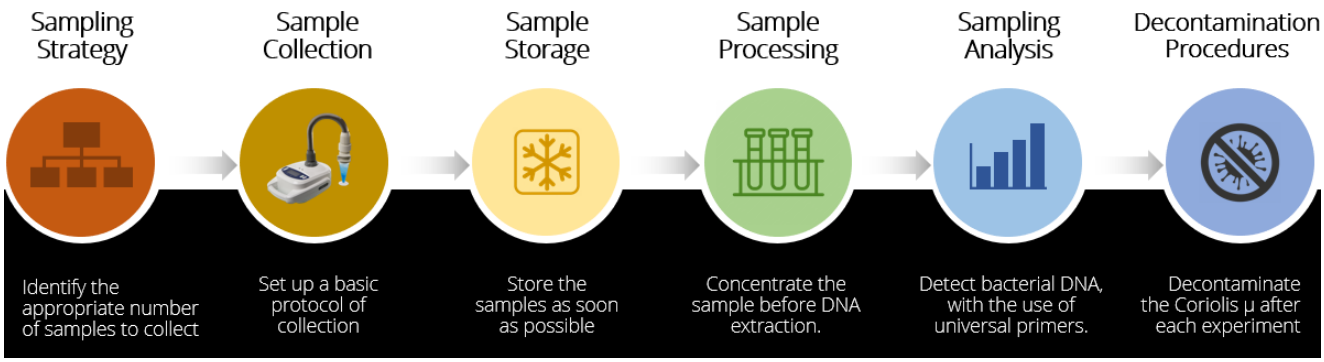
GUIDELINES FOR THE DETECTION OF BACTERIAL DNA IN THE AIR WITH CORIOLIS

The composition and concentration of bioaerosols in the air is a critical parameter to consider for the welfare of animals and humans. Monitoring bioaerosols by air sampling is one of the most efficient methods available. Nonetheless, the detection of DNA from bioaerosols can be challenging without the appropriate tools and the optimization of sampling and detection protocols. The use of the **Coriolis µ** air sampler itself is fairly simple thanks to its user-friendly design and cyclonic collection on liquid. Nonetheless, there are many other factors that need to be considered during the workflow in order to obtain the best results possible.

In this Application Note we provide, through examples, a guide on **How to analyze your Coriolis µ air sample for the detection of bacterial DNA.** The recommendations of this guide will allow you to verify the effectiveness of your air sampling protocol, by confirming that you have successfully collected bacterial DNA from the air.



Sampling strategy: Choose a sampling strategy adapted to the environment (room size, air flow patterns, etc). Place the **Coriolis µ** at least 1 meter above ground level. Depending on the size of the sampling area, plan to collect at several different points across the area, if possible.

Sample collection: As a basis for validating the technique for DNA extraction, set up a basic protocol of collection of 300L/min for 10 minutes using 15 ml of collection liquid, either indoors or outdoors. Then, based on the results obtained, adjust the collection time.

Sample storage: For the detection of DNA, we recommend to store the samples as soon as possible at 4 °C or -20 °C, for long term storage.

Sample processing: To increase the yield of DNA, given the relatively low concentration of bacteria expected in a sample of 1 m^3 of air, we highly recommend to concentrate the sample before DNA extraction. For sample concentration, use either a filtration (Amicon Ultra 15 100 Kda) or a centrifugation (>16000 rpm for 10 minutes) technique.

Sample Analysis

DNA extraction: The extraction of bacterial DNA can be carried out with the technique of your choice. We recommend the use of a kit specific for bacterial DNA.

qPCR: For the detection of bacterial DNA, the use of universal primers for the amplification of 16S rRNA is a current practice in molecular biology. For this reason, it is also important to select a mix that is sensitive and precise enough to perform reliable PCR assays.

Decontamination: Decontaminate the **Coriolis µ** after each experiment. The cane and the air intake can be autoclaved.

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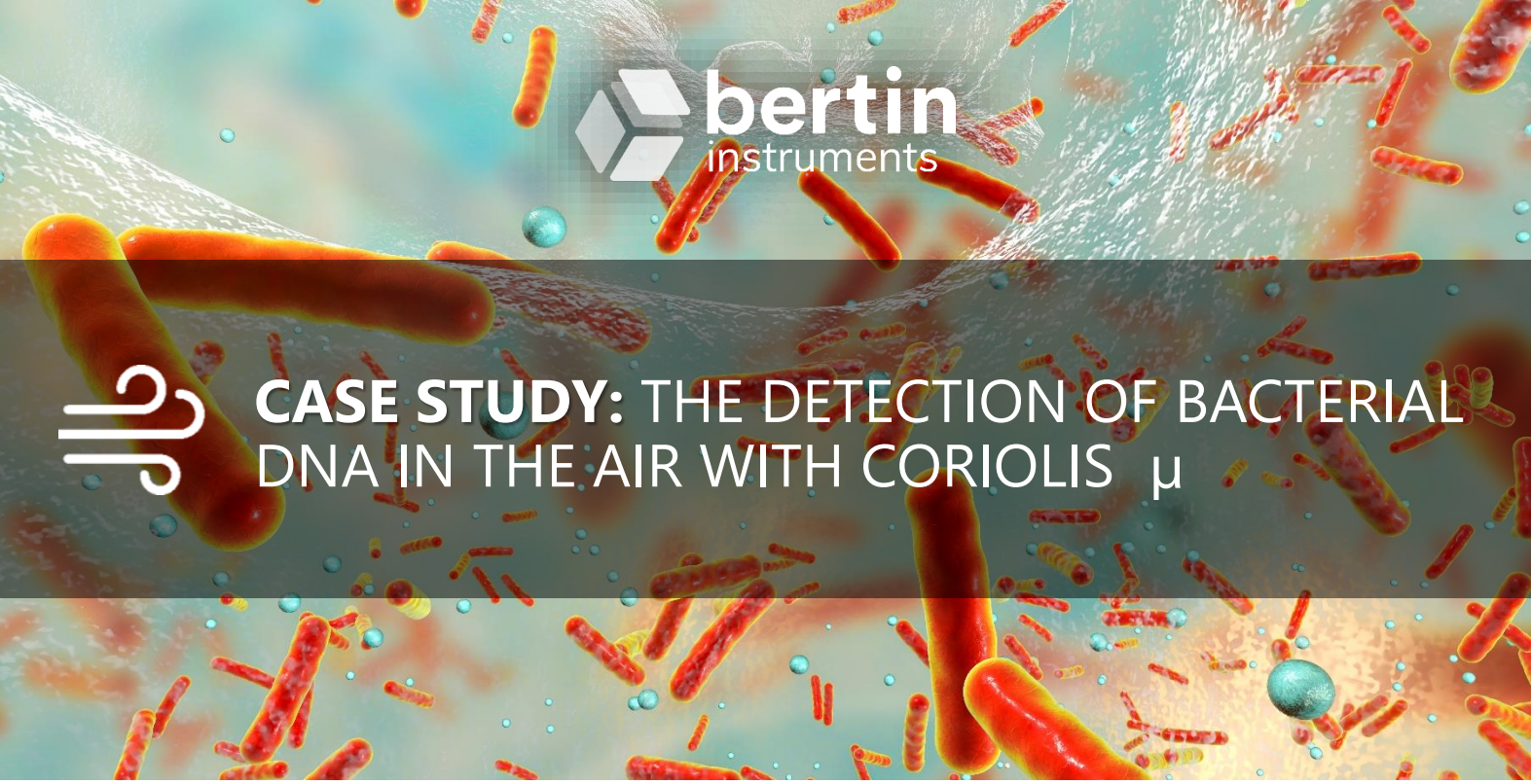

CASE STUDY: THE DETECTION OF BACTERIAL DNA IN THE AIR WITH CORIOLIS µ

DETECTION OF BACTERIAL DNA INDOORS AND OUTDOORS WITH THE CORIOLIS µ

Bertin Instruments, Montigny-le-Bretonneux, France

MATERIAL AND METHODS

Sampling strategy: The **Coriolis µ** was placed at approximately 1.2 m above ground level. Outdoors samples were taken on the common grounds of Bertin Technologies (France), while indoor samples were collected in the cafeteria.

Sample collection: Five different samples were collected with the **Coriolis µ**, using 15 ml of deionized water + 0,005% Triton as a collection liquid. Three samples outdoors and two samples indoors. In order to test the effect of collection time, the samples collected indoors and outdoors were obtained after 10 and 20 minutes of collection time.

Sample storage: All samples were frozen at -20 °C after collection.

Sample processing: To test the effect of the filtration technique, the samples from outdoors were concentrated using two different methods: centrifugation and filtration.

For centrifugation, two samples (10 and 20 minutes of collection) were centrifuged at maximum speed (16000 rpm) for 10 minutes. The supernatant was discarded, in order to obtain a concentrated sample of 400 ul.

For filtration, the remaining sample (10 minutes) was passed through a filter Amicon Ultra 15 100 KDa. A concentrated sample of 400 ul was recovered after 3 minutes of centrifugation at maximum speed (16000 rpm).

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- **Bacterial DNA extraction**

Bacterial DNA was extracted from the samples using the GenElute Bacterial Genomic DNA kit from Sigma. DNA concentration was then quantified with a NanoDrop (Table 1).

