Sampling Virus Aerosols Using the Gelatin Membrane Filter

Collection using a Membrane Filter at a High Air Sampling Rate

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During collection of experimentally generated T1 phage and influeza aerosols, the air inlet velocity at the Sartorius Gelatin Membrane Filter was able to be increased to 1.6 m/s, five times the speed for the standard method, without any inactivation of the virus particles. The results of this procedure were definitely confirmed by sampling influenza virus A in the room air of a children's polyclinic.

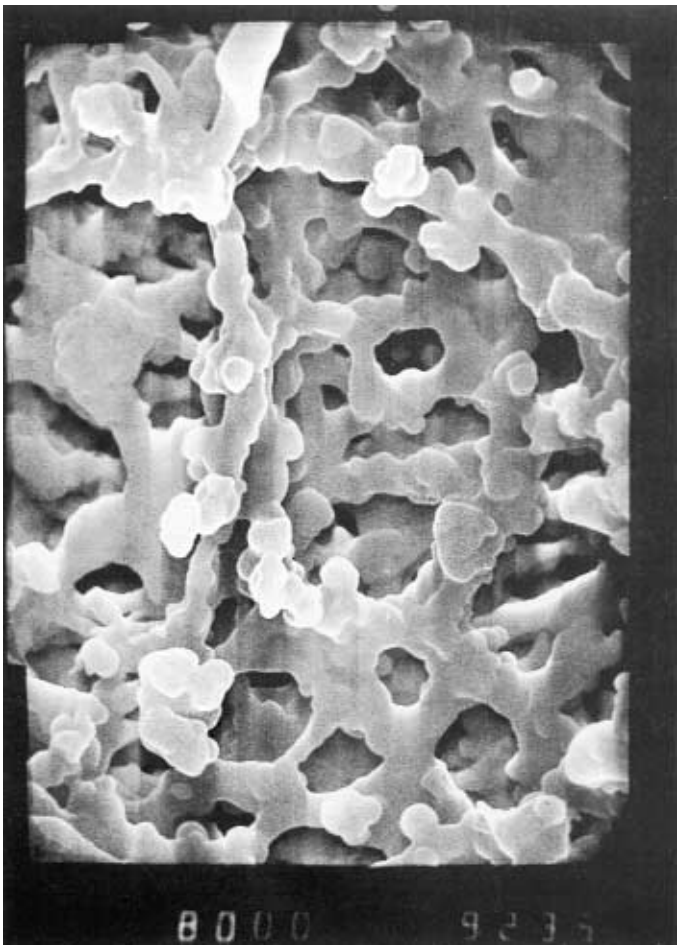


Fig. 1 Surface structure of a gelatin filter after being exposed for 15 min to the stress caused by an air stream (inlet velocity 1.6 m/s) at 30°C and 80–85% relative humidity. Scanning electron micrograph, magnified 5,600 times.

Of the sampling methods tested, the slit sampler, impinger and the filter collection methods, the gelatin filter method alone offered the possibility of increasing the air sampling rate within the standard time. Hence, it provides a second approach for sampling a large volume of air, in addition to the first method of prolonging the sampling time, in order to increase the lower detection limit for viruses in aerosols. However, the air inlet velocity at the filter had to be regarded as the most critical value for the biological stability of a bacterial or a virus aerosol [1]. Moreover, the earlier predictions about the suitability of the filter for large-volume sampling of virus aerosols could be classified as being in a range from conservative to completely negative [2, 3].

Tests to explore the amount of mechanical stress which the gelatin filter can withstand at high air inlet velocities, also under extreme ambient conditions, encouraged us to test the stability of virus aerosols during sampling under increased mechanical stress.

For these tests, the sampler SM 16711 (Collectron, predecessor model of the Sartorius MD8) could no longer be used. Instead, the filter holders of the unit were attached to two-speed, rotary vane vacuum pumps, either in an individual or in a parallel configuration. The MD8 sampler, which succeeds the former Collectron and is designed for high air sampling rates, was not yet available at the time the tests were conducted. A laboratory flow meter (Rotameter) connected between the instrument and the filter holder allowed the air flow rate to be measured. The air flow was regulated by a second tube behind the Rotameter and the tube clamp.

