Application Note:
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Isolation and purification of genomic DNA and viral RNA from clinical samples with Thermo Scientific KingFisher Flex

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Abstract
The purification of DNA and RNA from clinical samples requires rapid and reliable processing of large quantities of samples. Automated purification of nucleic acids, proteins and cells with Thermo Scientific KingFisher Flex is a reproducible and high-quality method, which produces excellent material for further analyses. KingFisher® Flex transfers and processes magnetic particles on microplates during purification. This offers a quick and easy solution for isolation of nucleic acids from various materials. Here, we discuss the purification of nucleic acids from clinical samples: The purification of DNA from blood samples and RNA from buccal swab or stool samples.

Introduction
The purifications of small particles, for instance DNA or RNA, are routine methods in clinical analyses to obtain high-quality starting material for further experiments. The preparation of the samples should be rapid, efficient and reproducible. However, the processing of large sample quantities for further analyses is often time consuming and subject to variability depending on the handler. KingFisher Flex is an automated purification system that is based on magnetic bead extraction, which provides a high-quality and reproducible system for purifying nucleic acids, proteins and cells. In the system, magnetic rods move particles attached to the magnetic beads through the purification steps, which enable high-quality processing with no cross-contamination between the samples or reagent carry-over. KingFisher Flex is a flexible and open system in which any available magnetic particle based purification kit suitable for the application can be chosen by the user. With KingFisher Flex, the hands-on work is minimised and productivity increased.

Here we present the extraction of nucleic acids from three common clinical sample types. DNA was isolated from large volume whole blood samples and viral RNA was analysed from buccal swab or stool

samples. Stool samples were used for the detection of norovirus RNA. The buccal swab sample generally removes only a few cells resulting in a low expected nucleic acid yield. The swab samples were analysed to detect the A/H1N1 influenza virus which causes swine flu. Clinical sample analyses require sensitive and reproducible methods for the purification of large quantities of specimens per day. To intensify the nucleic acid extraction, the sample purification was automated using KingFisher Flex. The result was rapid and efficient extraction of DNA and RNA.

Materials and methods
Instrument:
Thermo Scientific KingFisher Flex with a 24 well magnet head and a 96 deep well magnet head

Microplates/beads:
Thermo Scientific KingFisher Flex 24 deep well plate
Thermo Scientific Microtiter deep well 96 plate
Thermo Scientific KingFisher Flex 96 KF plate
Macherey-Nagel NucleoMag™ 96 Virus kit
Macherey-Nagel NucleoMag™ 96 Blood kit

Samples:
Clinical samples of whole blood, stool or buccal swab

Genomic DNA purification:
Human DNA was isolated from frozen pooled whole blood large volume (3ml) samples with King Fisher Flex using the NucleoMag 96 Blood kit (Macherey-Nagel, Germany). The purification protocol was optimised for large volume use. The modified protocol for King Fisher Flex handled 24 identical blood samples in 45 minutes. Isolated DNA was analysed using amplification and sequencing protocols.

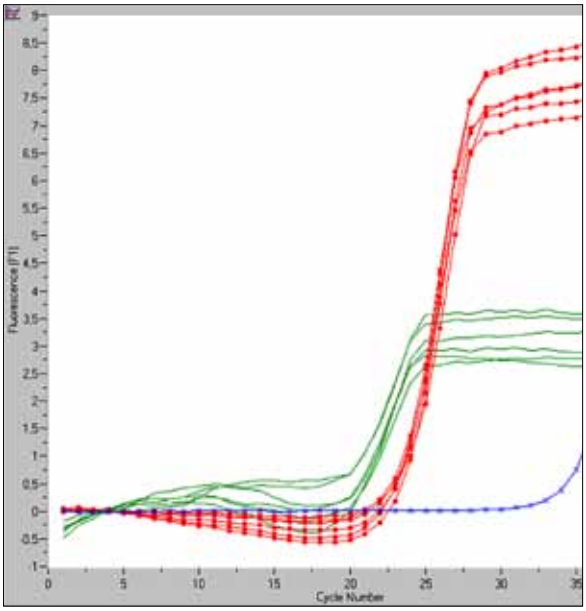


Figure 1. 2 µl of the isolated DNA was used without dilution (green lines) or diluted 10 fold (red lines) for real-time PCR (beta actin, Sybr green assay, 20 µl reaction volume).

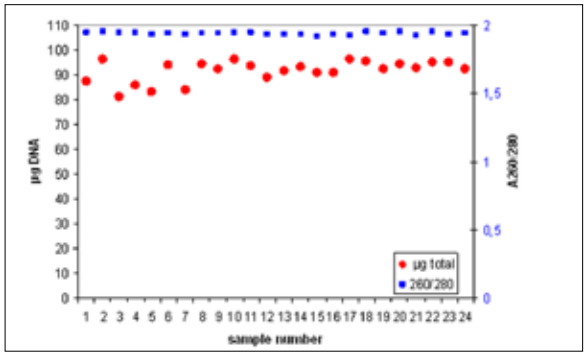


Figure 2. DNA yield and purity were determined by UV measurement. Total DNA yield 91.4 µg (mean value) and A260/280 1.94 (mean value).

