Application Note

Simultaneous Nucleic Acid Purification from Multiple Sample Types

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Key words

Total nucleic acid purification, feces, whole blood, nasal secretions, pathogen detection, KingFisher Flex, KingFisher Pure Kit

Introduction

Concern for animal welfare and for the safety of products of animal origin has increased the demand for veterinary medicine in recent years. As the treatment options and pathogen detection systems have developed, the need for veterinary genetics services has grown.

The sample materials used in veterinary analysis are highly variable. The starting substances are often various bodily fluids such as blood, feces or nasal secretions. Consequently, rapid, automated nucleic acid purification from multiple sample types is in high demand for different laboratory settings. At present, several alternative methods exist for total nucleic acid extraction. The procedures can either rely on the traditional techniques which utilize self-prepared solutions or on commercial nucleic acid extraction kits that employ, for example, spin column technology or magnetic particles.

Magnetic particles (also referred to as beads) are routinely used for nucleic acid isolation. One of the many benefits of the practice is the large overall surface area of the beads, which allows a higher yield of nucleic acid to be purified at a time. Automation of the magnetic particle processing increases reproducibility and reduces errors whereas the prospective number of processed samples is multiplied.

Thermo Scientific™ KingFisher™ instruments employ the magnetic particle technology. The instruments, coupled with the KingFisher Pure Kits, comprise a convenient, automated purification system for both DNA and RNA. Additionally, other magnetic bead based-kits can be optimized for the KingFishers.

Figure 1: KingFisher Flex Magnetic Particle Processor uses automated magnetic particle separation technology for DNA/RNA, protein or cell purification from virtually any source.

The KingFisher Flex can process up to 96 parallel specimens during a single run. The KingFisher Duo Prime is targeted for laboratories with a lower number of specimens and can extract nucleic acids from 12 samples per run. The input volumes of the buffer solutions and several other parameters—such as incubation times and mixing speeds—can be customized allowing for a wide range of flexibility for the extraction procedures.

In this note we describe how to simultaneously extract total nucleic acid from multiple sample types on the KingFisher Flex; and compare the performance of the KingFisher instrument to another nucleic acid extraction platform.

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Materials and Methods

Whole blood (n=30), feces (n=50) and nasal secretions (n=20) were obtained from several different species (canine, feline or equine). Total nucleic acids were extracted from the samples on the KingFisher Flex and on a vacuum based automated system that is currently used in our laboratory. On the KingFisher Flex, the purification was performed with the KingFisher Pure DNA Blood (Cat. No. 98010196) and the Viral NA Kits (Cat. No. 98070196). The samples were prepared according to the following instructions:

Whole blood and fecal samples (á 100 µl) were transferred to a KingFisher 96 deep-well plate designated as the "sample" plate. The specimens were then treated with the KingFisher Pure DNA Blood Kit reagents:

- 100 µl Lysis Buffer
- 20 µl Proteinase K

Whole blood and fecal samples were left at room temperature for approximately 10 minutes. After the lysis, the subsequent solutions were added to each sample:

- 400 µl Binding Buffer
- 25 µl Magnetic Beads

Nasal secretion samples (á 200 µl) were treated with the KingFisher Pure Viral NA Kit reagents:

- 200 µl Lysis Buffer with carrier RNA
- 50 µl Proteinase K

Nasal secretions were incubated for 10 minutes at 56 °C. Following lysis, the specimens were transferred to the sample plate and the subsequent solutions were added to them:

- 450 µl isopropanol (100%)
- 25 µl Magnetic Beads

During the incubation steps, the plates that contain the other necessary reagents for the extraction procedure were prepared (see Table 1).

The plates were set up as follows:

Step/Plate No.	Plate name	Amount in µl
1	Sample	Blood and feces (total 645 µl) OR Nasal secretions (total 925 µl)
2	1st Wash	900 µl Wash Buffer 1 (Pure DNA Blood Kit)
3	2nd Wash	800 µl Wash Buffer 1 (Pure Viral NA Kit)
4	3rd Wash	800 µl Wash Buffer 2 (Pure Viral NA Kit)
5	Elution	100 µl DEPC Water (Molecular grade)

Table 1: The plate numbers and solution volumes used for the total nucleic acid purification procedure with the KingFisher Blood DNA Kit and the KingFisher Pure Viral NA Kit.