Stability of the Gelatin Filter at a High Air Sampling Rate

Serial measurements based on increasing air flow rates were made using a Wright Respirometer connected upstream of the filter holder. The Respirometer allowed extremely accurate volumetric measurements of gas streams in a range of 2 to 300 l/min. It was demonstrated that a gelatin filter at

room temperature and an average humidity of 50–55% could be exposed to the mechanical stress of increasing air sampling rates up to 135 l/min without showing any signs of damage. Higher air sampling rates could not be achieved with the vacuum system available.

The filter proved to have the same mechanical stability also at temperatures of 25 and 30°C, even at a relative humidity of 85–90% and during a sampling period of 15 min. Changes in the consistency and the handling of the filter were not perceptible until after a temperature of 30°C and a 15-min sampling period were attained.

Collection Efficiency as a Function of the Air Sampling Rate and the Relative Humidity

It was not possible to carry out a parallel sampling procedure for comparison as for the tests in the air inlet velocity range of 0.1–0.4 m/s (see Ref. 1, Fig. 2). To determine the collection efficiency of the gelatin filter at increasing air inlet velocities, it was necessary to perform a parallel sampling procedure for reference using a standard air sampler under constant sampling conditions. For this purpose, the AGI-30 standard impinger was used, and the "yield" already introduced by Petras [4] was computed as the measure of the degree of the filter's efficiency:

$$\text{Yield} = 100 \times \frac{\text{PFU/l of air sampled through the filter}}{\text{PFU/l of air sampled by the impinger}}$$

The tests using a T1 aerosol were started at an inlet velocity of 0.6 m/s. The stress placed by this inlet velocity on the filter was increased at intervals of 0.2 m/s. The temperature and the relative humidity were approximately 20°C and 50–55%, respectively. Surprisingly, the inlet velocity could be increased to 1.6 m/s without having a significant effect on the filter's degree of collection efficiency. The average PFU yield was 140% (Fig. 2) for this procedure. This means that the collection efficiency of the gelatin filter was constant for sampling a T1 aerosol up to an air flow rate of 120 l/min, which therefore translates to a fivefold increase over the sampling rate under standard conditions (22.5 l/min), with the filter having a superior collection efficiency compared with that of the AGI-30 impinger.

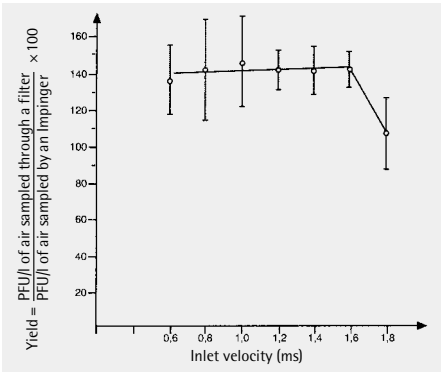


Fig. 2 Yield ol a gelatin filter, after sampling a T1 aerosol at 20°C and 55% relative humidity, as a function of the air inlet velocity at the filter. Titer of the suspension for aerosol generation (nutrient broth) $2.5 \cdot 10^9$ PFU/ml.

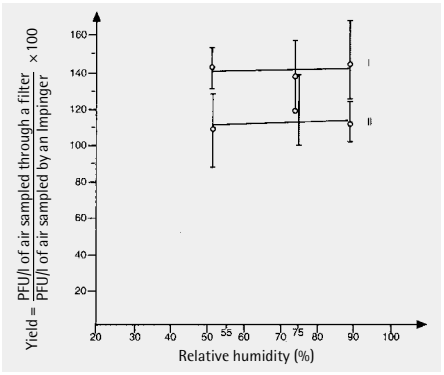


Fig. 3 Yield for the gelatin filter, after sampling a T1 aerosol, as a function of the relative humidity and inlet velocities of 1.6 m/s (I) and 1.8 m/s (II). T1 aerosol at 55%, 75% and 90% relative humidity. Titer of the suspension for aerosol generation (nutrient broth) $5.0 \cdot 10^9$ PFU/ml.

