



**Collecting Airborne
Viruses and Phages**
Using the
Gelatin Membrane
Filter Method

Collection of Viruses and Phages with the Gelatin Membrane Filter Method

Introduction

Based on experience gained in collecting airborne bacteria (Petras 1966, 1967; Rotter und Koller 1974), tests were run comparing the effectiveness of impaction collectors and standard impingers (model AGI-30) with the gelatin filter for sampling virus aerosols.

These studies had two objectives.

- To devise a standard method for filtration of virus aerosols and for processing the filter used to collect them
- To test the filter's suitability for large-volume sampling

Material and Methods

The studies were carried out with experimentally produced static aerosols of T1 coli phages (high stability over a wide humidity range), T3 coli phages and A/PR/8/34 (H1 N1) influenza viruses. The aerosols were generated in a 50 m³ experimental chamber, with an adjustable temperature and relative humidity, using a jet atomizer with a deflector (particle size $\leq 5 \mu\text{m}$). The aerosols were filtered using an 12602 Gelatin Membrane Filter installed in the holder of an 16711 Collector (Collectron, predecessor model of the MD8 airscan Air Sampler). At high flow rates, the device was used interchangeably with two parallel connected vacuum pumps (the current Sartorius MD8 airscan Air Sampler is designed for high flow rates).

The standard operating procedures and the sampling volume determined by the operating principle of the collector applied to both the impaction collector and the AGI-30 impinger.

Quantitative determination of the collected phage particles was done by the agar overlay method (plaque method), titration of the influenza virus using an incubated egg (Mayr et al. 1974, 1977) or by a hemadsorption test after culturing in ascites tumor cells (Adamczyk et al. 1975).

Results

I. Standard procedure for sampling virus aerosols by air filtration and processing of the filter (results for T1 and influenza virus aerosols)

The 0.1–0.4 m/s inlet velocity at the filter did not influence the filter yield of infectious units (I.U.'s)/l of air. For this reason, an inlet velocity of 0.3 m/s corresponding to 22.5 l/min is recommended for a sampling time of 1–2 min when collecting viruses of unknown stability. In the process, the filter, which is not affected by a relative humidity of up to 85%, shows a high retention rate of 99.9% or better.

The common method used to quantify airborne bacteria by direct incubation of the gelatin filter on agar plates is impracticable for virus aerosols. In the latter case, it is practical to dissolve the filter in a suitable medium, then to thoroughly mix the medium using a laboratory shaker to split up any microorganism | virus aggregates.

The following procedure has proved to be effective. To dissolve the filter, place 20 ml of $\text{m}/15$ phosphate buffer with a pH of 7.2 and approx. 40 glass beads with a 2.5 mm diameter in a 200 ml wide-necked Erlenmeyer flask. Place the flask on a laboratory shaker adjusted to an appropriate speed and shake the solution at room temperature for 5 minutes. The volume of the buffer solution can be reduced to as little as 2.5 ml in order to concentrate the infectious particles. The filter completely dissolves in the process.

II. Comparative sampling of T1 and T3 aerosols using a gelatin filter, an impaction collector, and an impinger according to the standard procedure

For both phage aerosols, the efficiency of the 3 collection methods fell into the order of $v_F > v_{I-C} > v_I$ (Fig. 1), which favored the filter. Differences resulted in the ratio of their effectiveness. For the T3 aerosol, $v_F : v_{I-C} : v_I$ was 4,02 : 3,76 : 1, where the difference between the filter and the impaction collector was insignificant. For the T1 aerosol, the ratio was 2,04 : 1,44 : 1 (at 50% relative humidity); there was significance at the 1% level between the values. The order of the collection efficiencies was confirmed for an aerosol concentration in the range of $10^3 - 10^5$ phage particles/ m^3 of air, which is also the lower detection limit for the phage aerosols for all 3 collection methods using the standard procedure.

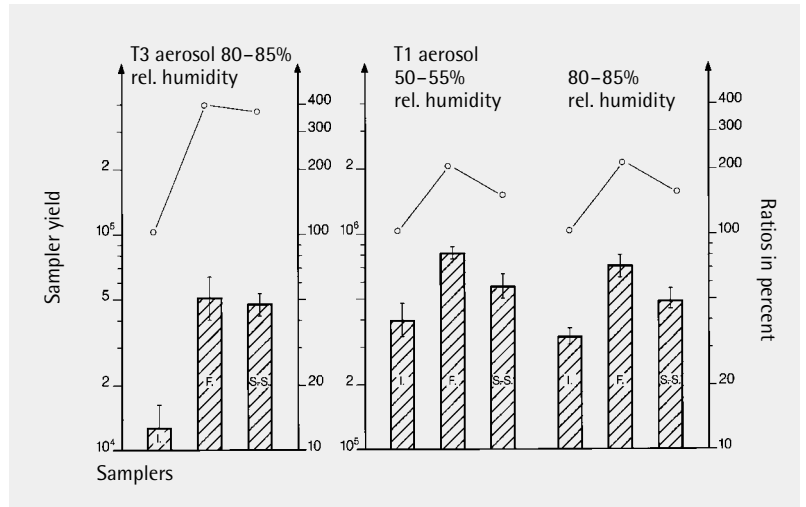
III. Large-volume sampling of virus aerosols with the gelatin membrane filter

