Sampling Virus Aerosols Using the Gelatin Membrane Filter

Effect of the Date of Manufacture on the Collection Efficiency, and the Stability of Virus Aerosol Particles After Collection on a Filter and Storage

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Original German title: "Sammlung von Virusaerosolen mit dem Gelatine-Membranfilter Einfluss des Herstellungsalters auf die Sammlungseffektivität und Stabilität von Virus-Aerosolpartikeln nach Filtersammlung und Lagerung"

The consistency of the collection efficiency over an undefined period is of special importance for a "disposable sampler," as represented by the membrane filter. Maintaining the stability of the collected microbes up until their assay is one of the basic requirements placed on a method for detection of airborne microbes.
The purpose of this study was to determine to which extent the Sartorius Gelatin Membrane Filter meets these requirements for virus aerosols.

With respect to the chemical nature and structure of membrane filters, the purpose of this study was to determine to which extent the date of manufacture and the storage conditions affect the collection efficiency of the filters. This issue is of special interest when filters are kept in stock for longer periods to have a sufficient supply for serial tests, for example. At the same time, this issue involves determining the consistency of the collection efficiency of different batches of filters that were manufactured on the same date. In literature, only one indication is found. Rotter and Koller [1] compared the CFU yields of filters of a four-week-old batch with filters that were 1/2 and 2 1/2 years old; the microbes were collected during a 15-min air sampling period at an inlet velocity of 0.1 m/s and then cultured on blood agar. No differences could be detected in the CFU yields.

For our own studies to test the effect of the age of the filter (eleven years maximum) on the collection efficiency, the following filter material from Sartorius was available:

Type	Control No.	Lot No.
SM 12602 308 F	48-53 3550	4/73
SM 12602	110/6 163	8/76
SM 12602 AC	110	81/80
SM 12602 AL (41)	1182	45/82

The filters manufactured in 1973 and 1976 had been stored at room temperature up to 1978, and then at 4°C up to the beginning of the studies in 1984; the filters of the lots from 1980 and 1982 were stored at room temperature (20° to 22°C).

To test the collection efficiency of various filter lots manufactured on the same dates, four filter lots of the year of manufacture 1981 were used:

Type	Control No.	Lot No.
SM 12602 AL (71)	981	68/81
SM 12602 AL (71)	981	73/81
SM 12602 AL (70)	1081	78/81
SM 12602 AL (25)	1281	90/81

Once the filters were received in 1982, they were stored at room temperature until they were used in the studies in 1984.

The apparatus setup allowed simultaneous sampling using four filters under standard sampling conditions, i.e., at an inlet velocity of 0.3 m/s. T1 virions were collected. An aerosol was generated from a suspension (nutrient broth diluted 1:1,000 with a solids content of 2 mg/100 ml) low in solids in order to keep the reaction between the virions and the filters as sensitive as possible.

The limited array of filter lots and the fact that no lot stored over several years had ever been tested in comparative studies did not permit all aspects to be assessed on a statistically valid basis.

However, the results obtained in our studies do allow the assessment to be made that the gelatin filter guarantees consistent collection efficiency over the years, provided it has been properly stored, and that there are no insupportable differences in effectiveness among the individual filter lots (Fig. 1).

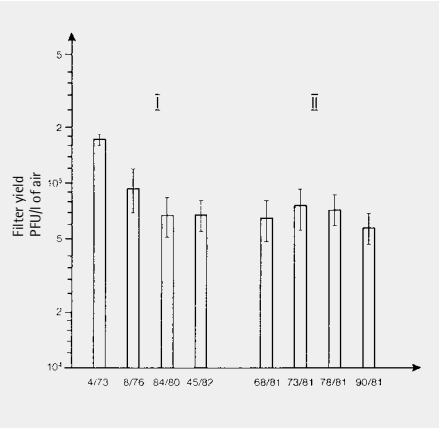

Fig. 1 Collection efficiency of gelatin filters as a function of the date of manufacture and of the filter lot. I: filters of various years of manufacture; II: filters manufactured in 1981; four different filter lots. T1 aerosol generated from liquid low in solids (titer $5.0 \cdot 10^9$ PFU/ml) at 55% relative humidity and 20°C.