At an inlet velocity of 1.8 m/s, the yield dropped significantly to an average of 107%. This abrupt decrease in yield raised doubts as to whether this was caused by virus inactivation. Samples taken at high air flow rates are subject to interference due to turbulence caused by shearing forces generated between the flow paths [5] – if the air enters the sampler through tubular channels or hoses. In the process, the particles can attain sufficiently high velocities near the walls of the tubes so that the particles are removed from the air stream on account of their moment of inertia before they reach the collection medium. Hence, these particles are lost and are therefore not assayed [6]. The occurrence of such turbulence can be defined by calculating the Reynolds number for an air stream (Re_f). Below a Reynolds number of $2.0 \cdot 10^3$, the air stream is laminar and meets the requirement for exact or isokinetic sampling. When Re_f begins to approach $(2–3) \cdot 10^3$, air turbulence must be expected. At $4.0 \cdot 10^3$, the air stream is nearly always turbulent [5, 7]. For the present test conditions, Reynolds numbers of $3.22 \cdot 10^3$ and $3.63 \cdot 10^3$ were calculated. The values show remarkable concurrence with the limits for air turbulence. Thus, it is highly probable that the decrease in the yield within the inlet velocity range of 1.6 and 1.8 m/s can be attributed to physical interference with the sampling procedure and not to an inactivation of the airborne phage particles. At the same time, this would mean that the limit of the gelatin filter's performance capability has not yet been reached at a sampling rate of 120 l/min.

The filter's high resistance to mechanical stress while providing a constant yield raised the question as to which extent this performance might be limited by the relative humidity. Increasing the relative humidity from 50–55% to 75% and 90%, both at 1.6 and 1.8 m/s, did not have any effect on the yield (Fig. 3).

This result also could not necessarily be expected considering the chemical nature of the filter. In this respect, Petras [4] already remarked that "Aerosols with a very high moisture content can adversely affect the efficiency of filters by causing them to soften and their pores to expand" [translation of the original German quote].

The studies at an inlet velocity of 1.8 m/s and a high relative humidity were conducted not only to test the gelatin filter's resistance to physical stress. According to the result depicted in Fig. 2 for 55% relative humidity, it could be postulated that increasing the relative humidity might reduce dehydration, in other words inactivation, of the collected virions by the air stream, an effect which at first appeared to be indicated by the drop in yield between 1.6 and 1.8 m/s. The constant difference in the average yield for the two inlet velocities, which is independent of the percent of relative moisture, proved this postulate to be wrong in fact. Hence, a more likely explanation of why the yield dropped is that physical interference occurred during sampling.

Effect of a Prolonged Sampling Period
The filter performance at a high inlet velocity for a 1-min sampling period inevitably prompted studies on prolonging the sampling period to explore the possibilities for further increasing the sampling volume. Similarly to the tests conducted under standard conditions, the sampling period was extended to 15 min; however, only for the filter sampling method. To maintain the actual comparison with the standard sampler under standard conditions as well, we continued to work with the AGI-30 impinger at a constant air flow rate of 12.5 l/min for a 1-min sampling period. In other words, the 15-min values obtained with the gelatin filter had to be related to the 1-min value yielded by the impinger. When each filter yield was compared with each impinger yield, the decrease in the aerosol concentration caused by aging and dilution during the prolonged sampling period had to be taken into account.

When exposed to extreme stress as a result of the prolonged sampling period, the filters proved to have a high mechanical stability. Their collection efficiency was not affected by the duration of sampling or by the relative humidity (Fig. 4). Calculations done to check the consistency of the values confirmed that the lower values of long-term sampling could be attributed to a decrease in the concentration of the virions in the aerosol caused by aging and dilution during sampling.

