Sampling Virus Aerosols

Comparative Studies on the Efficiency of Gelatin Membrane Filters, Impaction Collectors and Impingers

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English translation of the special reprint from "BioTec" No. October 1992
Original German title: "Sammlung von Virusaerosolen – Vergleichende Untersuchungen zur Effektivität von Gelatine-Membranfiltern, Schlitzsammler und Impinger"

The objective of the studies described in the following was to perform systematic test series to compare the collection efficiency of the Sartorius Gelatin Membrane Filter for phage aerosols with the two classical sampling methods involving the impaction collector and the impinger. Special importance was given to determining the detection sensitivity for a wide range of particle concentrations. One of the objectives of these studies was to discover to what degree long-term sampling can be used to extend the lower detection limit.

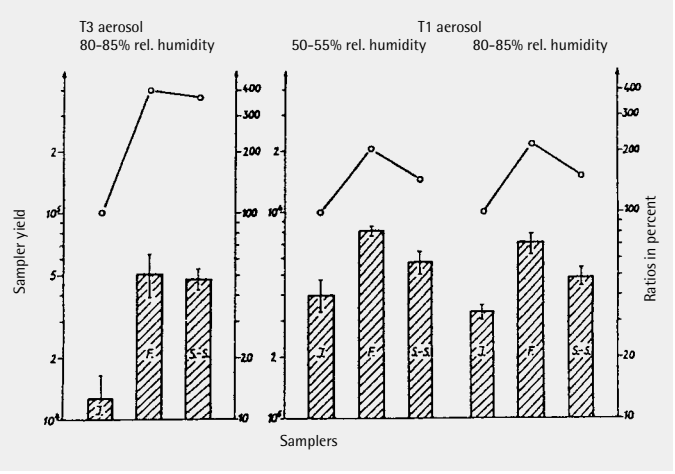


Fig. 1 Comparison of the collection efficiencies among the AGI-30 impinger (I), gelatin filter (F), and the impaction collector ["slit sampler"] (I-C) for a T3 aerosol at 80–85% relative humidity (titer of the liquid for aerosol generation: nutrient broth $2.5 \cdot 10^9$ PFU/ml) and for a T1 aerosol at 50–55% and 80–85% relative humidities (titer of the liquid for aerosol generation: nutrient broth $1.75 \cdot 10^{10}$ PFU/ml). Figures expressed as the number of PFU/l of air and the ratio in percent for the mean values (impinger = 100%).

For sampling bacterial aerosols, a number of comparative studies have been performed on the collection efficiency of the three most common sampling methods. The water-soluble gelatin filter (manufacturer: Sartorius AG, Goettingen, Germany) is gaining preference on account of its relatively high recovery compared with that of the impinger andlor impaction collector and – in cases where the recovery rates among all methods are similar – because of the benefits in practical handling.

Hecker et al. [1] point out that based on the results of their studies, it is not justified to underestimate the efficiency of cellulose membrane filters compared to the soluble gelatin filter. Summaries are found in [2, 3 and 4].

For virus aerosols, the available data almost exclusively refer to coli phages. The impinger sampling method proved to be the least destructive for T1 phages [5], followed by the "fritted bubblers" and filters. In the case of filters, these were so-called high-performance filters supplied by the following companies: Chemical Corps, type 6 filter paper (cellulose, asbestos fibers), which disintegrated into a thick paste when shook in liquid; and glass filter paper MSA 1106 BH (glass fiber material with organic binders, insoluble).

Even Happ et al. [6] obtained lower recovery rates for T1 phages using the Type 6 filter paper than they did using the AGI-4 impinger. In comparative studies between the impinger and the impaction collector for sampling T3 phages (Dahlgren et al. 1961 [7]), both recovery rates turned out to be comparable, even for a sampling period of 60 min using the impaction collector, also called a "slit sampler." Dahlgren et al. were the first to use a 12% phosphate buffer | gelatin medium for collection. For virus titration, the gelatin was melted or 45 min at 37°C in the collection plates.