Large-volume sampling, as a pre-requisite for detecting low viral particle (virion) concentrations, can be theoretically achieved by the following:

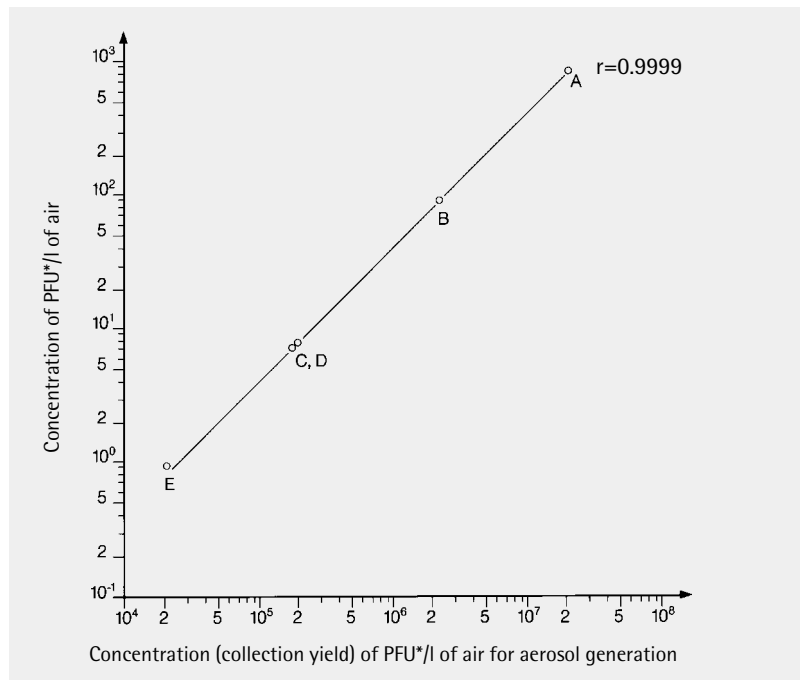
- increasing the flow rate within the specific time
- extending the sampling period

1. Extending the sampling period (results for T1 and influenza A aerosols):

Sampling the air for 15 minutes at an inlet velocity of 0.3 m/s through the filter (equivalent to 337.5 l of sampled air) did not yield an inactivation of the collected virions. This trial sampling procedure, along with a reduction of the buffer solution volume to obtain a higher virion | phage particle concentration, proved the mathematical theory that the lower detection limit can be shifted into the range of 10^2 I.U.'s/ m^3 of air (Fig.2).



Comparison of the collection effectiveness among the AGI-30 impinger (I), gelatin filter (F), and impaction collector (I-C) for T1 and T3 aerosols.



Relationship between concentration of the phage particles/ml of liquid for aerosol generation and the count of I.U.'s/l of air resulting from collection using a gelatin filter. T1 aerosols at 50-55% relative humidity and at 20°C.

A-E indicate the collection conditions:

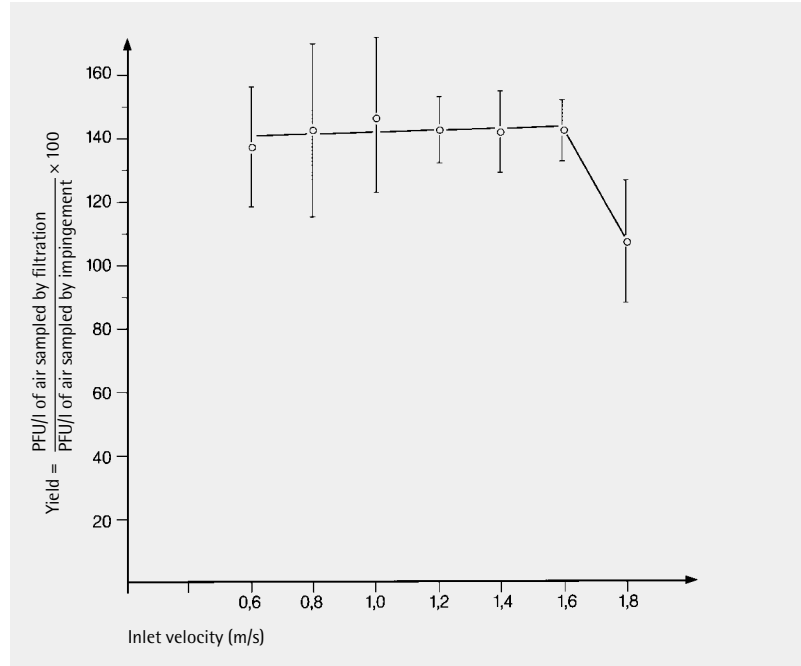
- A Standard procedure (see general information)
- B Sampling time: 5 min, solution volume: 20 ml
- C Sampling time: 15 min, solution volume: 20 ml
- D Sampling time: 5 min, solution volume: 5 ml
- E Sampling time: 15 min, solution volume: 5 ml

