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Abstract

A rapid and easily workable alternative (MEP: *Munich Extraction Protocol*) is outlined to our current RNA purification method for circumstances where there is limited availability of test kits. Details are available upon request.

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The outbreak of the new coronavirus SARS-CoV-2 is a significant challenge for the analytical performance of laboratories *(1, 2)*. In addition to the test capacity, this service also includes coping with requirements for increased supply of the necessary reagents.

We hereby present, in outline, a fast and easily workable alternative to our current RNA purification method for circumstances where there is limited availability of test kits. We also report on the increase of test capacity without modifying the equipment pool. This *Munich Extraction Protocol* (**MEP**) approach draws upon first principles of molecular biology that are in danger of being lost due to the normal availability of ready-to-use kits.

Nasal and throat swabs that are transported in a special liquid viral medium to the laboratory were used. An aliquot of the sample was transferred into a cavity of a 96-well plate. SARS-CoV-2 RNA extraction was performed either with two commercially available tests (MagNA Pure 96 Roche Diagnostics (Penzberg, Germany) and the Chemagic Viral DNA/RNA 300 Kit H96 (PerkinElmer Chemagen Technologie, Baesweiler, Germany); both tests require about 60 min per 96 samples). In addition we tested a fast method, which is based on a dilution step and heating to 92 °C for 10 minutes. The amplification was done in a Roche LightCycler® 480 II with an RIDA®GENE SARS-CoV-2 RUO assay from R-Biopharm.

Despite significant time saving and the use of readily available reagents, clinically acceptable sensitivity losses were seen with our “Munich Extraction Protocol (**MEP**)” (Fig 1).

With the commercial kits one dilution level better sensitivity could be measured. One reason is that a 10-fold higher amount of template RNA can be used in these kits. This reduction in the amount of RNA used is due to the fact that commercial kits use purified RNA, whereas we use matrix-contaminated RNA. In this case it is common practice to reduce the amount of material used in order to avoid inhibition. We noticed that while the MEP method perfectly performs with standard samples a delay of crossing point (CP) values is observed with samples contaminated with hemoglobin.

Due to the much shorter extraction time using MEP, the test capacity can readily be increased by a factor of four.

In a further experiment we transferred aliquots from four 96-well plates to one 384-well plate and adapted the real-time PCR protocol according to *(3)*. The number of positive cases remained the same for both methods.

The presented modifications in the analytical process enable laboratories to increase their independence from potential reagent supply bottlenecks and to increase the test capacity without major modifications in the laboratory workflow. The medical quality is maintained. We will be pleased to provide interested colleagues with the detailed methodological protocol.

References

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Figure 1

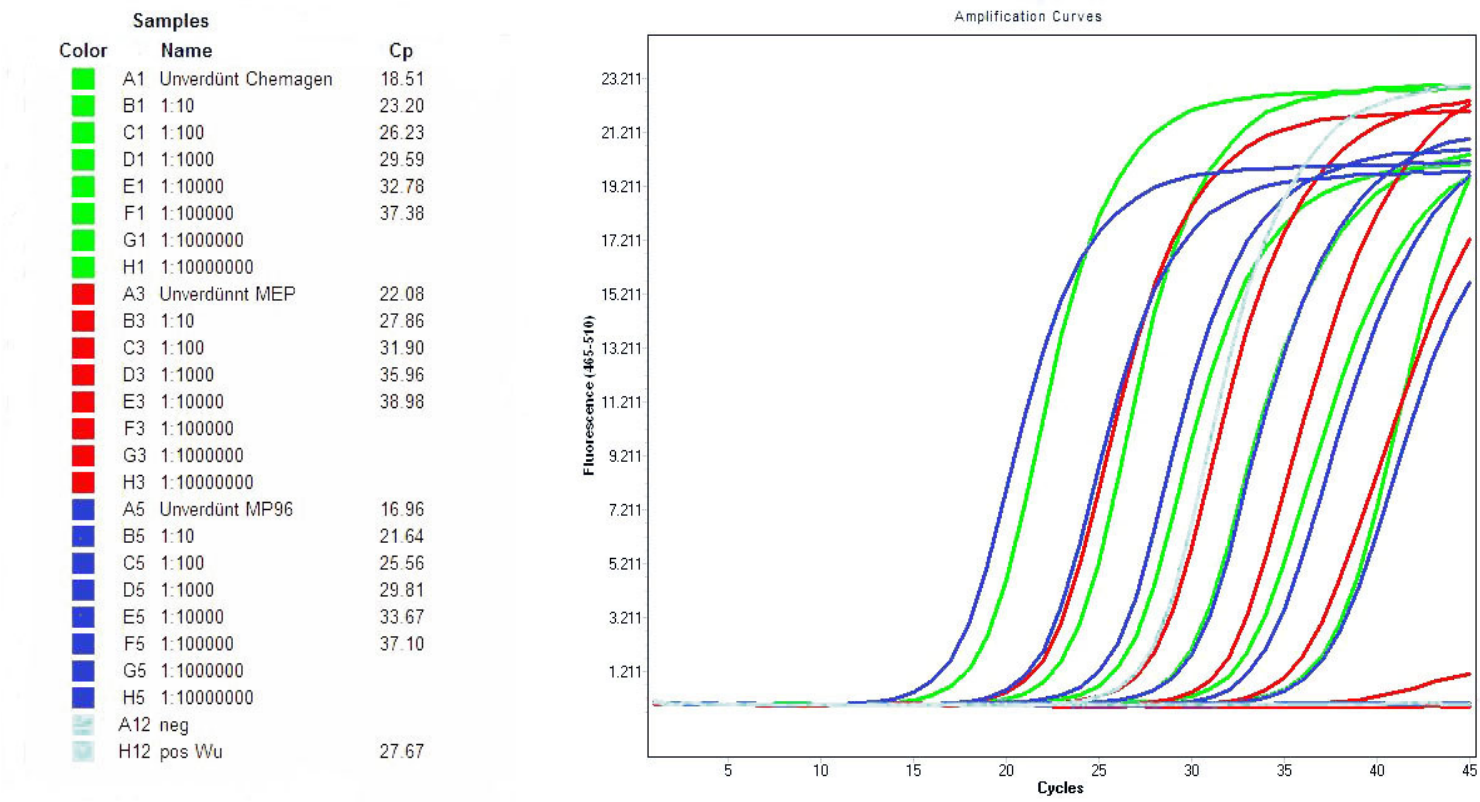

Figure captions

Figure 1. The amplification curves of the different preparation protocols (right side) and the corresponding dilution levels (left side; green: Chemagic Viral Kit, red: MEP; blue: MagNA Pure 96) are shown.
When using the undiluted sample, an increase in fluorescence was observed with the commercial kits from about 15 cycles. When using the MEP, this increase was observed after about 20 cycles. At this number of cycles an increase of the fluorescence signal of the first dilution stage (1:10) could be measured when using the commercial kits. When using the MEP, approximately 1:10 of the template RNA is used compared to the commercial kits.