At the end of the run, each sample was eluted with 100 µl of DEPC-water (not included in the kits). The extracts were analyzed with qPCR for the presence of nucleic acids originating from infectious agents such as bacteria, viruses, or parasites.

Results

In order to assess the effectiveness of the extraction procedures, the nucleic acid isolates were amplified with qPCR and the average quantification cycle (Cq) values were calculated. Generally, if a specific DNA molecule is present at higher quantities, the Cq value is obtained sooner during a qPCR run. Hence, a lower Cq value correlates with a higher initial copy number of the target. The comparison of the Cq values from different sample types demonstrated that the two purification systems produced comparable starting material for pathogen detection (Figure 2).

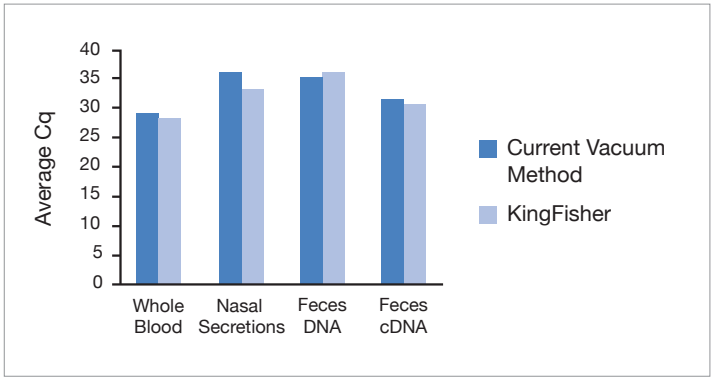

Figure 2: Detection of pathogens with qPCR - Average Cq values. From whole blood, Cq was approximately 28 with our current vacuum based method and with the KingFisher Flex. For nasal secretions, Cq was 35 with the current method and 32 with the KingFisher Flex. For feces, the Cq was broken down into DNA and cDNA; current method Cq = 34 / 31 respectively, and KingFisher Flex Cq = 35 / 30, respectively.

The total number of detected pathogen samples was similar between the two platforms (Figure 3). These results confirmed that the performance of the KingFisher Flex instrument was equivalent to our current vacuum based method. However, the extraction time with the KingFisher Flex was three times faster.

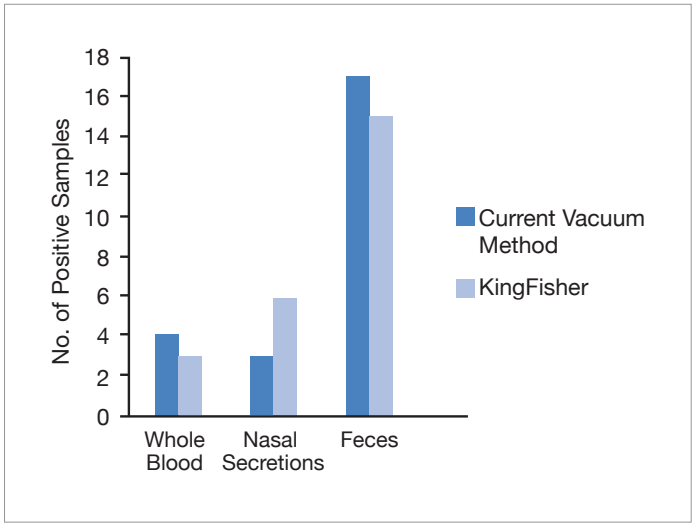

Figure 3: Comparison of the number of positive pathogen samples. Of the 30 whole blood samples, 4 were identified as positive with the current vacuum system and 3 were positive with the KingFisher. Of the 20 nasal secretions, 3 were positive with the current system and 6 with the KingFisher. Of the 50 fecal samples, 17 were positive with the current system and 15 with the KingFisher.

Conclusions

With minor modifications to the KingFisher Pure DNA Blood and the KingFisher Pure Viral NA Kits, total nucleic acids could be isolated from multiple specimen types during a single run on the KingFisher Flex instrument. The extraction procedure on the KingFisher Flex was three times faster and required less hands-on time compared to our current vacuum based method. The KingFisher Flex was as effective at isolating total nucleic acids from the studied sample materials as the vacuum system.

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