2. Sampling virus aerosols using an increased flow rate:

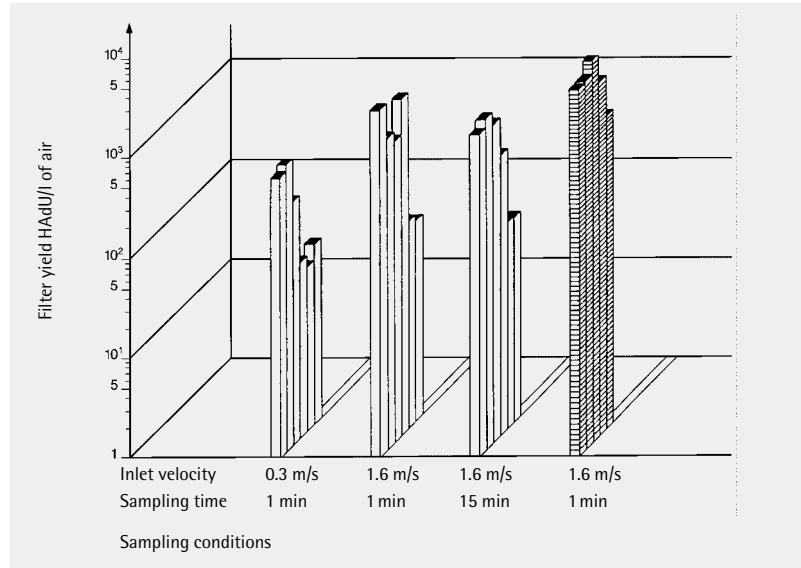
Mechanical stress tests performed on the gelatin filter at increased inlet velocities (up to 1.8 m/s, which is equivalent to 135 l/min) and under extreme ambient conditions (30°C max., 90% relative humidity), encouraged us to test the stability of virus aerosols under these tougher conditions during sampling.

Surprisingly, the inlet velocities were able to be increased up to 1.6 m/s without there being a significant influence upon the filter's efficiency. For a T1 aerosol, the yield of the filter in comparison to that of the standard AGI-30 impinger used as a reference quantity was 140% (Fig. 3). At 1.8 m/s, the yield dropped to 107% of the impinger.

Calculating the Reynolds number for the air current made it possible to trace the cause for the drop in yield to physical interference of the sampling procedure caused by air turbulence. Both the T1 coli phages and the influenza A viruses (Fig. 4) showed this high level of stability even during a 15-minute sampling period.



Yield of the gelatin filter after sampling of T1 aerosols at 20°C and 55% relative humidity as a function of the inlet velocity at the filter.



Collection effectiveness of the gelatin filter for influenza virus aerosols as a function of the inlet velocity at the filter and of the sampling time (6 trials).

Moreover, with T1 coli phages, it has been confirmed that the collection effectiveness of the filter remains constant under severe air sampling conditions, even at a 90% relative humidity. It is remarkable that the filter's retention capability for T1 aerosols still remained at an average level of 99.82% even under extreme stress. 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 T1 aerosols, the filter's retention rate was determined to be 99.76% on the average.

The consistency, stability, and handling properties of the filter clearly changed under these conditions.

The filter's periphery 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.

Scanning electron micrographs revealed that the web-like wall structures of the membrane swelled to two or three times their normal diameter under these extreme conditions. However, the basic structure of the filter remained stable.

Assessment in Summary

Following the successful use of gelatin filters for the collection of airborne bacteria, their suitability has now also been proved for sampling virus aerosols.

The gelatin filter provides a reliable collection method for the entire range of biological aerosols (bacteria, molds, and viruses).

The advantages of collection using a filter, which are of special importance to virus detection, are:

- Constant collection effectiveness over a wide range of flow rates/min.
- High retention capability of the filter even under extreme ambient conditions in terms of temperature and relative humidity.
- The gelatin in the filter acts like a capsid, a protective viral protein, by safeguarding viruses against surface inactivation.
- Use of the gelatin filter method is independent of the virus concentration.
- The method requires less work and materials both during preparation prior to collecting and during processing and evaluation afterwards.
- Collected virions can be cultured in parallel on different cell lines.

