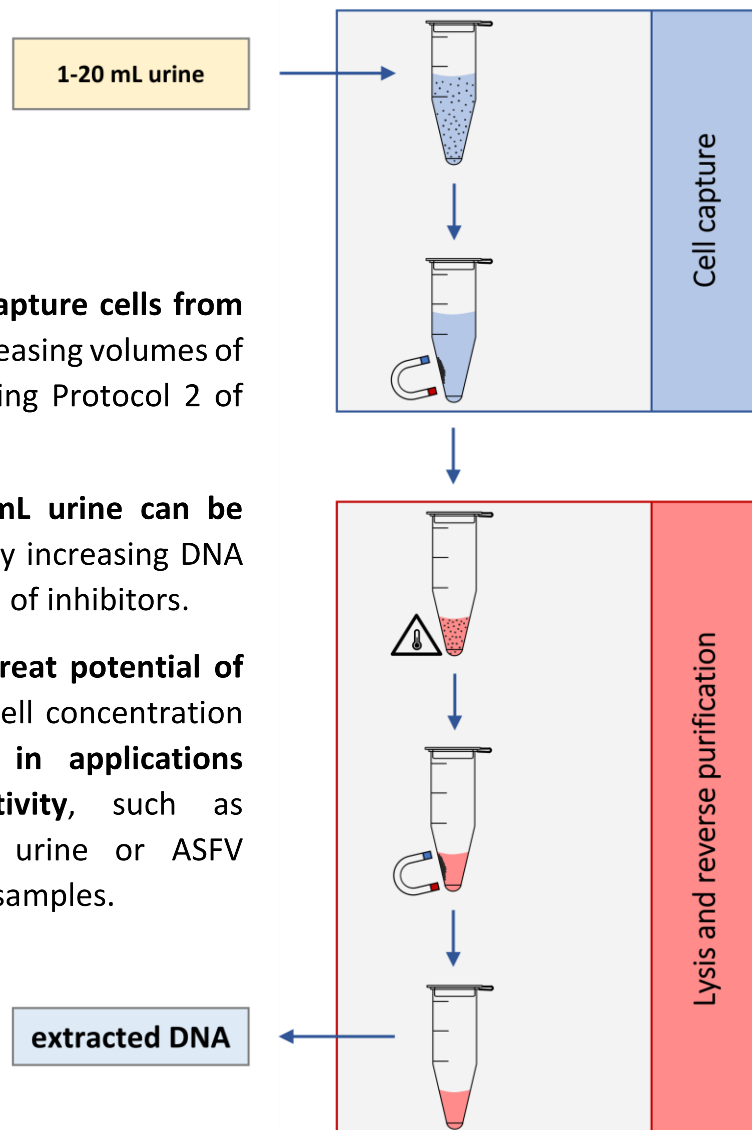
Processing of large volumes of urine for extraction of genomic DNA

Related product

This application note is related to **SwiftX™ DNA** (SXD-25).



Summary

SwiftX™ DNA was applied to **capture cells from urine to extract their DNA.** Increasing volumes of urine have been processed using Protocol 2 of the kit handbook.

It was shown that **up to 20mL urine can be processed** with a proportionally increasing DNA yield and without accumulation of inhibitors.

These data demonstrate the **great potential of SwiftX DNA** as a method for cell concentration and nucleic acid extraction **in applications requiring very high sensitivity**, such as Schistosoma detection from urine or ASFV detection from environmental samples.

Introduction

Urine specimens were obtained from volunteers and processed within 2 hours after sampling. Sample processing followed Protocol 2 of the handbook of SwiftX™ DNA (ca.no. SXD-25). The epithelial cells shed into the urine were taken as a model marker for demonstration of the cell capture and DNA extraction capabilities of SwiftX DNA. It should be noted here that the cell capturing mechanism can be used for other targets as well. One example is the capture of oocytes from *Schistosoma haematobium* in urine (Rostron *et al.* (2019) Parasites&Vectors 12:514). Another example is the capture of African Swine Fever Virus (ASFV) particles in various liquid specimens (internal data).

Results

Our study shows that SwiftX DNA enables the user to extract nucleic acids from samples of up to 20mL volume. The yield of extracted DNA increases proportionally with the input sample volume as indicated by decreasing Ct values (Figure 1).

