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Sampling of Human Pathogenic Viruses from Air by Gelatin Membrane Filters and Subsequent Detection by PCR Analysis

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1. Introduction

Outbreaks of infectious diseases represent an important global public health issue but also pose far reaching challenges and threats to global economies and healthcare systems. Viral infections without antiviral treatments are particularly menacing, hence understanding infection sources and routes of transmission and how these contribute to the spread of disease can facilitate effective prevention and adoption of control measures.

Currently, the COVID-19 pandemic demonstrates the importance of insights regarding transmission as well as infectiveness of human pathogenic viruses over time and space.

Besides inhalation of liquid droplets, close contact with infected persons or contact with contaminated surfaces, aerosol transmission of viruses is an important aspect to be considered[1, 4, 5, 8, 9-11].

At present, there is little information on the characteristics of airborne SARS-CoV-2 containing aerosols, their concentration patterns and behavior during airborne transmission due to the difficulties in sampling virus-laden aerosols and challenges in their quantification at low concentration. Such a lack of understanding limits effective risk assessment, prevention and control of COVID-19 disease outbreaks[8].

However, there are already several studies focusing on this aspect, on the importance of air as a transmission path for viruses[1,7,8].

The results of these studies indicate a certain stability of airborne viruses depending on species and emphasize the importance of monitoring air for the detection of potential infection sources as well as identification of transmission pathways[1].

Further, the effectiveness of quarantine measure as well as cleaning and sanitization procedures should be observed[8] as for example performed on cruise ships after norovirus outbreaks.

Van Doremalen 2020 pointed out, that pathogenic viruses show prolonged environmental presence persisting as aerosols for several hours. Persisting virus genome could be detected even up to more than 24 h after release[9,11]. Also long distance airborne transport of pathogenic virus can occur[3]. Therefore, the development and improvement of different methods for quantitative virus detection in air samples is important[4].

Even though, currently, there are no official regulations in place to monitor airborne viruses on a regular base, several scientists focusing on this issue are strongly advocating the need for air monitoring during outbreaks[1].

Fig. 1: Air sampling set-up using gelatin filter disposables with either MD8 Airscan or the portable AirPort MD8.

2. Gelatin Membrane Filters for Virus Sampling from Air

An easy, highly efficient and sensitive method for the monitoring of airborne pathogenic microorganisms as well as viruses is the gelatin filter method[1, 2, 4, 6, 8].

The suitability of the water-soluble gelatin filters especially for virus sampling has already been tested and proven[1, 4, 6, 7, 8, 10, 11].

The filters are particularly suitable as they show excellent virus collection efficiency with retention rates of up to 99.76%[2, 6].

Additionally, the air sampling procedure using gelatin filters allows for several possibilities for extending the lower detection limit down to as little as 10^2 particles/m^3[3,6].
It provides the option of prolonging the sampling period (sampling of high air volumes, such as 2000 l), a filter area of up to 50 cm^2 (Ø 80 mm), a flow rate of up to 50 l/min and the reduction of the solvent volume to a minimum of 80 – 100 µl/cm^2 filter area [4, 6, 8].

However, smaller filter sizes such as Ø 47 mm (filter area 17 cm^2) are also available, hence the solvent volume can be further reduced to as little as 1.5 – 2.0 ml.

The high suitability of gelatin filters has been demonstrated by Jaschhof (1992a, 1992b) as well as Friese, who observed that in individual cases, the gelatin filter is superior to other virus sampling methods.

This efficiency is attained without performing any additional preparatory or post processing steps for sampling virus aerosols, while maintaining the consistently high retentive capability of the filter[6].

Gelatin filters are available as pre-assembled and pre-sterilized ready-to-use filtration units.

Together with the portable, light weight AirPort® MD8 sampler, the gelatin filtration is a method, which is especially suitable for mobile applications and provides outstanding ease of use[4, 6].

Beyond that, Jaschhof (1992b) showed, that the sampling duration and a relative humidity of up to 80 – 85 % at 30°C did not negatively affect the retention of virus, which proved to be 99,76% on average.

Another benefit is that during sampling of human pathogenic viruses no liquid is needed, thereby reducing the risk of infection of the analytical testing staff.

The reported reduction of virus infectivity after sampling by gelatin filters[1, 4] proves to be advantageous as the samples can be considered as non-infectious material.