Reference Literature:

Adamczyk, B.; Adamczyk, C.; Millhahn, J.: Schnellnachweis von Influenzavirus A auf Ehrlich-Mäuse-Aszites-Tumorzellen mit der Hämadsorptionsmethode (Quick detection of the influenza A virus on mice ascites tumor cells using the hemadsorption method). Dtsch. Gesundh.wes, Berlin **30** (1975), pp. 2439–2443.

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Koller, W.; Rotter, M.: Weitere Untersuchungen über die Eignung von Filtern zur Sammlung von Luftkeimen (Further studies on the suitability of filters for collecting airborne microbes) Zbl. Bakt. I. Orig. B. Stuttgart **159** (1974), pp. 546–559.

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General Information about Collecting Viruses and Phages Using the Gelatin Membrane Filter Method

Steps of the Procedure

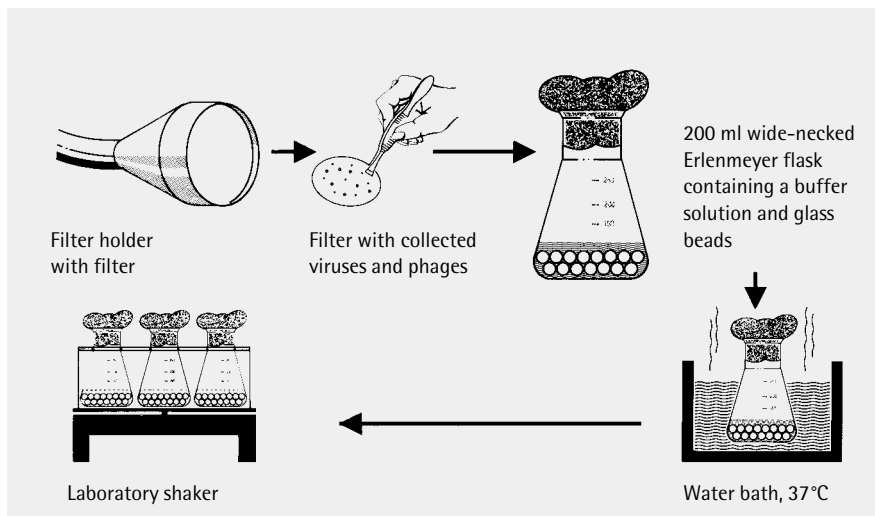
I. Collection of the viruses and phages:

MD8 airscan Air Sampler with Gelatin Filter
Disposables 80 mm Ø* Filter

* The application data given here were determined using the predecessor model 16711 with a 50 mm gelatin filter



III. Processing the gelatin membrane filter by dissolving it in a solution:



III. Virus and phage detection:

IV. Stabilizing the retained virus aerosols by storing the used filter until processing:

Standard Procedure

Comments about | Additional Steps for Standard Procedure

Flow rate through the 12602 gelatin membrane filter	22.5 l/min (50 mm Ø) 69.3 l/min (80 mm Ø) (corresponds to an outlet velocity of 0.3 m/sec.)	For selected virus aerosols, increase the flow rate up to at least 120 l/min for 50 mm Ø or 369 l/min for 80 mm Ø (corresponds to an inlet velocity at the filter of 1.6 m/s)
Sampling time	1–2 min	For selected virus aerosols and with a high flow rate/min, prolong up to at least 15 min
Permeability of the filter	10^{-1} – $10^{-2}\%$	This also applies to an increased flow rate/min
Humidity range	up to 90%	Tested only with the 50 mm Ø filter
Temperature range	up to 30°C	Tested only with the 50 mm Ø filter

Container for shaking	200 ml wide-necked Erlenmeyer flask with 40–10 glass beads with a 2.5 mm Ø	80 mm Ø filters are only to be placed in the flask after breaking them apart
Medium to dissolve the filter	m/15 phosphate buffer, pH 7.2	For acid-sensitive viruses, it is better to use pH 7.6 because the gelatin filter slightly lowers the pH.
Volume of the medium	20–5 ml	Tested for the 50 mm Ø filter up to 2.5 ml
Dissolving temperature	37°C in a water bath	
Dissolving time	5 min when left to stand	
Shaking time	5 min	For selected viruses, the shaking time can be prolonged up to 60 min without inactivation

Proven detection method using cell cultures: phage filtration based on the agar overlay method (plaque method); influenza virus by inoculating incubated eggs, FL cell cultures and ascites tumor cells and virus detection by HAT, CPE and HA₂T according to the usual techniques (Mayr et al. 1974, 1977)

Influenza virus Filter in a soaked condition, in 5 ml of a preservative solution with a pH of 7.6 at +4°C (200 ml Erlenmeyer flask)	Half-life of inactivation: 41.5 hours (30% survival rate after 72 hours)	Only 1.2% survival rate after the naturally moist filters have been stored for 72 hours at +4°C
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