These authors consider the slit sampler advantageous for sampling aerosols with low bacteriophage concentrations, although the relatively low air sampling rate of an impinger limits the overall number of phages recovered. In addition, they claim that the slit sampler offers an additional benefit in allowing long-term sampling. The same principle has also proved its efficacy for sampling Venezuelan equine encephalomyelitis viruses (VEE viruses) [8] so it appears that the impaction collector is especially suitable for long-term, large-volume phage sampling.

In Grigorieva's studies [9], soluble filters proved to be superior to a siphon-type sampler and porous filters. Soluble filters showed the highest adsorption and minimal passage. Kewitsch [10] reports a definitely lower collection efficiency (27.1%) with a slit sampler for sampling ΦX 174 phages and influenza viruses compared to that of the Porton impinger (60.3%). Based on the results with T3 aerosols, Haferkorn et al. [11] rate gelatin filters as the most suitable for sampling viruses, if low passage and high detection sensitivity are to be achieved. Equating the detection capability of the impinger with 100%, a detection sensitivity of 74% was yielded for gelatin filters and 15% for membrane filters, with the passage rates being 30% for the impinger, 10^{-2}% for gelatin filters and 10^{-5}% for membrane filters, respectively.

Comparative Sampling of a T1 and a T3 Aerosol

For sampling according to the standard method, the following conditions applied: gelatin filters: 22.5 l/min air flow rate; 0.3 m/s inlet velocity; 1-min sampling period; the filters were dissolved in 20 ml of phosphate buffer.

Impaction collectors (slit samplers): 30 l/min air flow rate; 1 min sampling time; retention of the phages in 30 ml of phosphate buffer | gelatin.

AGI-30 impinger: 12.5 l/min air flow rate; 1 min sampling period; retention of the phages in 20 ml of m/15 phosphate buffer.

The T3 phages were tested for their stability only at 80-85% relative humidity; the T1 phages were tested, taking both relative humidity ranges into account. For both phage aerosols, the collection efficiencies η_F; η_S and η_I fell into the order of $\eta_F > \eta_S > \eta_I$ (Fig. 1). Differences resulted in the ratio of their effectiveness. For the T3 aerosol, $\eta_F : \eta_S : \eta_I$ was 4.02 : 3.76 : 1, whereas the difference between the filter and the slit sampler was insignificant. For the T1 aerosol, the ratio was 2.04 : 1.44 : 1 (50-55% relative humidity) and 2.14 : 1.50 : 1 (80-85% relative humidity); there was significance at the 1% level between the values.

The results firmly support the assessment made by Haferkorn et al. [11] as to the suitability of gelatin filters for sampling virus aerosols and, moreover, demonstrate that in individual cases, the gelatin filter is superior to the impaction collector (slit sampler) and the impinger.

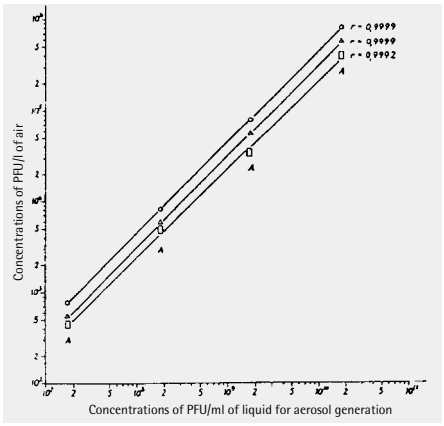


Fig. 2 Relation between the concentration of PFU/ml of liquid for aerosol generation (nutrient broth) and the number of PFU/l of air for sampling using gelatin filters (○), slit samplers (△) and impingers (□). r = correlation coefficient. T1 aerosol at 50-55% relative humidity and 20°C. "A" designates sampling under the conditions of the standard method.

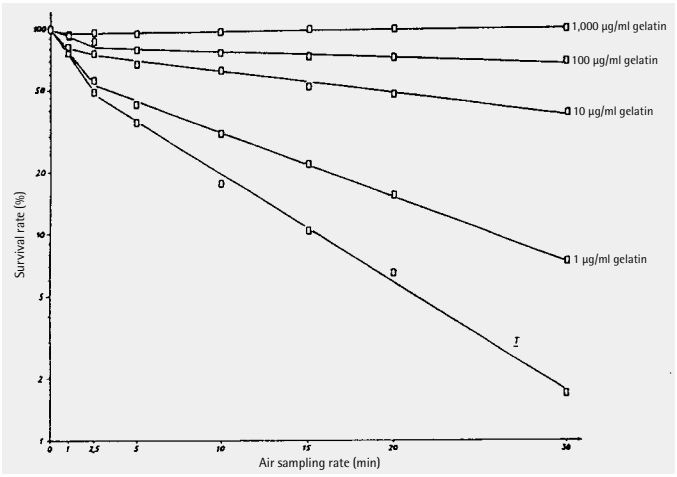


Fig. 3 Inactivation of T1 phages in 20 ml of m/15 phosphate buffer in the AGI-30 impinger when sterile air was sampled by the unit under the conditions for the standard impinger method (air flow rate 12.5 l/min (I); and effect of the graduated gelatin concentrations (1–1,000 µg/ml) on inactivation.

Collection Efficiency as a Function of the Aerosol Concentration

The purpose of the present studies was to provide information on the reliability of the three types of samplers in assaying different phage concentrations in an aerosol. The different aerosol concentrations were obtained by reducing – or diluting – the concentration of phage particles in the suspension used to generate the aerosol. Differences among the phage particle concentrations in the suspension and in the aerosol are also caused by the collection efficiency of the samplers in addition to dilution of the particles in the air and biological and physical disintegration.

Couch et al. [12] demonstrated a close correlation between both quantities for a coxsackie A21 virus aerosol in a tube-shaped chamber of approx. 214 cm × 15 cm (38 l). With a titer in the range of 10^{11} to 10^{6} $TCID_{50}$/l (tissue culture infective dosis) for generating the aerosol, the average difference between the concentration of viruses in the suspension and in the aerosol was $10^{6.3}$ $TCID_{50}$/l. Haferkorn et al. [11] confirmed such a correlation for a T3 aerosol in a 6 l test chamber (glass round-bottomed flask). Couch et al. used the Shipe impinger as a sampler, and Haferkorn et al. the Djakanow apparatus as well as an all-glass impinger, presumably of the type AGI-30.

Our studies were performed with a T1 aerosol in nutrient broth in the range of 10^{10} to 10^{7} PFU/ml of suspension for generating the aerosol. Parallel recovery of the viruses from the aerosol using samplers was done at three sampling locations under the conditions of the standard sampling method.

As a result, a high correlation among the phage concentrations in the suspension for aerosol generation and in the aerosol was able to be demonstrated not only for the AGI-30 sampler introduced as standard equipment (comparative sampler in terms of Brachmann et al. [13]) but also for the impaction collector and the gelatin filter (Fig 2).

The average differences measured between both concentration ranges were $10^{7.34} \pm {}^{0.1}$ for the soluble filter, $10^{7.49} \pm {}^{0.01}$ for the impaction collector and $10^{7.63} \pm {}^{0.06}$ PFU/l for the impinger. Thus, the order of the collection efficiencies of the three samplers (see above) was confirmed for the entire concentration range. There were no significant variances among the collection efficiency ratios. Therefore, in searching for a solution to the problem posed at the beginning of this paper, it can be deduced that all three sampling methods not only provide for reliable detection of phages in an aerosol in the range of 10^{8} to 10^{5} PFU/m^3 of air but also ensure consistent results. As already substantiated in [14], the lower detection limit for phage aerosols was also attained at the same time by applying the standard sampling methods.