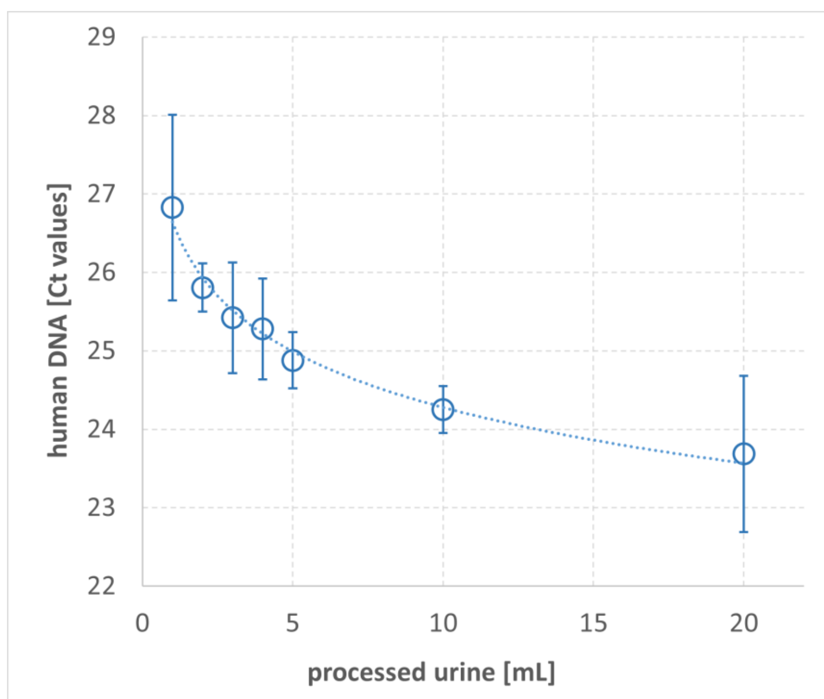


Fig. 1: Ct values of the real-time PCR for human DNA depending on the volume of urine that was processed.

Figure 2 illustrates that the increase in DNA yield is almost linear for volumes of up to 5mL urine and follows a non-linear relationship for volumes above 5mL. The reason for the non-linear behavior at higher volumes can be a saturation of the surface of the magnetic particles by the constituents of the urine samples. Further experiments are required to examine (a) if the DNA yield can be further increased by use of larger amounts of cell-capturing Beads A, and (b) if the yield-to-volume ratio follows a longer

linear trend if not urine but less complex liquid specimens such as PBS or environmental water is analyzed.

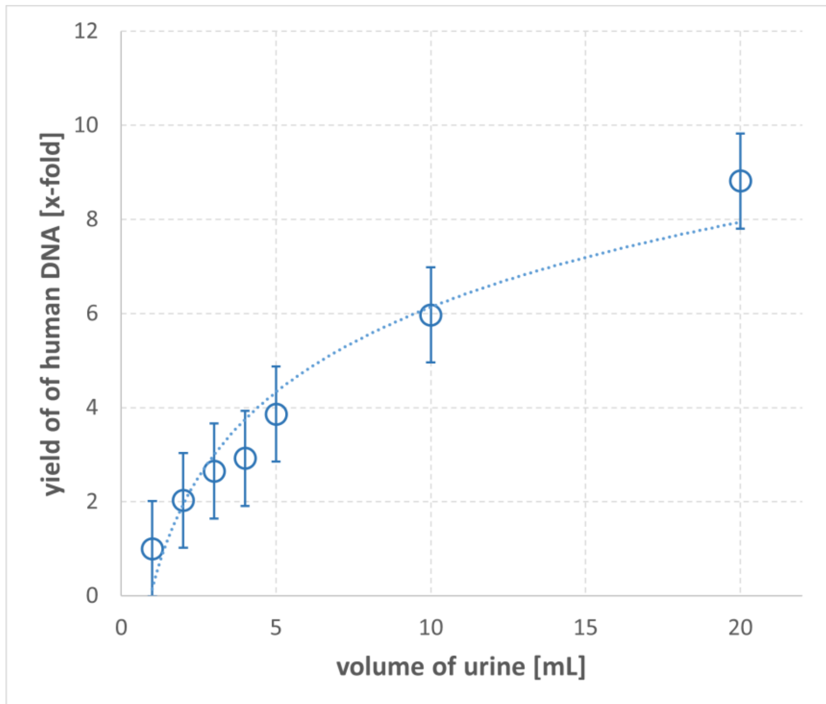


Fig. 2: Relative DNA yield of SwiftX DNA extraction in relation to the amount of urine used for cell capture and DNA extraction.

