ThermoFisher
SCIENTIFIC

Sample prep

MagMAX CORE Nucleic Acid Purification Kit on the KingFisher Apex Purification System

Purpose

To demonstrate performance of the Applied Biosystems™ MagMAX™ CORE Nucleic Acid Purification Kit on the Thermo Scientific™ KingFisher™ Apex Purification System with 96 Deep-Well Head.

Methods

Test 1: Porcine epidemic diarrhea virus (PEDV) samples

1. Eight fecal samples and eight environmental samples were prepared following the "complex" workflow in the user guide of the MagMAX CORE kit.

    a. For fecal samples:

        i.   0.2 g fecal material plus 1 mL PBS

        ii. 200 µL of resulting supernatant combined with 450 µL of Lysis Solution

        iii. 500 µL input

    b. For environmental samples:

        i.   200 µL of samples combined with 450 µL of Lysis Solution

        ii. 500 µL input

2. 4 µL of Applied Biosystems™ VetMAX™ Xeno™ RNA control (10,000 copies/µL) was spiked into each isolation.

3. All processing plates were loaded on the KingFisher Apex and KingFisher Flex systems according to the user guide of the MagMAX CORE kit for nucleic acid isolation on the KingFisher Flex system.

4. KingFisher Apex and KingFisher Flex systems were each run using their respective heated scripts for the MagMAX CORE kit.

    a. KingFisher Apex system: MagMAX_CORE_heated_v1.kfx

    b. KingFisher Flex system: MagMAX_CORE_Flex.bdz

5. Recovery of PEDV was analyzed using the Applied Biosystems™ VetMAX™ PEDV/TGEV/SDCoV Kit.

Test 2: Porcine reproductive and respiratory syndrome virus (PRRSV) samples

1. Eight PRRSV-positive samples from porcine processing fluids, serum, tissue, blood (seven samples), and oral fluids were prepared following the user guide of the MagMAX CORE kit.

    a. For processing fluid, serum, tissue (homogenized lung), and blood samples:

        i.   "Simple" workflow followed

        ii. 200 µL input

    b. For oral fluid samples:

        i.   "Complex" workflow followed

        ii. 300 µL of samples combined with 450 µL of Lysis Solution

        iii. 600 µL input

2. 4 µL of VetMAX Xeno RNA control (1,000 copies/µL) was spiked into each isolation.

3. All processing plates were loaded on the KingFisher Apex and the KingFisher Flex systems according to the user guide of the MagMAX CORE kit for nucleic acid isolation on the KingFisher Flex system.

4. KingFisher Apex and KingFisher Flex systems were each run using their respective heated scripts for the MagMAX CORE kit.

    a. KingFisher Apex system: MagMAX_CORE_heated_v1.kfx

    b. KingFisher Flex system: MagMAX_CORE_Flex.bdz

5. Recovery of PRRSV was analyzed using the Applied Biosystems™ VetMAX™ PRRSV NA & EU Reagents Kit.

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Test 3: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) samples

1. Six MAP-positive bovine fecal samples were prepared according to the "high-input" workflow in the user guide of the Applied Biosystems™ MagMAX™ CORE Mechanical Lysis Module.

   a. 1 µL of Applied Biosystems™ VetMAX™ Xeno™ DNA control (5,000 copies/µL) was spiked into the Lysis/Binding Mix to serve as a process control.

   b. Each sample was processed in triplicate on the KingFisher Apex system with heated and non-heated scripts.

   c. Lysates were prepared as described in the user guide of the MagMAX CORE Mechanical Lysis Module.

2. All processing plates were loaded on the KingFisher Apex and KingFisher Flex systems according to the user guide of the MagMAX CORE kit for nucleic acid isolation on the KingFisher Flex system.