There were no differences in the degree of retention efficiency among the filter lots from 1976 to 1982 (I), and the recovery yields of the four filter lots from 1981 (II) were also on this level. Indeed, one is rather tempted to assume that the filter material "improves with age." It was statistically proven that the eleven-year-old filters of lot 4/73 (I) had the highest collection efficiency compared to the two-, four- and eight-year-old filters, where the filters stored for eight years attained the next higher average recovery yield. For actual use of the gelatin filter, the decisive factor is that it ensures a consistent collection efficiency even for long-term serial tests. The exact reproducibility of the sampling results obtained seven months later can be evaluated as strongly confirming this assessment [2].

Stability of Microbial Aerosols
The stability of microbes recovered from aerosols on the gelatin filter touches an essential aspect, chiefly when sampling is done in the field: How long can the time be extended between exposition of the filters to an air stream and quantitative and | or qualitative assay using culturing techniques for diagnosis, and what storage conditions must be observed to keep inactivation within reasonable limits? These questions are raised particularly for virus aerosols. Virological procedures involving cell cultures require special techniques and laboratories that conform to the appropriate safety standards. The necessity of transporting exposed filters to a central laboratory will become a common practice.

For bacterial aerosols, the critical time interval just mentioned has proved to be very long. Rotter and Koller [1] found a reduction in the number of microbes to 76% of the initial count after the filters had been stored for 24 h at 24°C ± 3 K and 28 ± 7% relative humidity. There was a further reduction after 192 h, although it was only 10%. Petras [3] demonstrates that "the normal components of the airborne microflora can survive all day long on gelatin filters without any significant loss in vitality" [English translation of the original German quote]. Until now, no data have been available for virus aerosols.

Experimental Procedure
The tests were first performed with T1 and T3 phage aerosols, then with influenza virus strain A/PR/8/34/(H1N1).

For the phage sampling tests, four filters were simultaneously exposed to an air stream at separate sampling sites at an inlet velocity of 0.3 m/s for a 1-min sampling period. Following the sampling procedure, the first filter was immediately assayed quantitatively; the other filters were assayed after 24, 48 and 72 h of storage on filter paper in sterile petri dishes at 4°C in the refrigerator and at 22°C ± 2 K and 29 ± 1% relative humidity under room conditions, respectively. In preliminary trials, it had been statistically proven that parallel sampling at four sites in the experimental chamber gave the same yields of PFU counts | filter (Fig. 2 (a)).

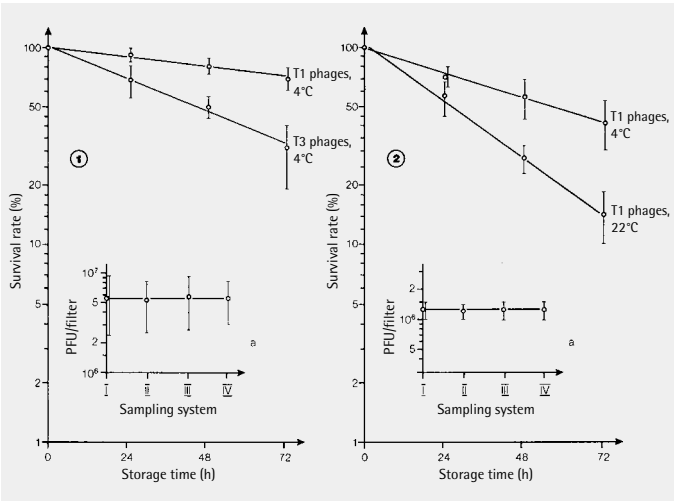

Fig. 2 Stability of T1 and T3 aerosol particles after collection on filters and storage of the filters at 4°C and 22°C, resp. 1: Phages from an aerosol generated from nutrient broth. 2: Phages from an aerosol generated from nutrient broth diluted 1:1,000. (a): Number of PFU/filter of four filters used in a simultaneous sampling procedure at four sampling sites and assayed immediately after sampling.

In the case of influenza virus A, the air volume had to be increased by sampling at an inlet velocity of 1.6 m/s for 2 min on account of the lower detection threshold of the virus determined using the hemadsorption test (HAdT) and mouse ascites tumor cells [4].