Our data also demonstrate very clearly that the processing of increasing amounts of urine with SwiftX DNA does not lead to an increased inhibitory potential of the DNA extract, which may potentially impair the PCR analysis (Figure 3).

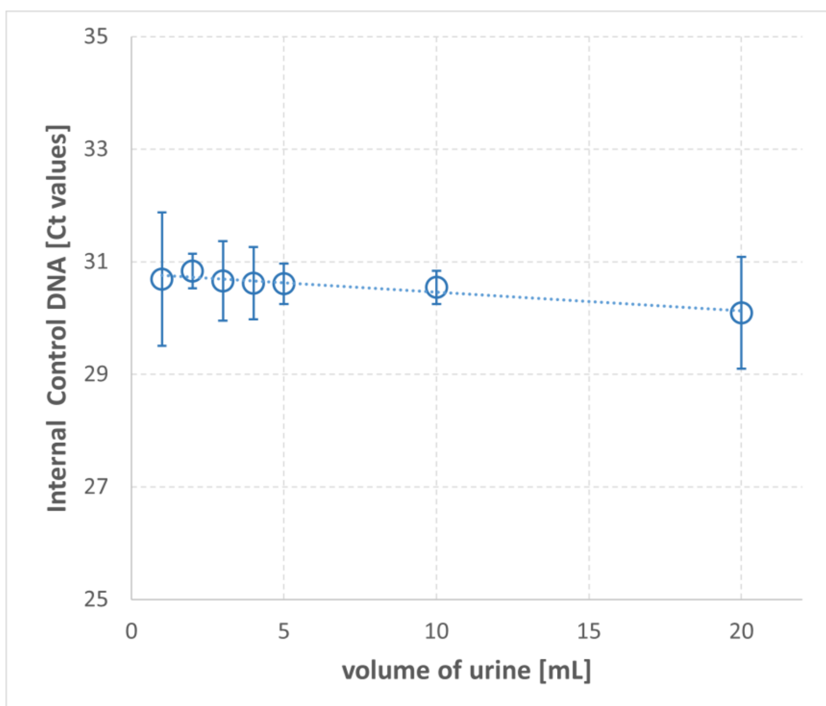


Fig. 3: Signal of the internal control of the real-time PCR kit.

Experimental details

Urine samples of volumes ranging from 1mL up to 20mL have been extracted following the instructions in Protocol 2 of the SwiftX DNA kit. For the cell capture step, the volume of Buffer EN was adjusted to the sample volume to be processed, e.g. 2mL Buffer EN was used to capture cells from 2mL urine. In contrast, the amount of the magnetic particles "Beads A" has been kept at 30µL throughout all extractions. The size of the vessel for conducting the cell capture was chosen according to the urine volume to be processed, e.g. a 15mL sample tube was used for cell capture from 5mL urine. Beads A were first mixed with the appropriate volume of Buffer EN. This beads/buffer suspension was mixed with the urine and processed as instructed in Protocol 2.

If the cell capture was performed in larger vessels, the bead suspension was first magnetically separated for 1 minute in the large vessel, then the supernatant was poured off and discarded. The remaining volume of beads/buffer suspension was mixed with a micropipette, transferred to a 1.5mL microtube, and magnetically separated again for 30 seconds. The supernatant was completely removed and discarded. Finally, the magnetic beads were further processed as described in Protocol 2 of the handbook of SwiftX DNA.

The captured cells were resuspended in 100µL Buffer DL and lysed by 10 minutes incubation at 95°C in a heat block. This releases the cellular human DNA as well as any potentially present bacterial and viral DNA. After the lysis, cell debris and potential inhibitors were removed from the lysate by reverse purification as described in the handbook. The amount of extracted human DNA was quantified using a commercial real-time PCR kit (QIAGEN Investigator Quantiplex).

Additional **Buffer EN (cat.no. BEN-25mL)** for processing higher sample volumes can be purchased from Xpedite Diagnostics.

For this work, the **magnetic separation rack (cat.no. MAG-12)** was used, which is available for a competitive price from Xpedite Diagnostics.