Sample preparation methods for an integrated diagnostic POC-system

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Goals and introduction

The goal of this work was to find sample pre-treatment methods that are suitable for integration into a fully automated point-of-care (POC) molecular diagnostic device utilizing PCR for analysis.

The methods have to work well on different types of clinical samples, as well as on different types of pathogens.

Automation and integration of sample pre-treatment methods is important in order to minimize human error and guarantee correct and consistent treatment of the sample every time – and thereby guarantee correct test results. It also saves time and requires less training of personnel.

Methods

Lysis

Lysis of the pathogens is necessary to obtain the nucleic acids inside the micro-organisms. Micro-organisms that are difficult to lyse need mechanical lysis methods, e.g. bead beating.

Bead beating (figure 1) can be done by using an external electromagnet that rotates a small magnet in the test tube. The rotation makes the small beads added to the tube move vigorously, thus crushing the pathogens in the liquid sample.

With bead beating, the used micro-organism was *Bacillus subtilis* spores.

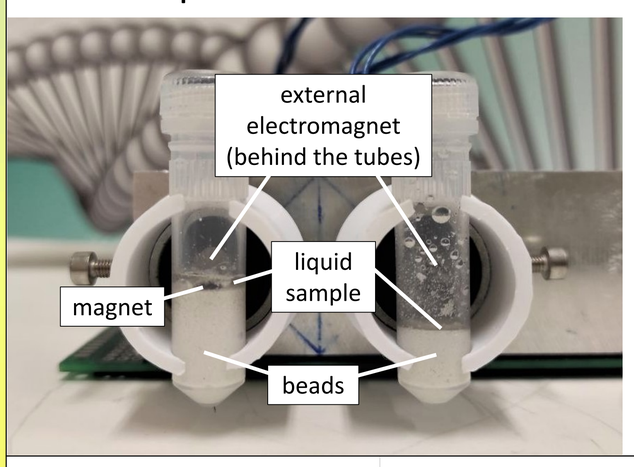

Figure 1. Electromagnetic bead beater with two slots for test tubes. An external electromagnet is behind each tube. The magnet in each tube is buried in the beads.

Purification

After lysis, the obtained nucleic acids need to be purified for analysis. Comparisons were done between silica matrix and gel filtration (figure 2).

The samples were nasopharyngeal swab samples spiked with inactivated SARS-CoV-2.

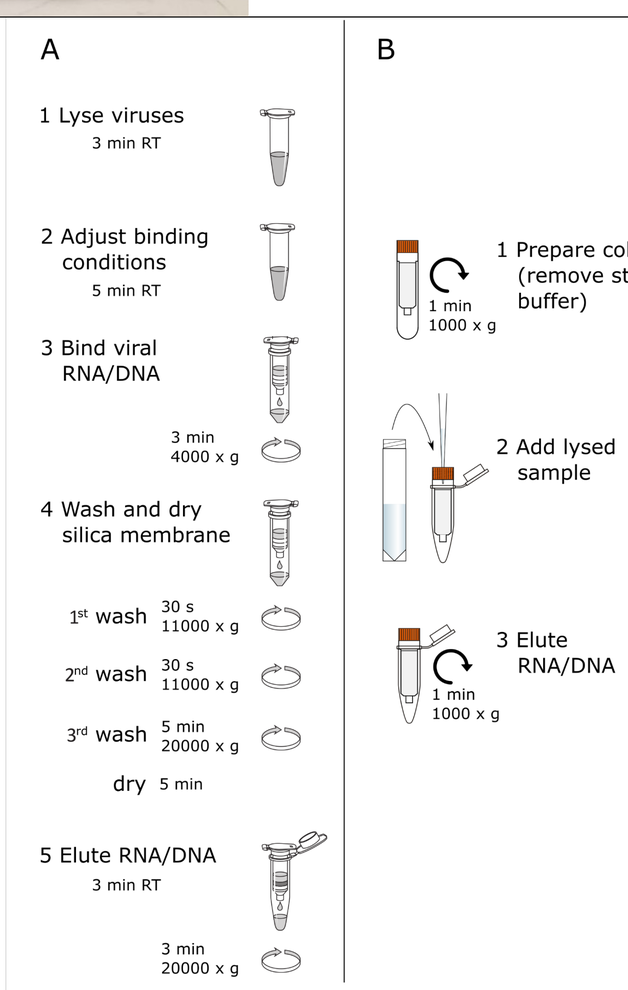

Figure 2. Purification of nucleic acids with a silica column (A) is more complicated and time-consuming than using a gel column (B).

Results and discussion

Lysis

Bead beating with an external electromagnet has produced promising results (figure 3). To obtain the same level of lysis as the reference vortex method, optimization is still needed. A problem with a high bead to sample ratio is that the sample is more difficult to pipette.

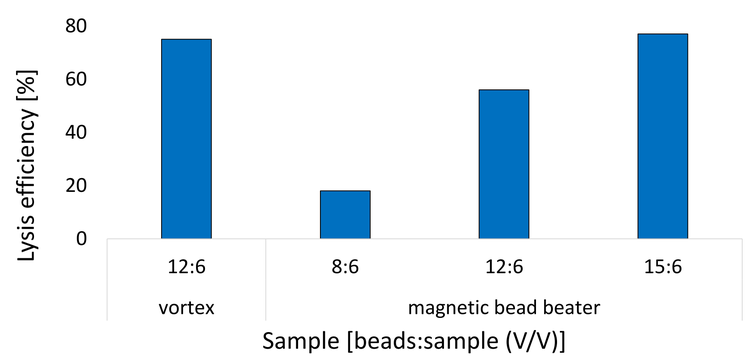

Figure 3. Lysis efficiencies with bead beating. The more beads there are relative to the liquid volume, the more efficient the lysis.

Purification

With the silica column the sample is concentrated, which results in an earlier Ct (threshold cycle) than with the gel column (figure 4).

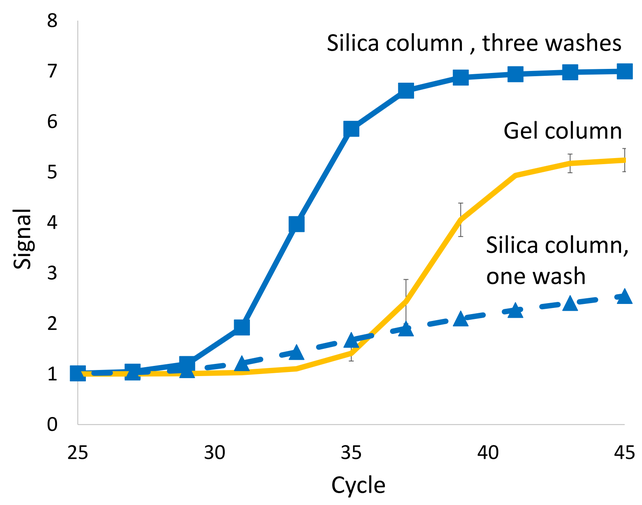

Figure 4. PCR-curves of samples purified with a silica column using one and three washes, as well as using a gel column. With only one wash of the silica column, too many inhibitors are eluted with the nucleic acids for the PCR to work without interference.

Conclusions

For a method to be suitable for integration into a diagnostic device, it needs to be as simple and amenable to automation as possible. There is, however, always a compromise between simplicity and performance. Several parameters need to be carefully considered before method selection.

References

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