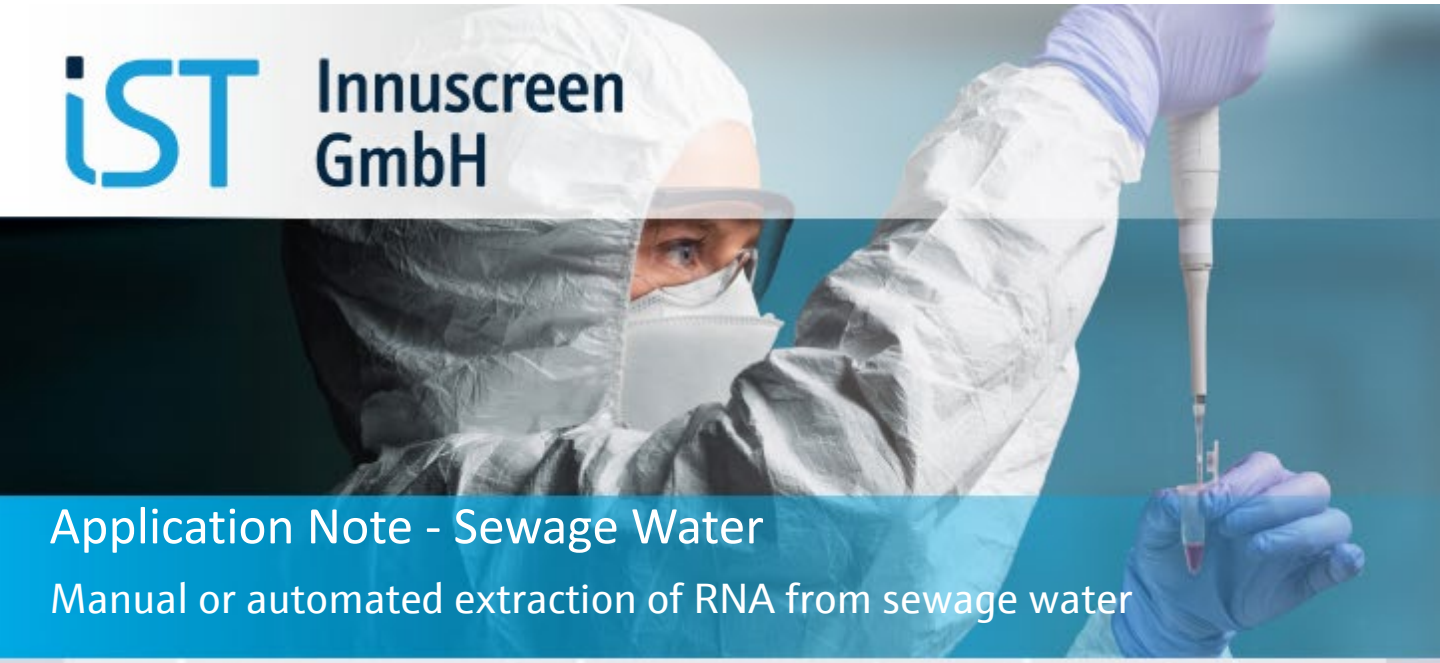
Innuscreen GmbH



Application Note - Sewage Water

Manual or automated extraction of RNA from sewage water

Introduction

The investigation of sewage water for the detection of Covid-19 as an epidemiological early warning system is becoming increasingly important. In this context, it is important to have a simple and quickly practicable method for extracting amplifiable viral RNAs from sewage water. IST Innuscreen GmbH has developed a new, simple, fast and efficient process for extracting viral RNA from sewage water. The process can be carried out manually or automatically for small to large numbers of samples. It does not require any filtration, ultracentrifugation or complex PEG precipitation methods. The process is based on a new, patented technology for the enrichment of viral particles or viral fragments and the subsequent extraction of the viral RNA. The viral nucleic acid is ready for further analysis within approx. 1.5 hours.

Workflow:

PME-Enrichment

Sample mixed with two components (Reagent 1 and Reagent 2) – forming a biomolecule/polymer-complex

Centrifugation of the biomolecule/polymer-complex

Dissolving of the biomolecule/polymer-complex

Low to high throughput extraction

Magnetic Particle-Based Extraction

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Automated Protocols:

Low to Medium Throughput Automated Extraction with InnuPure C16touch (845-00020-2, Analytik Jena GmbH):

20 ml of sewage water sample is first processed with the PME Sewage Water Enrichment Tool (845-IR-0010050, IST Innuscreen GmbH). The dissolved biomolecule/polymer complex is transferred to the reagent plastic of the innuPREP Anipath DNA/RNA Kit-IPC16 (845-IPP/PPP/IPS-8016016/96/480) followed by extraction on the InnuPure C16touch.

