MAGNETIC NANOPARTICLES IN NUCLEIC ACID EXTRACTION FROM VIRUSES AND BACTERIA IN DIAGNOSTICS AND RESEARCH

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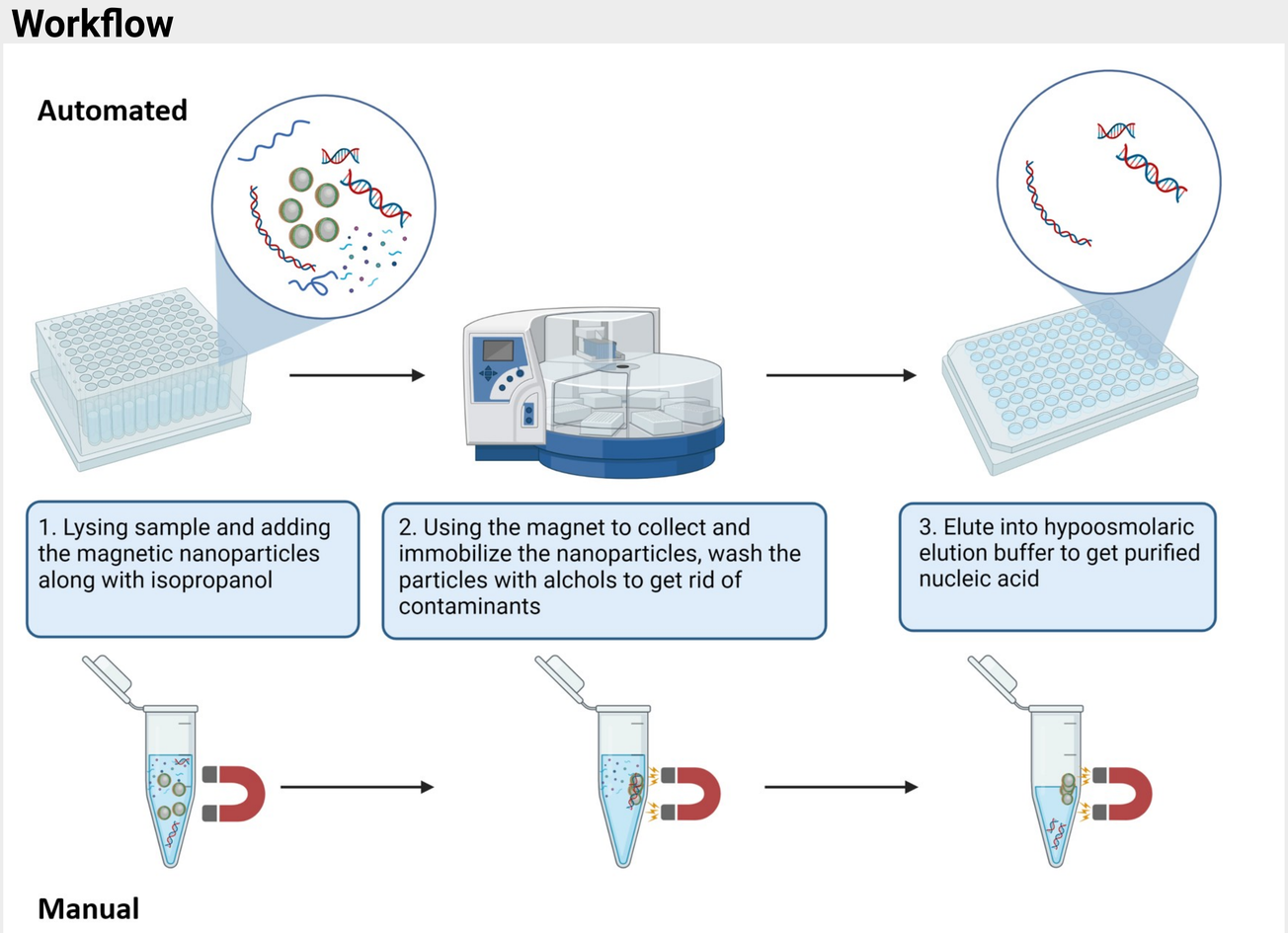
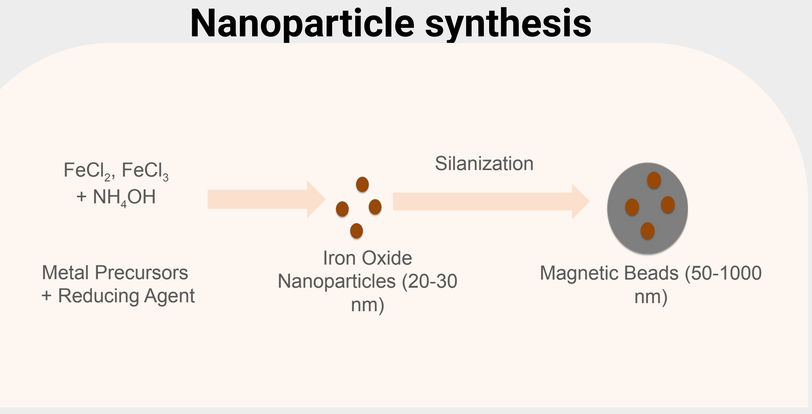
Abstract

During the pandemic caused by the SARS-CoV-2 virus which was first identified in Wuhan, there was a sudden and drastic increased demand for nucleic acid (NA) extraction kits. This caused shortages and hampered the accurate tracking of the pandemic within countries. The NAxtra method was first developed at NTNU to provide the local hospitals with such kits. Using magnetic nanoparticles covered in silica, it was possible to isolate RNA from patient samples and test them for viral-RNA with qPCR. The method was further tested on other viruses and showed promise as a robust diagnostic method for several pathogens. With small amounts of preparatory work, the method also proved useable for NA from bacteria and mammalian cells. Herein, we demonstrate the NAxtra™ methods versatility as a NA extraction method, showing extraction results from differing types of viruses and bacteria. We also discuss the importance of the varying preparatory work relating to the different samples. Such factors include whether to homogenize a sample, enzymatically aided lysis, heat-mediated enzyme treatment, and longer lysis times.

Workflow



Nanoparticle synthesis



Results

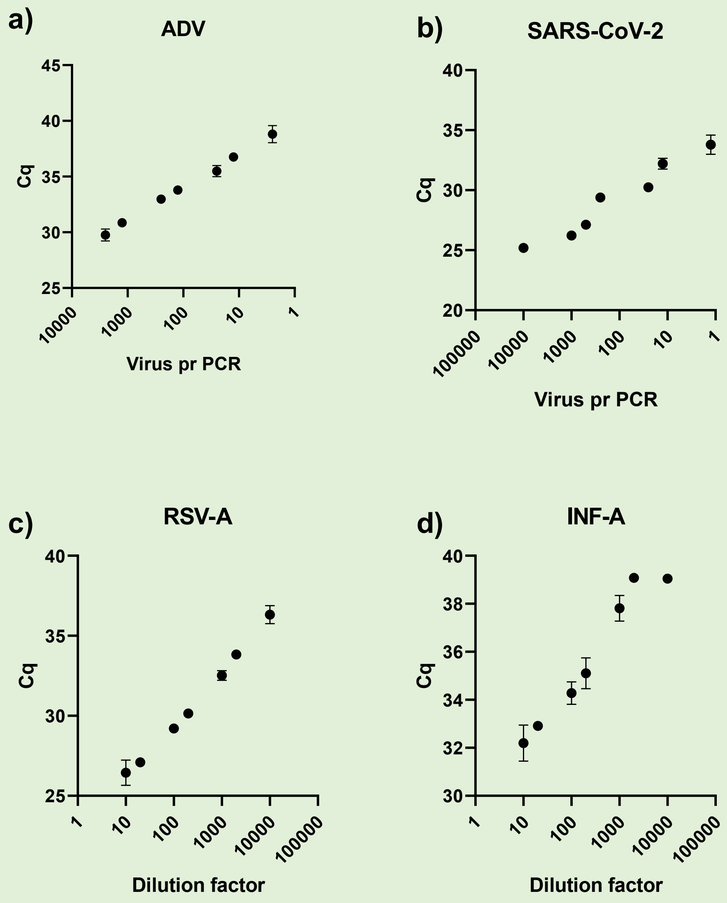
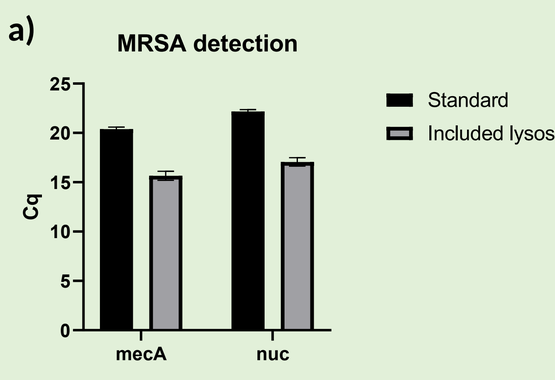
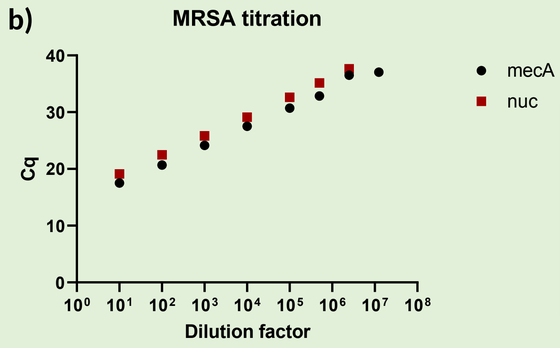


Figure 1: Application of the NAxtra™ method on four Qnostics analytical panels. Panels a) and b) had quantified number of viruses, while prearranged dilutions were provided for c) and d). Eluates were analyzed via qPCR. Error bars show the standard deviation from 3 technical replicates for ADV, INF-A & SARS-CoV-2 and 4 technical replicates for RSV-A.



	Standard		Lysostaphin	
Target	Avg.	SD.	Avg.	SD.
mecA	20,39	0,21	15,66	0,45
nuc	22,17	0,18	17,06	0,43



Number of MRSA per PCR		
Dilution factor	Avg.	SD.
1x10^5	116	12,17
5x10^5	20,67	4,62
2,5x10^6	4	2,83
12,5x10^6	NA	NA

Figure 3: Application of the NAxtra™ method on MRSA. **a)** Result from experiment on MRSA comparing the use of the standard NAxtra™ lysis method versus inclusion of 2 µl lysostphin. Resulting eluates were measured on qPCR. **b)** Extraction using the NAxtra™ method on a titration of MRSA to test limit-of-detection.

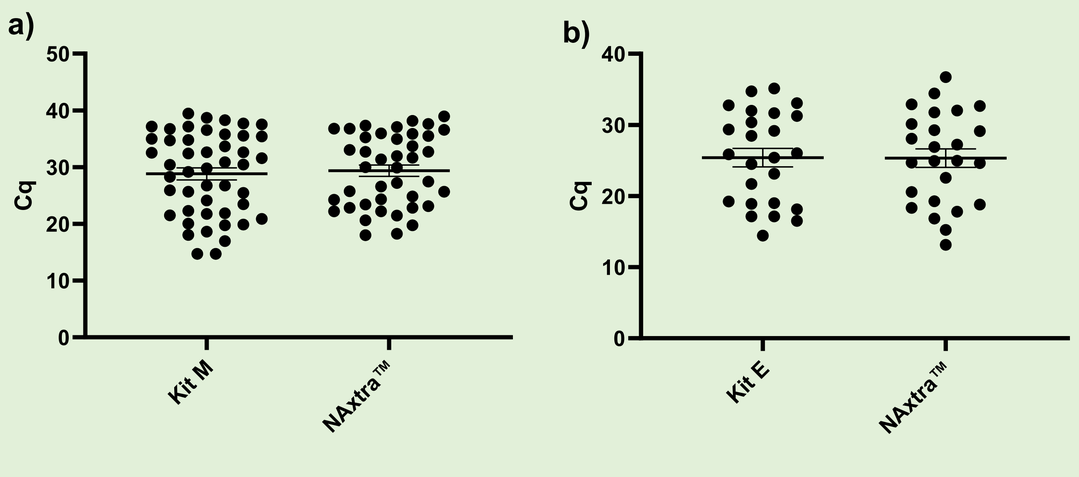


Figure 2: a) Validation data of the NAxtra™ protocol on a Tecan Fluent® 1080 robot platform. 47 samples from COVID-19 patients were used to evaluate nucleic acid extraction using kit M and NAxtra™ methods. Experiment performed by Oslo University Hospital. Eluates were analyzed using commercially available PCR reagents (Superscript). Error bars represent standard error of the mean (SEM). **b)** Validation data of the NAxtra™ protocol on the KingFisher™ Flex system. Tested on 25 different COVID-19 patient samples. Experiment performed by Trondheim University Hospital. Error bars in SEM.

Summary

We have demonstrated that the NAxtra™ method can extract nucleic acid from several types of viruses and bacteria, showing the method is robust in tackling a variety of different biology. It's ease-of-use and sensitivity is shown in the implantation of the method at both Oslo University Hospital and St. Olavs Hospital during the pandemic when available RNA extraction methods were scarce. The Qnostics panels also prove the sensitivity of the method, with NAxtra™ being able to detect down to 2 viruses per PCR reaction.

Future work

Further development is already ongoing, and application of the NAxtra™ method on other biological samples will be expanded. Further work will also be done on the nanoparticles themselves to be able to target biological samples of choice.