmax**