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Coronavirus Detection from Aerosols

PCR based Detection of SARS-CoV-2 from Gelatin Membrane Filter

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Abstract

The outbreak and distribution of the acute respiratory syndrome coronavirus (SARS-CoV-2) has emerged as one of the biggest challenges to global healthcare and economic systems. Since the main routes of SARS-CoV-2 transmission via aerosols are becoming more and more evident, scientific, political and health institutions are taking their stance, emphasizing the urge and need for further research on airborne pathways of the virus either as droplets or aerosols. With the ongoing pandemic, reliable air monitoring solutions will become crucial to measure and manage pandemic dynamics. In this study we demonstrate the successful detection of SARS-CoV-2 reference virus from gelatin filters and provide guidance on how to integrate gelatin air samples into a virus detecting PCR protocol.

Background

Increasing scientific evidence suggests airborne transmission as a key pathway of the coronavirus[7, 8, 9, 11, 12, 13, 14, 15, 16]. As a consequence, the World Health Organization (WHO) updated their implications for infection prevention precautions[a]. The Centers for Disease Control and Prevention (CDC) updated their scientific brief on SARS-CoV-2 air borne transmission[b].

As Europe currently faces a second epidemiological wave of SARS-CoV-2 infections, health care organizations and governments are exploring strategies to prevent national healthcare systems from collapsing while at the same time balancing the economic loss and social side effects. Although, official regulations to monitor airborne viruses on a regular base are not yet implemented, the scientific and medical community strongly emphasizes the need for air monitoring during outbreaks[1, 5, 7, 8, 9].

Yet, an easy, efficient and sensitive method to monitor airborne microorganisms and viruses is the gelatin filter method[1, 2, 3, 4, 5]. The suitability of the water-soluble gelatin membrane filters (GMF) specifically for coronavirus sampling has already been demonstrated[1, 3, 4, 5, 6, 7, 8, 11]. With retention rates of up to 99.8 %, gelatin air filters show excellent virus collection efficiencies[2, 4]. Coupled with a sensitive detection method such as real-time reverse transcriptase-polymerase chain reaction (RT-qPCR), the gelatin air sampling method contributes to various applications that enhance surveillance and the management of epidemics caused by airborne viruses. Several studies demonstrated that a PCR analysis subsequent to air sampling using gelatin filters provides recovery results superior to other detection methods and supplies a robust workflow for the detection of airborne viruses[3, 6]. Briefly, all virus particles retained on the water-soluble filter during air sampling are recovered in particularly small volumes of deionized water or any other appropriate buffer or medium. This is ideal for the subsequent detection by nucleic acid amplification assays[1, 3, 5, 7]. To obtain fast, sensitive and specific results for the presence or absence of microorganisms, quantitative real-time PCR is the method of choice[2, 3, 5, 10]. In the case of SARS-CoV-2, RNA extractions and reverse transcription are preceding the amplification of viral cDNA during quantitative PCR. National and international health organizations provided consented and consistent instructions for PCR-based diagnostics of SARS-CoV-2 which were applied here to detect SARS-CoV-2 surrogate virus from gelatin filters.

Materials and Methods

We assessed the detectability of a AccuPlex™ SARS-CoV-2 surrogate virus (Verification Panel Sera Care) from air samples on gelatin filters via the CDC recommended RT-qPCR protocol[10]. The reference material was designed to serve as a full molecular control for the validation of PCR-based SARS-CoV-2 diagnostic assays. The quantified viral surrogate coat contains recombinant material that allows for monitoring of both the RNA extraction prior to reverse transcription and the amplification of the CDC published consensus sequences.

Gelatin filters were spiked with surrogate virus particles and dissolved resulting in liquid samples with loads of 60, 600, 6,000 and 60,000 reference particles per mL. Dissolved samples were aliquoted and either used for immediate RNA extraction or stored for up to ten weeks at +4 °C after which they were dissolved a second time. Amplification and data anaylisis were carried out on a BioRad Touch CFX96 Deep Well as well as a Roche Light Cycler 480 according to CDC instructions ("Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel-Instructions for use"[10]).

For SARS-CoV-2 sample preparation a 47 mm Sartorius gelatin membrane (12602–47—-ALN) was mixed with 1.7 mL 0.2 % Tween and AccuPlex™ SARS-CoV-2 particles of the respective concentration. The later were added before the filter was dissolved by shaking for ten minutes at 37 °C. A pure 47 mm filter dissolved in 1.7 mL 0.2 % Tween served as Extraction-Negative-Control (NEC). Samples were equally aliquoted and subsequent detection of SARS-CoV-2 specific target regions via PCR were performed by two independent laboratories.