Similarly as described for airborne bacteria (see above), the T1 and T3 aerosol particles proved to have a prolonged stability when stored on gelatin filters. The number of phages inactivated over time corresponded to a reaction of the first order (Fig. 2). Apparently, however, stability cannot be understood as being due to a stability-promoting effect of the filter material. The response of both of the phage aerosols studied was identical to that generally known of T phages and their environmental stability. At 4°C, the T1 phage had a half-life of 147 h, which was more than three times the stability of the T3 phage with a half-life of 44 h (Table 1). These values were obtained for aerosols generated from nutrient broth.

When the concentration of the stabilizing components was dramatically reduced in the nutrient broth by diluting it 1:1,000 for aerosol generation, the half-life for the T1 aerosol particles was decreased two and a half times to 58 h (Fig. 2 (2), Table 1). A storage temperature of 4°C caused less inactivation than did 22°C, just as expected. For non-stabilized T1 particles (nutrient broth diluted 1:1,000, Fig. 2 (2)), the half-life decreased to 25 h when the filters were stored under room conditions.

Petras [3] confirms that the stability of microbes recovered from bacterial aerosols (Serratia marcescens), during storage on gelatin filters depends on the composition of the liquid used to generate the aerosols. The addition of powdered skim milk considerably prolongs the life of part of the Serratia marcescens bacteria collected compared with those in aerosols generated with distilled water.

In the case of the influenza virus aerosol, storage of the filters in an air-dry condition (on filter paper in petri dishes) resulted in a rapid inactivation of the virus within the first 24 h (Fig. 3), even when the filters were stored at refrigerator temperature. The half-life was approx. 4.6 h. During the following 48 h, inactivation yielded a curve with a definitely flatter slope. The half-life increased nearly tenfold to 45.5. h. After 72 h of storage under these conditions, 1.2% surviving infectious units on average were able to be detected.

This result agrees with the general view that myxoviruses have low environmental stability [5]. Apparently, the degree of the general environmental stability of a virus for filters stored under dry conditions also can be regarded as the measure of stability for long-term storage of virus aerosol particles retained on filters.

Wlodawetz et al. [6] studied the persistence of viruses on diverse surfaces (white cotton fabric, linoleum, wood with an oil-based paint coating) under comparable conditions involving contamination of the model surfaces by sedimentation of viruses from artificially generated virus aerosols. Parainfluenza virus type 3 and RS virus were inactivated the fastest: Parainfluenza virus could be detected on cotton fabric only for 6 h, and on wood and linoleum in the course of 24 h. RS virus could no longer be detected on cotton fabric even immediately after contamination, but was still found on linoleum and wood in low concentrations; however, after 3 h, the tests for detection of the virus were negative for all surfaces.

Increase in Stability

The result obtained after storing the exposed filters in swollen condition indicated an approach for increasing the stability of influenza virions sampled from the aerosol. For this purpose, the filters were placed, immediately after sampling, in 200-ml Erlenmeyer flasks with 5 ml of Adamczyk's survival medium [4] to retain the virions' ability to replicate, and the flasks were kept at +4°C. The filters immediately spread out over the shallow layer of liquid and, after a few minutes, they took on the appearance of swollen filter slabs.