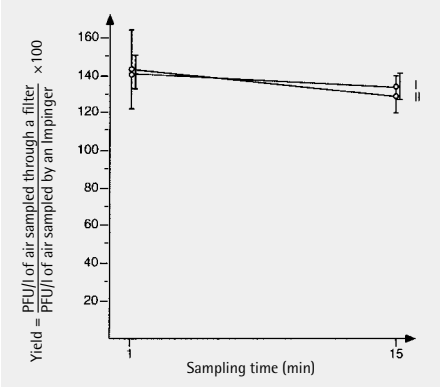


Fig. 4 Effect of a prolonged sampling period on the collection efficiency of the gelatin filter at an inlet velocity of 1.6 m/s and a relative humidity of 50–55% (I) and 80–85% (II) (air temperature 20°C) for a T1 aerosol generated from nutrient broth. Titer of the suspension for aerosol generation $5.9 \cdot 10^9$ PFU/ml.

For 90-minute T1, T3 and f_2 phage aerosols, the overall aging and physical aging were determined at 20°C for the humidity ranges of 50–55% and 80–85% using uranine as a tracer. It must be emphasized that the retention capability of the filter still remained at an average of 99.82%, even when the filter was exposed to these extreme conditions, and therefore did not differ statistically from the values yielded under standard conditions (Fig. 5). The tested maximum stress of the filter reached an inlet velocity of 1.6 m/s over a sampling period of 15 min at 30°C and at 80–85% relative humidity. For a T1 aerosol, the filter's retention rate was determined to be 99.76% on average (Fig. 5).

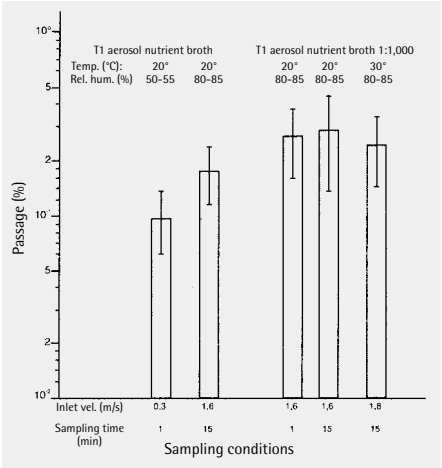


Fig. 5 Passages in percent through a gelatin filter at an inlet velocity of 1.6 m/s as a function of air temperature, relative humidity and sampling time for a T1 aerosol generated from a liquid high in solids (nutrient broth) and a liquid low in solids (1:1,000 diluted nutrient broth). Titer in the liquid for aerosol generation $2.0 \cdot 10^9$ PFU/ml.

The consistency, stability and handling properties of the filter clearly changed under these conditions. The outer ring of the filter holder was wet by droplets of condensed moisture. The filter showed a rubber-like change in consistency, although it did not stick to the filter holder base and was easily removed. No difference could be determined in the manner in which the filter dissolved. Scanning electron micrographs revealed that the web-like wall structures of the hollow "cells" in the membrane swelled to two or three times their normal diameter under these extreme conditions. However the basic structure of the filter, the system of hollow "cells," remained stable (Fig. 1) – according to Schröder [8], the breaks in the walls of the hollow "cells" are the pores which are effective in filtration.

Effect of Inlet Velocity and Sampling Time
Until now, the results presented for sampling airborne virions using gelatin filters were only for T phages. The present experiment using the determinative parameters of the gelatin filter method was performed to check the validity of this method for assaying a pathogenic virus in order to assess the degree of general applicability of these results. Another aspect of decisive importance for the general applicability of the gelatin filter sampling method was to prove that the dissolved filter material does not have any effect on the cell culture during the assay of the collected viruses.