3. The KingFisher Apex system was run using the appropriate heated and non-heated scripts. The KingFisher Flex system was run with its heated script.

   a. KingFisher Apex system: MagMAX_CORE_heated_v1.kfx

   b. KingFisher Apex system: MagMAX_CORE_Noheat_v1.kfx

   c. KingFisher Flex system: MagMAX_CORE_Flex.bdz

4. Recovery of MAP DNA was analyzed using the Applied Biosystems™ VetMAX™-Gold MAP Detection Kit.

Test 4: Cross-contamination test

1. 300 µL of North America PRRSV vaccine solution was added in a checkerboard layout (24 wells) to a KingFisher deep-well plate as shown in the results section. To the remaining 72 wells, 300 µL of PBS was added. This plate served as the lysis plate.

2. Samples were processed according to the "simple" workflow in the user guide of the MagMAX CORE kit.

3. All processing plates were loaded on the KingFisher Apex system according to the user guide of the MagMAX CORE kit for nucleic acid isolation on the KingFisher Flex system.

4. The KingFisher Apex system was run using its heated script.

   a. KingFisher Apex system: MagMAX_CORE_heated_v1.kfx

5. Recovery of PRRSV RNA was analyzed using the VetMAX PRRSV NA & EU Reagents Kit.

Results

Tests 1 and 2

Consistent results were achieved for the MagMAX CORE kit on the KingFisher Apex and KingFisher Flex systems for PEDV-positive fecal and environmental samples as well as PRRSV-positive processing fluid, serum, tissue, blood, and oral fluid samples. Consistency was determined by mean C_t values within 1.0. Results for the VetMAX Xeno RNA control, which serves as the positive and inhibition control, also met this criterion. RT-qPCR results for each sample are shown in Figures 1–7.
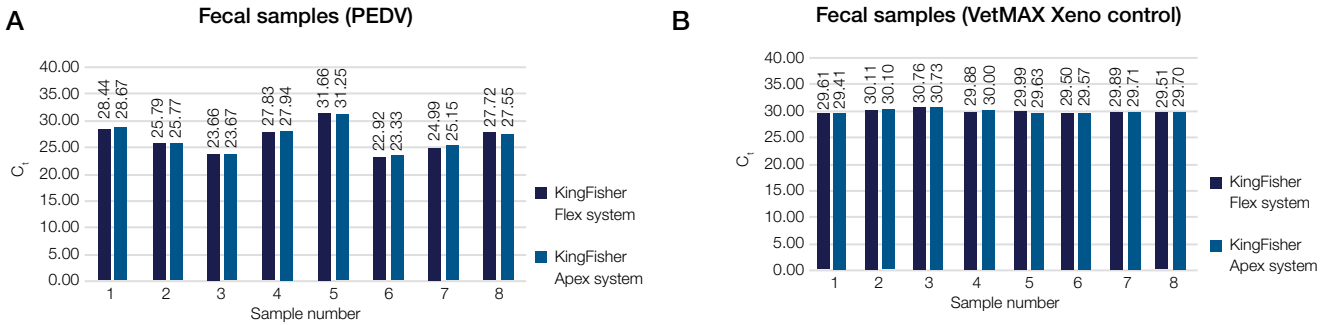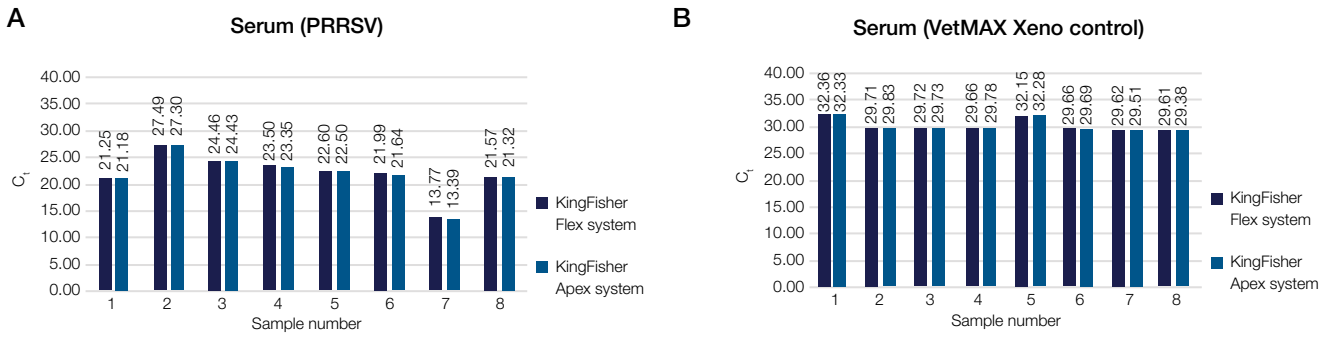

