Tech Note – Wastewater Workflow
SpeedMill PLUS

Evaluation of the nucleic acid preserving Zymo Research DNA/RNA Shield as elution and conservation medium for Analytic Jena's nucleic acid extraction and PCR analysis workflow for wastewater samples

Introduction

In the early phase of the COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2, the possibility to detect the virus in wastewaters was evaluated. Based on the corresponding results and data inferred from other pathogens, the European Commission recommended the implementation of a wastewater-based epidemiology (WBE) approach for pandemic monitoring in its member states in March 2021. In parallel, Analytik Jena developed a functional workflow to purify and analyze wastewater samples.

This Tech Note presents the use of Zymo Research DNA/RNA Shield as filter extraction and conservation medium, which not only allows interim storage of samples and their transport at ambient temperature, but also increases the sensitivity of the workflow to improve pathogen detection in diluted samples such as wastewater. In addition, it inactivates bacteria, viruses, fungi, and parasites and thus increases application security of wastewater-derived samples.

Your Benefits

- Improved process stability for DNA/RNA-based molecular biology workflows using an established preservation solution
- Flexible experimental scheduling and logistics
- Improved analytical sensitivity
- Improved user safety through inactivation of infectious agents

Challenge

Wastewater is among the most challenging matrices for molecular analysis. First, analytical targets can be highly diluted, which usually requires a concentration step during sample purification. Second, PCR inhibitors must be removed for a reliable detection. Third, the sample is biologically and enzymatically active, which complicates the detection of RNA, which is prone to fast degradation. The Analytik Jena WBE workflow developed for SARS-CoV-2 detection addresses the first two challenges (Link). By inclusion of Zymo Research DNA/RNA Shield, the process not only gains stability through preservation of the sample – results show that the process sensitivity is significantly improved using the medium.

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Materials and Methods

The analytical workflow, required instrumentation, kits, and reagents are described in a separate publication (Link). The experimental adaptation which is subject of this note concerns the treatment of the electro-negative filters (HAWP04700, Merck Millipore) used to collect and concentrate the nucleic acids contained in wastewater:

After filtration, one half of the membrane is folded and vigorously extracted using a homogenizer (SpeedMill PLUS, 845-00007-2, Analytik Jena) and beaded tubes (innuSPEED Lysis Tubes J, 845-CS-1120100, Innuscreen GmbH). This process releases filter-bound organisms, virions, and adsorbed nucleic acids through liquid elution and mechanical force.

Originally, PBS (phosphate-buffered saline) was used to mediate elution/desorption. Here, PBS was replaced by DNA/RNA Shield (R1100-250, Zymo Research). A representative, SARS-CoV-2-positive wastewater sample was filtered in ten replicates. Five of the samples each were allocated to the two different treatment groups. One series of replicates was directly extracted using PBS, the other series was stored for 6 days in DNA/RNA Shield and then processed. Other process steps remained unchanged. In the downstream PCR, each of the five extracts was measured in duplicate. The local 7-day incidence within the corresponding catchment area was 38 per 100,000 residents at the time of sampling.

Results

In the case of PBS extraction, the wastewater sample was at the limit of detection. At Ct values above 35, amplification becomes successively inconsistent because of the low number of targets per reaction. At the same time, standard deviations increase. Two extractions resulted in negative PCR, and only one extraction showed consistent amplification in both PCR replicates. In total, six of the ten PCR reactions failed.

In contrast, all extractions and their corresponding PCR duplicates were clearly PCR positive when DNA/RNA Shield was used for the filter extraction. Moreover, the comparably low Ct values indicate a substantially improved nucleic acid extraction efficiency. Also, the process adaptation leads to improved stability when comparing replicates, indicated by the Ct standard deviation of less than half a PCR cycle.

Conclusion

Zymo Research DNA/RNA Shield has two major effects on the WBE workflow quality:

First, the preservation effect adds substantial process flexibility at critical points which may impair the feasibility of WBE in practice, namely limited cooling, storage, and transport capacities. A filter stored in DNA/RNA Shield requires little space compared to a wastewater sample bottle.

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Extraction replicate	PCR replicate	PBS extraction	Zymo Research DNA/RNA Shield extraction
		Ct	Ct
1	1	34.52	30.7
	2	38.02	30.51
2	1	37.03	30.05
	2	no Ct	31.13
3	1	34.46	29.49
	2	no Ct	29.93
4	1	no Ct	30.78
	2	no Ct	30.51
5	1	no Ct	30.3
	2	no Ct	29.98
Average Ct ± SD		36.01 ± 1.8	30.34 ± 0.48

Figure 1: Results of the sample preservation/transport experiment (explanation: see text).

In addition, residual enzymatic and microbial activity limits sample storage even at refrigerate temperature, which complicates sample logistics, e. g., at remote locations. The pressure-driven filtration equipment is mobile, which enables the initial sample concentration step to be executed at the sampling site. The use of DNA/RNA Shield permits convenient shipment of such samples to the analytical lab.

Second, the SpeedMill PLUS extraction of the filter turned out to be substantially more efficient using Zymo Research DNA/RNA Shield. For the use in wastewater monitoring for flexible pandemic management, reliable and sensitive detection is crucial for early measures. At nucleic acid sample concentrations which occur during low-prevalence periods, this efficacy improvement can make the difference between a positive and negative result. In average, the sensitivity gain was more 5-6 PCR cycles, or an approx. 50-fold increase in sensitivity, respectively. The consistent detection of SARS-CoV-2 at a regional 7-day incidence below 50 per 100.000 renders the improved workflow a useful tool for pandemic management, because during past pandemic waves, political decisions such as lockdowns happened at those incidence values.

Concluding, DNA/RNA Shield has been shown to be a key element for the improvement of the Analytik Jena WBE workflow flexibility and sensitivity.

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