Shift in the Lower Detection Limit for Phage Aerosols

Extending the lower detection limit for aerosol concentrations using samplers could be theoretically achieved by the following:

- increase the flow rate
- prolong the sampling period
- reduce the volume of collection medium
- increase the filter area (for gelatin filters)

For the impaction collector and the impinger, the air flow rate and the volume of the collection medium are functional parameters and determine the biological collection efficiency, either directly or indirectly to a substantial degree. These parameters as such are either fixed for operational reasons or may only be varied within narrow limits.

Prolonging the sampling period caused problems in both sampling methods. For the impaction collector, the low stability and the sensitivity of the gelatin collection medium to temperature were limiting factors. Even when the gelatin plates were allowed to set and were then precooled at 4°C, they barely withstood an 8-min sampling period at an air temperature of 20°C and were destroyed by the strong air stream (the slit was blocked by the melted gelatin). Therefore, the assessment made by Dahlgren et al. [7], among others, on the special suitability of the impaction collector for long-term sampling could not be supported, at least if gelatin medium is used with this collector [15–18].

In the case of sampling using the impinger, the phenomenon entailing inactivation of virions, or virus particles, at the water | air interfaces must be taken into account [19-21]. It occurred quantitatively during collection in phosphate buffer even when virions were recovered from nutrient broth aerosols of a complex composition.

In any case, long-term sampling of viruses requires proteinaceous collecting liquids to saturate the interfaces with a second protein that acts like a capsid. These protein liquids, in turn, necessitate the additional use of biologically inert antifoaming agents. Fig. 3 illustrates the effect of surface inactivation of T1 phages in a nearly protein-free system – 10^{-7} dilution of a phage suspension in phosphate buffer – and the effectiveness of increasing concentrations of gelatin which acts like protective protein. With a concentration of 1 mg/ml – of gelatin, the effects of inactivation were able to be entirely cancelled out even for a 30-minute sampling period.

Antifoaming agents based on silicon and supplied as "antifoams" by Dow Corning, Chemical Products Division, Midland, Michigan, USA, are commonly used in a concentration of 0.5-1%. In our own studies, polypropylene glycol D_{10} in a concentration of 0.001% proved to be the antifoaming agent of choice. One of the primary reasons was that the polypropylene glycol itself has a strong stabilizing effect: below 0.001% PPG without a gelatin additive, 97% of T1 phages survived 30-minutes' exposure to an air stream in the impinger. This stabilizing effect could be confirmed for T3 and Q_β phages as well.

As a result of these studies, the impinger offers the following potential possibilities for long-term sampling: If a collecting liquid is used with at least 0.1% gelatin and 0.001% polypropylene glycol as an antifoaming agent, the sampling time can be extended to at least 30 min without the risk of inactivating the collected virus particles at the water | air interfaces. This means an increase in the sampling volume from 12.5 l under standard conditions (1 min) to 375 l of air and a shift in the lower detection limit by more than one power of ten.

However, there are some reservations about the use of the impinger, in this case the AGI-30: the increased passage rates of virions and phages must be stressed. In tests designed to provide passage percentages as a guide, a passage of 50-57% ± 1.90% was yielded for a 13 aerosol at 80-85% relative humidity during 1-min sampling. Haferkorn et al. [11] confirm this value based on their results of 22-50% passages rates, 30% on the average.

Finally, sampling using filters allowed three possibilities for extending the lower detection limit, provided this procedure was done at a constant inlet velocity under standard conditions – directly by prolonging the sampling period as for the impinger; indirectiy by reducing the volume of solvent for the exposed filters; and both directly and indirectly by combining these two steps.