The prerequisites for carrying out the gelatin filter sampling method were fulfilled for working with strain A/PR/8/34 (H1N1) influenza virus. The virus stored at -70°C was diluted with 19 ml of Adamczyk medium [9 (1975)] immediately before use to retain the viruses' ability to replicate, and an aerosol was generated from this suspension. The aerosol was conditioned at a temperature of 18–20°C and a relative humidity of 40–45%. Following cultivation on Ehrlich mouse ascites tumor cells, the virus was titrated according to the hemadsorption test (HAdT) method. The following variants were chosen as the sampling parameters:

- 0.3 m/s inlet velocity, 1-min sampling period (sampling under standard conditions was done to obtain a value for comparison)
- 1.6 m/s inlet velocity, 1-min sampling period (sampling at maximum inlet velocity)
- 1.6 m/s inlet velocity, 15-min sampling period (max. inlet velocity combined with max. sampling period)

As a result, the data obtained with T phages used as virus models were able to be confirmed for influenza virus as well and hence for a virus that is pathogenic for humans (Fig. 6): In an inlet velocity range of 0.3 to 1.6 m/s, neither the air sampling rate nor the sampling time prolonged up to 15 min had any effect on the collection efficiency of the gelatin filters. With all parameter variants, approximately the same recovery rates were attained.

This means that for selected viruses, a 50-mm filter can be used to sample at least 1,800 l of air to collect airborne particles. If the filter is dissolved in only 5 ml of medium [1], the lower detection limit for a virus titrated in an embryonated, incubated egg [10] can be computed on the order of 10^2 infectious units per m^3 of air.

If the Sartorius MD 8 Air Sampler is used with a 80-mm filter, the conditions for sampling virus aerosols are even more favorable. An air volume of 1,800 l is sampled within 15 min at an inlet velocity of 0.5 m/s. The inlet velocity of 1.6 m/s tested with the 50-mm filter cannot be applied for the 80-mm filter, however, because the maximum sampling rate of the MD 8 is 8 m^3/h. An air volume of 2,000 l is attained for a 15-min sampling period.

The results of the virus sampling method using the gelatin filter were strongly confirmed by those obtained by sampling influenza virus in the first practical application of this method. Influenza virus A was able to be isolated during the third subculture transfer from one of three samples of room air taken in a children's polyclinic in Greifswald, Germany. These samples were taken at an inlet velocity of 1.6 m/s during a 15-min period during the seasonal rise in morbidity for acute respiratory diseases (ARD) at the beginning of 1990. In assaying the viruses isolated from the room air and the virus isolated from the nasal secretions of children, who had a fresh case of ARD and had been in the waiting room during air sampling [11], it was found that the migration rates of the isolated viruses' proteins and the band patterns of their v-RNA following polyacrylamide gel electrophoresis matched. This is the first time in practice that the suitability of the gelatin filter for sampling airborne respiratory viruses has been proved.

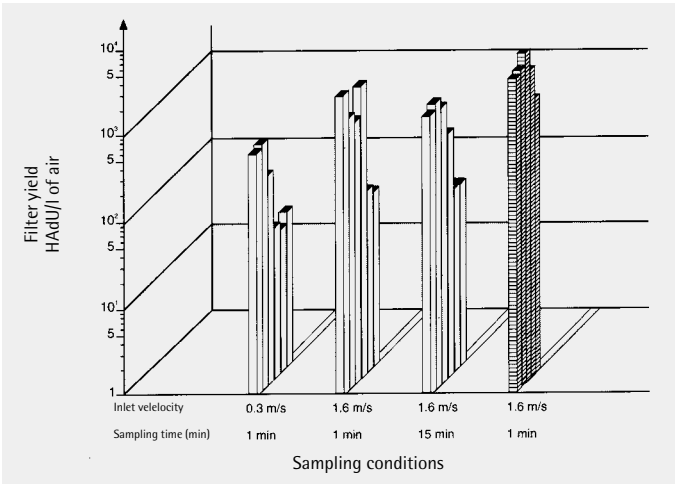


Fig. 6 Collection efficiency of the gelatin filter, for influenza virus aerosol, as a function of the air inlet velocity at the filter and the sampling time (six trials). Columns with hatching: supplementary data from sampling trials to test the stability of collected influenza virus aerosol particles when stored. Titer of the suspension for aerosol generation $2.2 \cdot 10^8$ HAdU/ml. Aerosol sampled at 20°C and 40–45% relative humidity.

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