Viral RNA purification:
The viral RNA was purified from buccal swab samples from patients potentially carrying the A/H1N1 virus or from stool samples for norovirus detection with KingFisher Flex using the NucleoMag 96 Virus kit (Macherey-Nagel, Germany). Swab samples were first lyzed in 500 µl of modified lysis buffer with agitation. 400 µl of this solution was chosen as a suitable amount for use in RNA extraction. The suspended stool samples were centrifuged for 15 minutes at 13.000 rpm and the supernatants were used for the extraction.

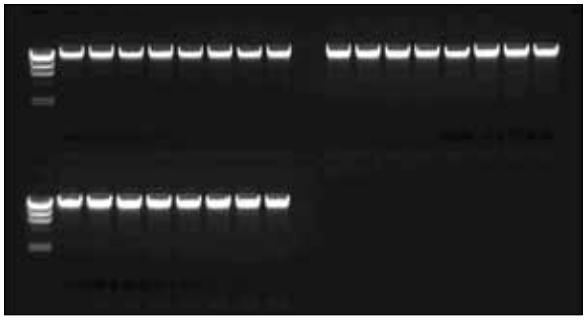
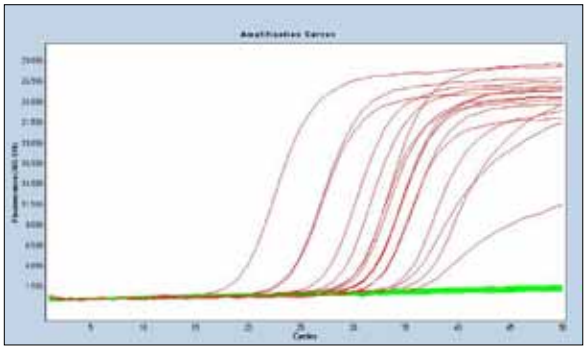


Figure 3. 10 µl from each purified DNA sample was loaded onto a 0.8% agarose gel. λ Hind III DNA ladder as a marker.

4a



4b

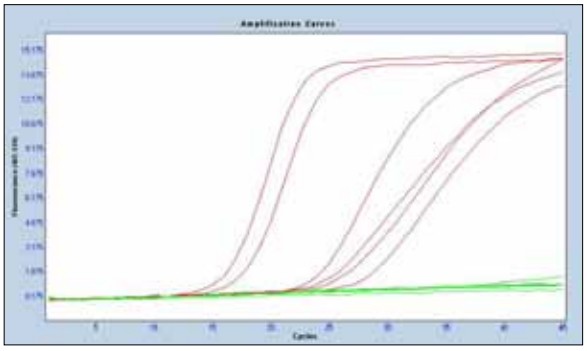


Figure 4.
A. A/H1N1 real-time PCR amplification plot.
B. Norovirus real-time PCR amplification plot.

Results

Genomic DNA purification:
All the isolated DNA samples showed reliable results using both diluted and undiluted eluates in real-time PCR (LightCycler 480, Roche) (Fig. 1). The concentration of DNA ranged from 25–40 µg/100 µl in a final volume of 500 µl DNA eluate and the sample quality was excellent (Fig. 2). Purified DNA samples were also run on an agarose gel (Fig. 3). Neither carryover nor cross-contamination was observed during the purifications.

Viral RNA purification:
The presence and quality of viral RNA was confirmed by amplification with quantitative real-time PCR. All the analysed speciments contained amplifiable RNA and gave similar Ct values, and no significant differences between the samples were detected (Fig. 4A, 4B). The extraction time of viral RNA was reduced to 60 minutes to accelerate the purification. This enabled the extraction of as many as 500–800 swab samples or 150–200 stool samples per day without any changes in the normal working routines. Even with a large quantity of stool samples, the norovirus analyses took less than four hours which shortened the delay before obtaining the results.

Conclusions

The high-quality nucleic acids were obtained from the blood, stool and swab samples. The blood samples were pooled because the amount of DNA depends on the quantity of white blood cells in the sample. Good yields of purified DNA were obtained in 45 minutes (24 samples). All DNA extracts were analysed with amplification and sequencing methods, and the results indicated an effective purification of the samples and the removal of PCR inhibitors with the KingFisher Flex.

Rapid tests for detecting viruses are often based on antibodies recognising the virus proteins. These tests are not always reliable, however, and are less sensitive than real-time PCR analyses. PCR analyses nevertheless require the purification of the samples which increases the number of procedure steps and the time required to perform the analyses. Extensive extraction protocols are necessary especially for the efficient purification of stool samples due to the complexity of the material. The purified RNA samples offered a good starting material for sensitive real-time PCR analyses of A/H1N1 influenza virus and norovirus.

In conclusion, KingFisher Flex is a reliable automated system for high-throughput DNA and RNA extraction from clinical specimens. Including hands-on work, 96 clinical samples were processed in less than 60 minutes and 24 high-volume samples in 45 minutes. The system offers efficient and reproducible purification of high-quality nucleic acids from varied and often difficult clinical samples. Automation and elimination of liquid handling results in an authentic and robust purification process.

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