High Throughput Automated Extraction with KingFisher Flex 96 (845-KF-1196000, IST Innuscreen GmbH):

20 ml of sewage water sample is first processed with the PME Sewage Water Enrichment Tool (845-IR-0010050, IST Innuscreen GmbH). The dissolved biomolecule/polymer complex is transferred to the reagent plastic innuPREP KFFLX Plate Set (845-KF-1296010, IST Innuscreen GmbH) of the innuPREP Anipath DNA/RNA Kit-KFFLX (845-KF-5296096/480/9600, IST Innuscreen GmbH) followed by extraction on the KingFisher Flex 96.

High Throughput Automated Extraction with CyBio FeliX (OL5015-24-100 & OL5015-25-120, Analytik Jena GmbH):

20 ml of sewage water sample is first processed with the PME Sewage Water Enrichment Tool (845-IR-0010050, IST Innuscreen GmbH). The dissolved biomolecule/polymer complex is transferred to the reagent plastic of the innuPREP Anipath DNA/RNA Kit-FX (845-FX-2396096/480, IST Innuscreen GmbH) followed by extraction on the CyBio FeliX.

Application Example Manual Extraction (data kindly provided by Tamara Härpfer, Endress+Hauser BioSense GmbH)

20 ml of sewage water sample, taken from the Abwasserzweckverband Breisgauer Bucht, was processed with the innuPREP Sewage Water DNA/RNA Kit (845-KS-4920050, IST Innuscreen GmbH) according the user manual.

Reagents and Instrumentation

- innuPREP Sewage Water DNA/RNA Kit (845-KS-4920050, IST Innuscreen GmbH)
- AcroMetrix™ Coronavirus 2019 (COVID-19) RNA-Kontrolle (RUO) (954519, Thermo Fisher)
- SARS RT-PCR test kit (40-0776-96; TIB MOLBIOL)
- Standard lab equipment
- CFX Connect Real-Time PCR Detection System (BIO-Rad)

Results and Discussion

After manual RNA extraction of 10 ml supernatant with the innuPREP Sewage Water DNA/RNA Kit the RNA was eluted in 50 µl RNase-free water. 5 µl of the extracts were used for qRT-PCR-based detection of SARS-CoV-2 in the FAM channel. Four replicates were processed without any additions and the 5th replicate was processed with 2500 copies of an artificial Covid-19 RNA to monitor the extraction efficiency. In order to detect inhibitory effects of substances contained in the extracted RNA an internal control was added to each sample and detected in the Cy5 channel.

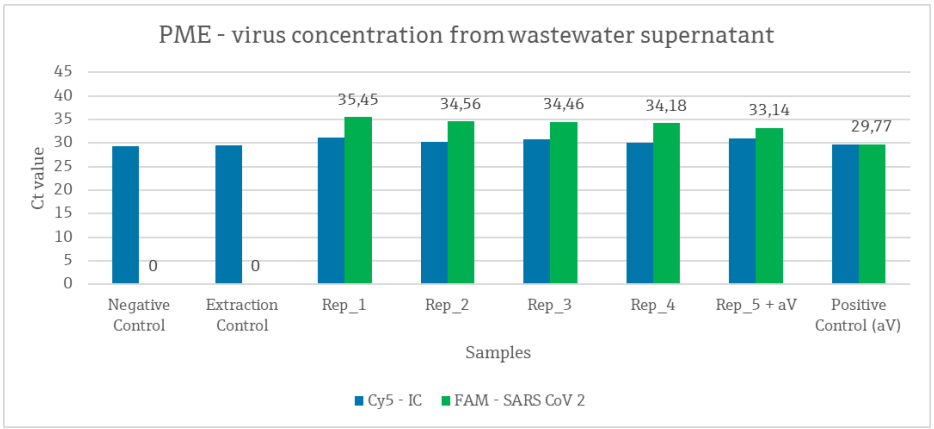

Figure 1: Extracted RNA, amplified and detected with qRT_PCR

The results show that the innuPREP Sewage Water DNA/RNA Kit can be used to prepare viral RNA from wastewater. Quality and quantity are sufficient for subsequent qRT-PCR application as depicted in Figure 1. Sample 5 shows that the artificial RNA was extracted successfully, resulting in a higher RNA concentration and thus a lower ct value. The internal control shows no inhibition in each sample.