Thus, 140 µL of the dissolved membrane were used for RNA extraction utilizing the QIAamp® Viral RNA Mini Kit following the instructions of the manufacturer's manual. For RNA elution, 60 µL of AVE Buffer provided in the kit was used. Subsequently, five µL of the resulting extracts were transferred into a TaqPath™ 1-Step RT-qPCR Master Mix containing the 2019-nCoV_N1/N2/RP CDC EUA combined Primer/Probe Mix for the targeted amplification of respective SARS-CoV-2 *N1*, *N2* or *RP* control genes. For PCR controls, 2019nCoV2_N_positive control (PC) and PCR grade water were used (NTC), respectively. PCR reactions were performed in triplicate. For general quick instructions see also Table 1.

Table 1: Qick guide of Material and Methods

Instruction	Material
Mix 47 mm GMF and surrogate SARS-CoV-2 virus (or alternative) in 1,7 mL 0,2 % Tween (or alternative) and dissolve for 10 min at 37 °C	Gelatin filter 3.0 µM, 47 mm, Sterile Sartorius 12602–47—-ALK (50)/ 12602–47—-ALN (100) AccuPlex™ SARS-CoV-2 Verification Panel Sera Care 0505-0129
Transfer 140 µL dissolved sample into RNA extraction	QIAamp® Viral RNA Mini Kit Qiagen 50 extractions 52904
Transfer 5 µL RNA extract (or PC/NTC) each into N1, N2 & RP RT-PCR Master Mix	TaqPath™ 1-Step RT-qPCR Master Mix, CG ThermoFisher A15299
Component / **Amount for 1 reaction** Nuclease free water 8.5 µL TaqPath 1-Step MMix 5 µL Combined Primer/Probe (N1, N2 & RP) 1.5 µL Sample or control 5 µL Total 20 µL	2019-nCoV_N1/N2/RP Combined Primer/Probe Mix CDC EUA Kit Integrated DNA Technologies 225390262 2019nCoV2_N_positive control Integrated DNA Technologies 225390258
Temp. / **Time** / **Cycles** 25 °C 2 min 1× 50 °C 15 min 1× 95 °C 2 min 1× 95 °C 3 sec 45× 55 °C 30 sec 45×	

Results

We were able to detect the reference virus from gelatin membrane filters. Applying CDC protocols for RT-qPCR based SARS-CoV-2, the Ct-values for the amplification of the SARS-CoV-2 specific targets *N1* and *N2* for concentrations of 60, 600, 6,000 and 60,000 virus particles per mL gelatin sample are shown in Table 2. Ct-values were considered valid since PC, NCT and RP gene controls (data not shown) exhibited the expected performance. Pure gelatin NEC showed no amplification of *N1* and *N2*. Although, samples with lower virus concentrations are challenging the efficiency of the RNA extraction and sensitivity of the PCR, all tested virus concentrations showed an amplification for *N1* and/or *N2* with the expected increase in variation. Unsurprisingly, the robustness of detection increased with increasing viral concentrations and seemed dependent on viral target regions (Table 2). However, as we aimed toward proof-of-principle, the limit of detection remains to be determined to deliver compelling data.

Table 2: Ct values for SARS-CoV-2 *N1* and *N2* amplification of dissolved gelatin (GMF) with indicated reference virus concentrations.

Instruction	Ct *N1*	Ct *N2*
	n=6	n=6
GMF NEC	No Amp	No Amp
GMF+ 60 surrogate virus/mL	No Amp	40*
GMF+ 600 surrogate virus/mL	37	40**
GMF+ 6,000 surrogate virus/mL	36	41
GMF+ 60,000 surrogate virus/mL	32	36

*1 out of 6 replicates **2 out of 6 replicates

Ct cut-off setting and acceptance criteria may vary from diagnostic interpretations and may be optimized for environmental air monitoring to further increase the sensitivity of the detection method.

Conclusion

Here we demonstrated the successful integration of gelatin membrane filters for air sampling into established real-time PCR-based SARS-CoV-2 detection systems. Reference AccuPlex™ SARS-CoV-2 material was amplified at all tested concentrations.

Viruses such as SARS-CoV-2 or other microorganisms retained on soluble gelatin membranes during air sampling can be concentrated in particularly small volumes ideal for further processing by nucleic acid extraction. Sartorius gelatin filters therefore enable the merging of sensitive air monitoring methods to sensitive and rapid real-time PCR-based viral detection and, hence, can contribute to the understanding of pandemic dynamics and furthermore facilitate effective spread and contamination controls and the adoption of prevention measures.

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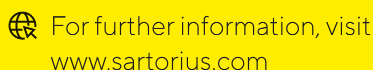

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