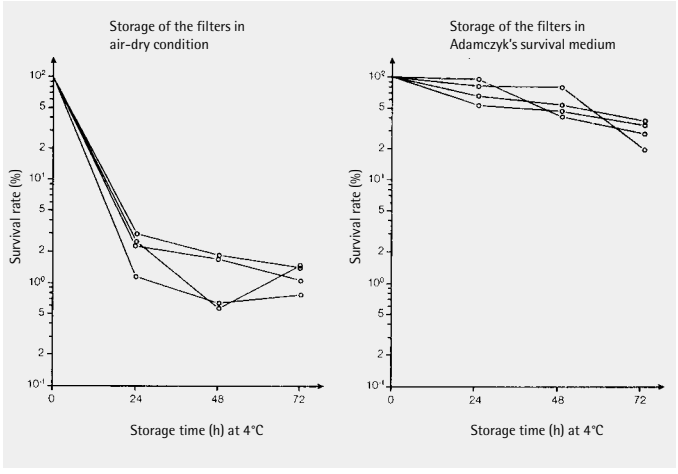

Fig. 3 Stability of influenza virus aerosol particles, following collection on filters, during 72 h of storage of the filters at 4°C in an air-dry condition and in swollen condition after soaking in 5 ml of Adamczyk's survival medium (right side of the graph). Virus aerosol with survival medium used as liquid for aerosol generation (titer $2.2 \cdot 10^8$ HAdU/ml) at 20°C and a relative humidity of 40 to 45%.

Under these conditions, inactivation took place at a significantly slower rate. Therefore, the kinetics of this inactivation corresponded to a reaction of the 1st order. The half-life was 41.5 h, and after 72 h, only 30% surviving infectious units could be detected compared with 1.2% when the filters were stored in dry condition (Fig. 3). Whether the increase in the stability of the virions is the result of the swollen filter gelatin and | or the components of the survival medium (buffering, yeast extract) cannot be decided based on the setup of these tests. However, this issue is insignificant for the actual virus sampling procedure. What is indeed important is the pronounced increase in the stability of a virus when stored in this manner, even if it has a low environmental stability. This increased stability upon storage can be attained even when sampling is done in the field, as will be shown later.

Immediate transfer of the exposed filters to a survival or preservation medium for transport is therefore recommended as a standard procedure. However, it is advisable to test the influence of the filter on the reaction of the medium chosen in order to prevent negative effects on the virus and on the cell culture. Gelatin filters have a weakly acidic reaction. In the present case, control measurements of the pH showed that the initial pH of the survival medium was lowered from 7.2 to 6.1. Increasing the initial pH to 7.6 compensated for such acidification.

Table 1: Half lives ($t_{½}$) of inactivation of phage aerosol particles, when stored on gelatin filters, as a function of the composition of the liquid for aerosol generation and of the storage temperature.

		Liquid for aerosol generation	
Phage	Temp.	Nutrient Broth	Nutrient Broth 1:1,000
T1	4°C	t½ 147.26 h	58.25 h
	22°C	t½ –	25.58 h
T3	4°C	t½ 44.60 h	–

(–) = not testet

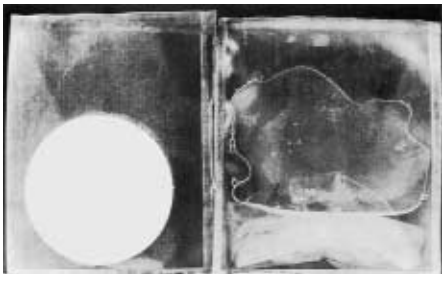

Fig. 4 Filter pocket made of polyethylene for storage and transportation of virus-laden gelatin filters. On the left is a filter sealed in a naturally moist condition in the pocket for comparison to the filter on the right, which received 5 ml of survival medium and is swollen.

Summary

By selecting optimal protective solutions yet to be tested, the survival rate of sensitive viruses can certainly be further increased. Obara et al. [7] developed a routine procedure for preservation and transportation of infectious material by using, for example, dextrose, skim milk and Na ascorbate as stabilizers and gelatin as an "embedding medium." However, they did perform a redrying step in a desiccator as part of the procedure. Enterobacteriacea, Neisseria, and Haemophilus, among others, survived in the gelatin discs, which acted as a preserving medium, for one to five years.

For field sampling work, a simple-to-perform technique has been tested using filter pockets made of polyethylene. The filter pockets with an interior edge length of 60 × 70 mm were made by sealing seams on a 0.2-mm thick and colorless double-layer film. The interior lumen was sterilized by UV radiation.

At the sampling site, the exposed filters were placed inside the sterile filter pockets, 5 ml of survival medium were added to each pocket, and each pocket was completely sealed. The survival medium contained 400 IUs of penicillin and 400 µg of streptomycin sulfate/ml of medium to suppress infection. The filters were temporarily stored and transported in a portable cooler.

This procedure optimally proved its efficacy in practice for detection of influenza virus in room air of a children's polyclinic [8].

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