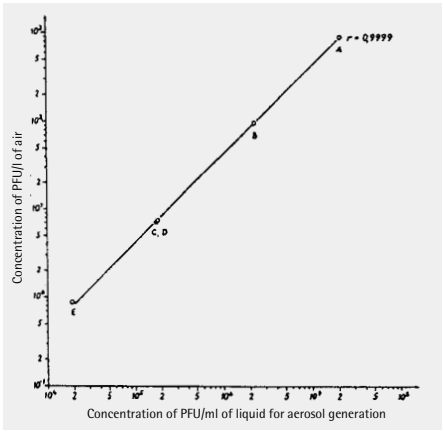


Fig. 4 Relation between the concentration of phage particles/ml of liquid for aerosol generation (nutrient broth) and the number of PFU/l of air for sampling using gelatin filters; r = correlation coefficient. T1 aerosol at 50-55% relative humidity and 20°C. The sampling conditions are described in the text below.

Assay of Aerosol Concentrations in the Range of 10^4 to 10^2 Phage Particles/m^3 of Air

To test the effectiveness of sampling procedure using the gelatin membrane filter and the range of steps just mentioned, the following experimental variants were designed:

Variant (A): Titer of liquid for aerosol generation in the range of 10^7 PFU/ml, 1-min sampling period, 20-ml volume for dissolving the filter (these parameters are equivalent to the conditions for standard sampling and were chosen for a study on the reproducibility of the 10^7 value of Fig. 2 after 7 months had elapsed!).

Variant (B): Titer of liquid for aerosol generation in the range of 10^6 PFU/ml, 5-min sampling period, 20-ml volume for dissolving the filter.

Variant (C): Titer of liquid for aerosol generation in the range of 10^5 PFU/ml, 15-min sampling period, 20-ml volume for dissolving the filter.

Variant (D): Titer of liquid for aerosol generation, likewise 10^5 PFU/ml, but with a 5-min sampling period and a 5-ml volume for dissolving the filter.

Variant (E): Titer of liquid for aerosol generation in the range of 10^4 PFU/ml, 15-min sampling period, 5-ml volume for dissolving the filter.

Based on the validity of the partial results of these test variants obtained so far, it could theoretically be expected that the lower detection limit could be shifted by three powers of ten, thereby providing demonstrable evidence of the reliability of sampling aerosols with low phage particle concentrations using the gelatin filter method.

The practical results agreed with this theoretical model (Fig. 4) to an unexpectedly great extent. Prolonging the sampling period to 15 min (C) and reducing the volume of medium for dissolving the filter (D) both resulted in the expected titers/l of experimental chamber air. Even the results of variant (E) with the combination of the maximal, tested sampling period and reduced volume for dissolution of the filters showed a linear dependence between the titers/ml of suspension for aerosol generation and titers/l of air. The difference between the two concentrations was calculated as $10^{7.38} \pm {}^{0.02}$. Therefore, it matched the average for the concentration range of 10^{10} to 10^{7} PFU/ml of suspension for aerosol generation (see previous page).

Summary

As a result of these studies, the following can be deduced, expanding our level of knowledge about the reliability of gelatin filters for sampling virus aerosols:

- The lower detection limit for a selected T1 virus aerosol can be extended down to as little as 10^2 particles/m^3 of air for sampling at an inlet velocity of 0.3 m/s (air flow rate of 22.5 l/min) for 15 min ≅ 337.5 l of air and by using a 5-ml volume for dissolving the exposed filter. If the Sartorius MD8 Air Sampler is used together with an 80-mm gelatin filter, the air volume of 337.5 l can be attained within 5 min. Compared with the slit sampler and the AGI-30 impinger, including the gelatin filter employed under the conditions for standard sampling, the results for the gelatin filter used on an MD8 air sampler translate to an extension of the lower detection limit by three powers of ten.

- The results demonstrate the consistent reliability of the gelatin filter for collecting viruses from a selected aerosol in the range of 10^8 down to 10^2 PFU/m^3 of air. This requires a 15-min sampling period.

This filter efficiency is attained without performing any additional preparatory or post processing steps for sampling virus aerosols, with the consistently high retentive capability of the filter being 99.9%.

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Printed in Germany on paper that has been
bleached without any use of chlorine.
W4A000 · G
Publication No.: SM-8031-e04042
Order No.: 85030-520-42