Figure 1. Fecal samples. (A) PEDV: KingFisher Apex system (n = 8; C_t mean = 26.67) and KingFisher Flex system (n = 8; C_t mean = 26.63). ΔC_t of KingFisher Apex vs. Flex systems: mean = 0.04, SD = 0.25. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 29.86) and KingFisher Flex system (n = 8; C_t mean = 29.91). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.05, SD = 0.19.
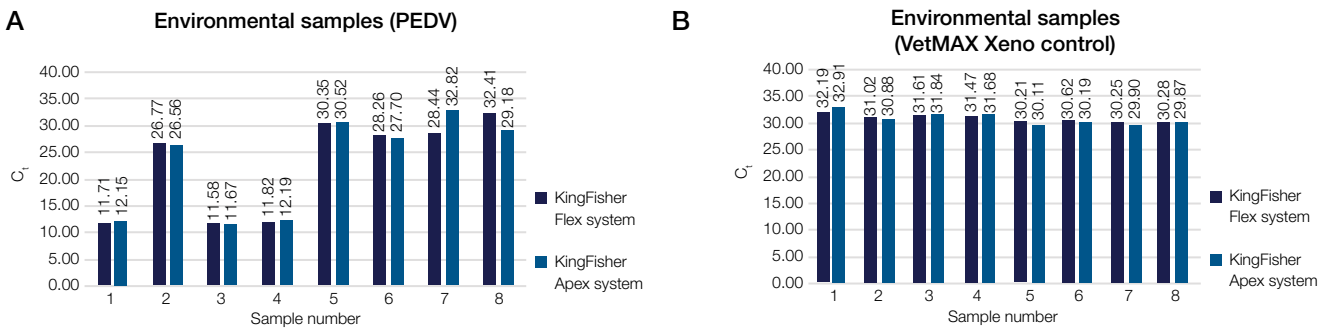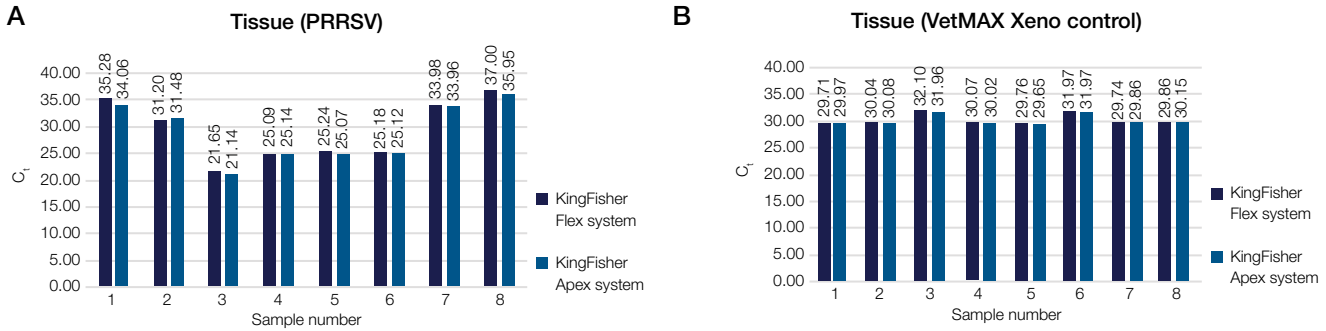