Further, the option to store the gelatin filters after sampling[1] as well as the option to ship the gelatin filters to a laboratory for further analysis before dissolution can be beneficial.

3. Processing of Gelatin Membrane Filters for Virus Detection by PCR Analysis

As soon as the gelatin filter has been dissolved in deionized water or any other appropriate buffer or medium, all virus particles retained on the filter can be further processed and detected using PCR[1, 4, 8, 10, 11].

In order to obtain fast, sensitive, highly specific and reliable results on the presence of specific viruses the quantitative real-time PCR (qPCR) is the method of choice[1, 2, 4, 8].

Even sensitive RNA material can be detected by the combination of gelatin filtration and qPCR[1, 4, 8, 11].

Yet, it should be considered that RNA extraction is a crucial step within sample preparation. Therefore, to reduce loss of genetic material particular attention should be paid to this step[2, 4].

Several studies showed that a PCR analysis subsequent to air sampling using gelatin filters provides recovery results superior to other detection methods and hence can be considered as a precise and practicable method for detection of airborne viruses[4, 7].

4. Typical Air Sampling Procedure Using Gelatin Membrane Filters and Subsequent Sample Preparation for Detection of Airborne Viruses by PCR

1) Unpack the Ø 80 mm gelatin filter disposable (ref-no. 17528--80----ACD)
2) Mount the gelatin disposable onto the filter holder for the Sartorius MD8 Air Sampler (either AirPort MD8 (ref-no. 16757) or MD8 Airscan (ref-no. 16748))
3) Contamination of the filter by touching it should be avoided
4) Start sampling of air at a speed of e.g. 50 l/min for 20 min (1000 l is a typical air sampling volume)
5) After sampling, detach the filter holder along with the disposable from the MD8 by turning it anti-clockwise
6) The top part of the filter holder is detached from the base by turning it anti-clockwise
7) Insert the Ø 80 mm filter into a 15 ml Falcon tube – the filter can easily be broken (optional a Ø 47 mm can be used)
8) Optional storage period and | or shipment to analytical laboratory
9) Add 4 – 5 ml solvent or 1.5 – 2 ml for a Ø 47 mm filter respectively (e.g. sterile deionized water or appropriate buffer medium)
10) Spin at 3000 × g in Centrifuge
11) Incubate in a thermal shaker (120 rpm) or a heating block for 10 min at 37°C to dissolve the gelatin
12) Optional virus inactivation
13) Follow RNA extraction according to the manufacturer's instructions
14) Dissolve RNA
15) Optional addition of RNase inhibitor
16) Process immediately on ice or optional storage period at -80°C
17) Synthetize cDNA by reverse transcription using a commercially available kit with primer and oligo dT primer in a thermal cycler
18) Optional storage of cDNA at -20°C
19) Carry out PCR analysis (e.g. qPCR)

5. Application Areas for the Detection of Human Pathogenic Viruses in Air

Monitoring of the effectiveness of cleaning and sanitization procedures

Monitoring of aerosol transmission of virus in different hospital areas, such as:
- Patient areas
- Medical staff areas (especially changing rooms)
- Adjacent rooms to quarantine areas
- Public areas
- Deposition areas
- Room ventilation
- Patient toilets

Monitoring of public means of transportation, such as:
- Airplanes
- Cruise liners
- Trains
- Railways vehicles
- Ferry boats

Monitoring of public areas with high visitor traffic, such as:
- Airports
- Train stations

Identification and monitoring of potential infection sources, ways of transmission or hot spots, such as:
- Barns and stables
- People gatherings

6. Key Features of Gelatin Membrane Filters for Virus Sampling and Subsequent Detection by PCR

- High sampling efficiency (retention of up to 99,9% of virus particles)
- Sampling of large air volumes possible (adjustable flow rate, filter diameter of up to Ø 80 mm), thus providing highest sensitivity
- Effective retention at environmental conditions up to 30°C and 80 – 85% rH
- Soluble in small liquid volumes (minimum 80 – 100 µl/cm² filter area), thus providing high sensitivity
- High recovery rates of nucleic acids
- No liquids needed for sampling
- Reduced infectivity of human-pathogenic viruses
- Storable after sampling
- Dry shipment after sampling possible without loss of recovery
- Dissolved Gelatin filters can be further processed with rapid tests methods such as PCR
- Outstanding ease of use

Fig. 2: Single packed gelatin filter disposable.

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