- **qPCR of bacterial 16S rRNA**

In order to quantify the 16S rRNA gene from bioaerosols collected with the **Coriolis** indoors and outdoors, a SYBR-Green-based qPCR assay was performed on the **Q qPCR machine (Quantabio, Beverly, MA).** The universal primers set targeting bacterial 16S rRNA gene included forward primer: 5'-TCCTACGGGAGGCAGCAGT-3' and reverse primer: 5'-GGACTACCAGGGTATCTAATCCTGTT-3' with an amplicon size of 466 bp on the reference *E. coli* genome.

The PCRs were performed in a total volume of 25 µl containing 12.5 µl of 2X **PerfeCTa SYBR Green FastMix (QuantaBio, Beverly, MA),** 1 µl of each 500 nM primer, 10 µl of template DNA, and 0.5 µl of molecular-grade water. The thermocycler was programmed for 10 minutes of denaturation at 95 °C and 40 cycles of 15 s of denaturation at 95 °C and 1 min of annealing/extension at 60 °C, with an additional extension step of 30 s at 72 °C. Upon completion of PCR amplification, a melt curve analysis was performed to check the purity of generated amplicons. A 10-fold serial dilution of bacterial DNA was amplified with samples as a standard curve on reaction plate.

The qPCR products were also visualized with an E-Gel 1% agarose with SYBR Safe (Invitrogen).

RESULTS

Bacterial DNA was recovered from all the samples, both indoors and outdoors.

Table 1. *DNA concentrations in air samples obtained with the Coriolis µ*

Sample name	Sample type	Air collection protocol with Coriolis µ	Concentration technique	DNA conc (ng/µl)	Ct
COR1	Indoor	300L/min for 10 min	Centrifugation	0.9	26,04 ± 0,34
COR2	Indoor	300L/min for 20 min	Centrifugation	1.7	30,83 ± 0,70
COR3	Exterior	300L/min for 10 min	Filtration	1.0	32,56 ± 1,05
COR4	Exterior	300L/min for 10 min	Centrifugation	2.1	13,35 ± 0,21
COR5	Exterior	300L/min for 20 min	Centrifugation	2.5	13,50 ± 0,08

The PCR performed on bacterial DNA obtained from bioaerosol samples was very sensitive, and even 100-fold dilutions of the original samples could be detected. The results of each PCR are indicated by a threshold value cycle (C_T) value in Table 1. The Ct value of the NTC was 35,99 ± 0,55.

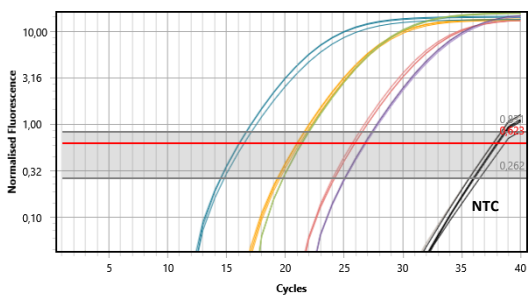

Figure 1. *qPCR amplification of bacterial 16S rRNA with the* ***PerfeCTa SYBR Green SuperMix (QuantaBio).***

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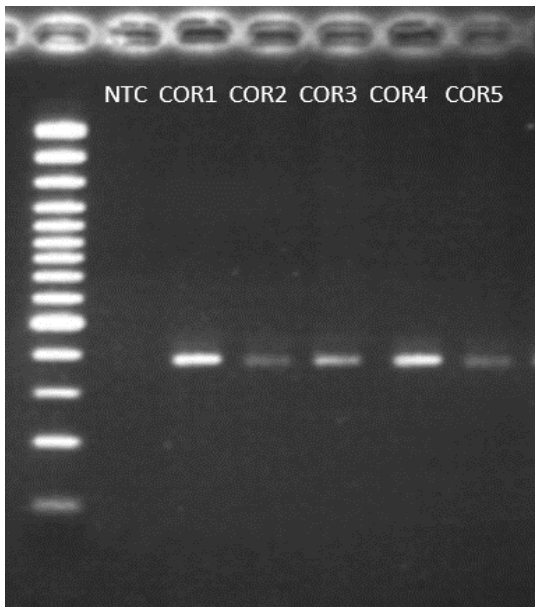
The amplification of the 466 bp fragment of 16S rRNA was also confirmed for all samples (except NTC) by gel electrophoresis.



CONCLUSIONS

This case study demonstrates how to validate a protocol of collection for the detection of bacterial DNA, using the **Coriolis µ**. Furthermore, it allows to identify key recommendations to improve bacterial DNA yield:

- For sample concentration, the centrifugation technique is more efficient than filtration.
- Increasing the collection time does have a positive impact on the concentration of total bacterial DNA obtained, particularly indoors.