Figure 2. Environmental samples. (A) PEDV: KingFisher Apex system (n = 8; C_t mean = 22.85) and KingFisher Flex system (n = 8; C_t mean = 22.67). ΔC_t of KingFisher Apex vs. Flex systems: mean = 0.18, SD = 2.07. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 30.92) and KingFisher Flex system (n = 8; C_t mean = 30.96). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.04, SD = 0.40.
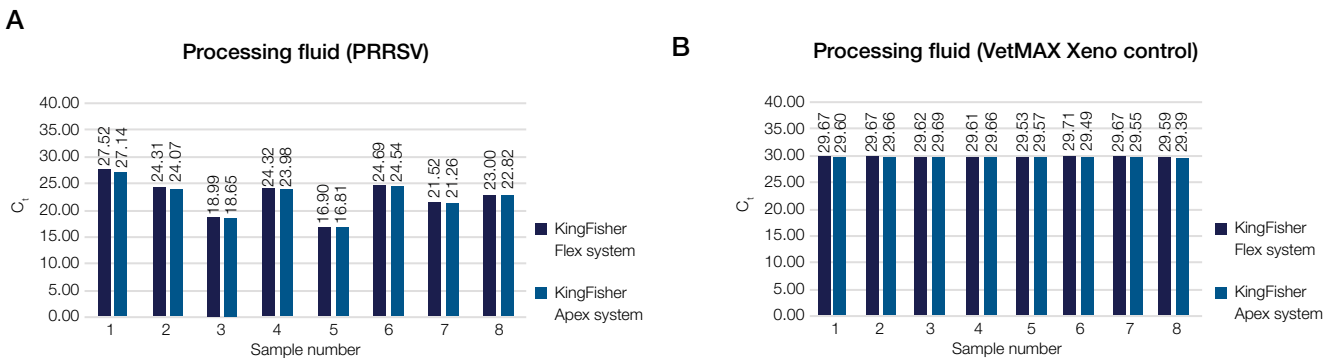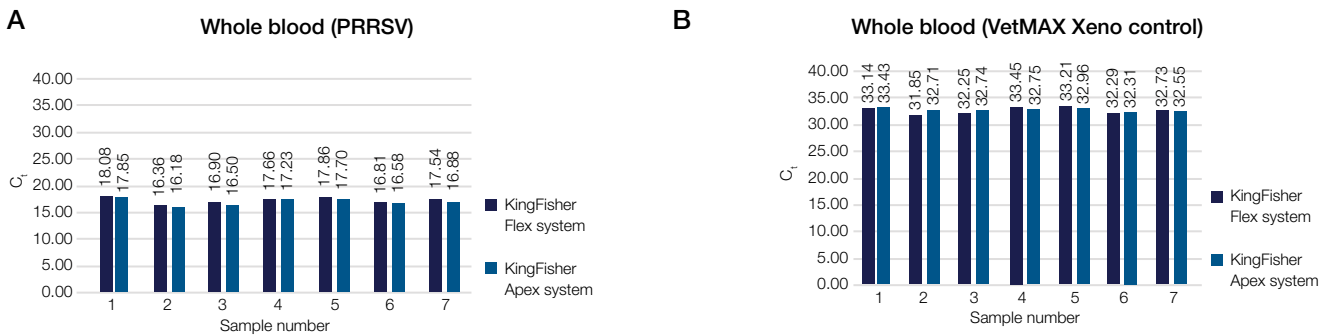

Figure 3. Processing fluid samples. (A) PRRSV: KingFisher Apex system (n = 8; C_t mean = 22.41) and KingFisher Flex system (n = 8; C_t mean = 22.66). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.25, SD = 0.10. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 29.58) and KingFisher Flex system (n = 8; C_t mean = 29.64). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.07, SD = 0.12.
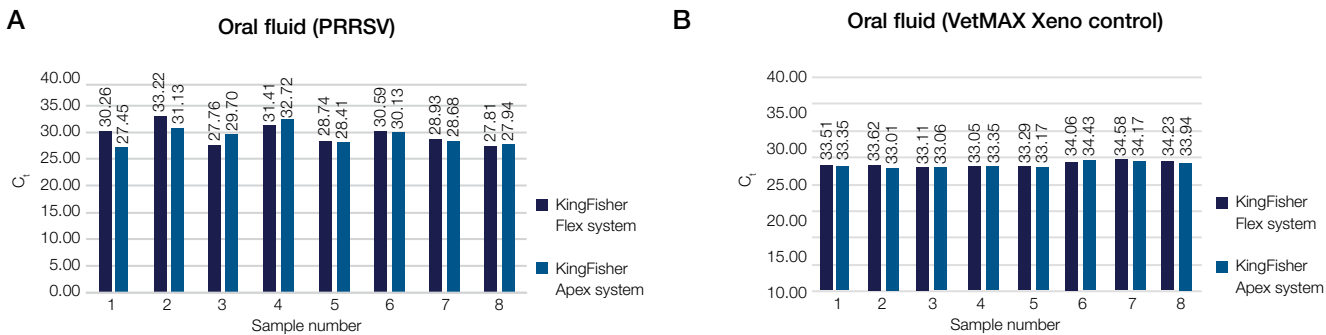
Figure 4. Serum samples. (A) PRRSV: KingFisher Apex system (n = 8; C_t mean = 21.89) and KingFisher Flex system (n = 8; C_t mean = 22.08). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.19, SD = 0.13. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 30.32) and KingFisher Flex system (n = 8; C_t mean = 30.31). ΔC_t of KingFisher Apex vs. Flex systems: mean = 0.01, SD = 0.13.

Figure 5. Tissue samples. (A) PRRSV: KingFisher Apex system (n = 8; C_t mean = 28.99) and KingFisher Flex system (n = 8; C_t mean = 29.33). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.34, SD = 0.54. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 30.46) and KingFisher Flex system (n = 8; C_t mean = 30.41). ΔC_t of KingFisher Apex vs. Flex systems: mean = 0.05, SD = 0.16.


Figure 6. Whole blood samples. (A) PRRSV: KingFisher Apex system (n = 7; C_t mean = 16.99) and KingFisher Flex system (n = 7; C_t mean = 17.32). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.33, SD = 0.18. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 7; C_t mean = 32.78) and KingFisher Flex system (n = 7; C_t mean = 32.70). ΔC_t of KingFisher Apex vs. Flex systems: mean = 0.07, SD = 0.52.

Figure 7. Oral fluid samples. (A) PRRSV: KingFisher Apex system (n = 8; C_t mean = 29.52) and KingFisher Flex system (n = 8; C_t mean = 29.84). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.32, SD = 1.58. **(B)** VetMAX Xeno control: KingFisher Apex system (n = 8; C_t mean = 33.56) and KingFisher Flex system (n = 8; C_t mean = 33.68). ΔC_t of KingFisher Apex vs. Flex systems: mean = −0.12, SD = 0.33.

Test 3

Performance of the MagMAX CORE kit on the KingFisher Apex and KingFisher Flex systems with heated scripts was consistent for the six MAP-positive bovine fecal samples tested (Figure 8). The non-heated script produced C_t values approximately 1.5 C_t later than the heated scripts for MAP detection, and there was no difference for the VetMAX Xeno DNA control. Recovery of the VetMAX Xeno DNA control was again consistent between the KingFisher Apex and KingFisher Flex systems.

A   Fecal samples (MAP)

B   Fecal samples (VetMAX Xeno control)
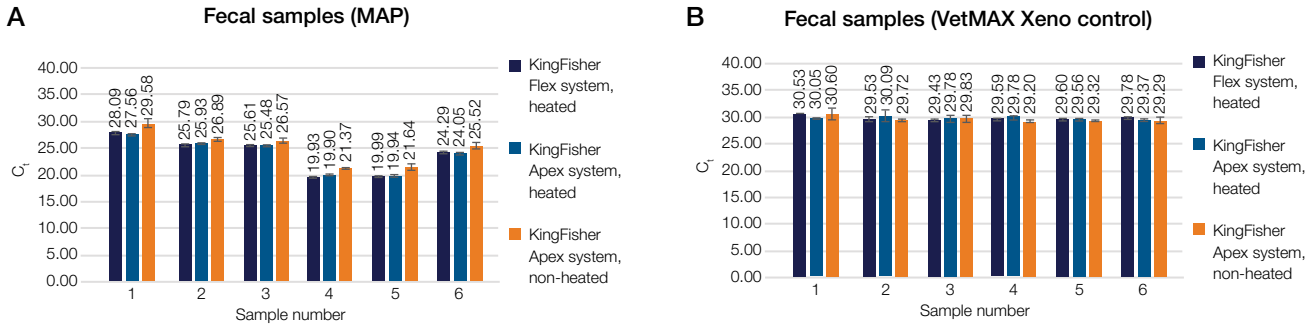
Figure 8. Fecal samples processed using heated and non-heated scripts. (A) MAP and **(B)** VetMAX Xeno control compared between the KingFisher Flex system (heated script) and KingFisher Apex system (heated and non-heated scripts). Data represent triplicate qPCR reactions.

Test 4

No cross-contamination was observed. Figure 9 shows that PRRSV was detected only in wells that had received a spike-in of PRRSV vaccine. All adjacent wells that had only received PBS buffer remained negative even in proximity to the high-titer positive samples (average C_t value of 16.4). Note: the first column of the plate was reserved for controls and therefore excluded from analysis.

	1	2	3	4	5	6	7	8	9	10	11	12
A		–	–	16.78	–	–	–	16.62	–	–	–	16.58
B		16.69	–	–	–	16.49	–	–	–	16.66	–	–
C		–	–	16.47	–	–	–	16.59	–	–	–	16.34
D		16.71	–	–	–	16.63	–	–	–	16.58	–	–
E		–	–	16.36	–	–	–	16.43	–	–	–	16.61
F		16.23	–	–	–	16.32	–	–	–	16.30	–	–
G		–	–	16.21	–	–	–	16.31	–	–	–	16.20
H		16.13	–	–	–	16.20	–	–	–	16.16	–	–

Figure 9. Evaluation of cross-contamination. C_t values are shown for samples processed on the KingFisher Apex system and analyzed by RT-qPCR. All wells displaying C_t values received a high-titer concentration of North America PRRSV vaccine (24 total). All adjacent wells in columns 2–12 received only PBS buffer (64 total).

Conclusions

This testing shows that the MagMAX CORE Nucleic Acid Purification Kit is compatible with the KingFisher Apex Purification System. Extractions from feces, environmental swabs, processing fluids, serum, tissue, blood, and oral fluids yielded consistent performance with the KingFisher Apex and KingFisher Flex systems for PEDV, PRRSV, and MAP targets. Additionally, no cross-contamination occurred during the test using high-titer samples, a critically important result.

Ordering information

Product	Quantity	Cat. No.
MagMAX CORE Nucleic Acid Purification Kit	100 reactions	A32700
MagMAX CORE Nucleic Acid Purification Kit	500 reactions	A32702
MagMAX CORE Mechanical Lysis Module	100 reactions	A32836
VetMAX-Gold MAP Detection Kit	100 reactions	A29809
VetMAX PRRSV NA & EU Reagents	100 reactions	4468465
VetMAX PRRSV NA & EU Controls	1 kit	4405548
VetMAX PEDV/TGEV/SDCoV Kit	100 reactions	A33402
VetMAX Xeno Internal Positive Control DNA	100 reactions	A29764
VetMAX Xeno Internal Positive Control DNA	500 reactions	A29762
VetMAX Xeno Internal Positive Control RNA	100 reactions	A29763
VetMAX Xeno Internal Positive Control RNA	500 reactions	A29761
KingFisher Apex Purification System with 96 Deep-Well Head	1 instrument	5400930
KingFisher Flex Purification System with 96 Deep-Well Head	1 instrument	5400630

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

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