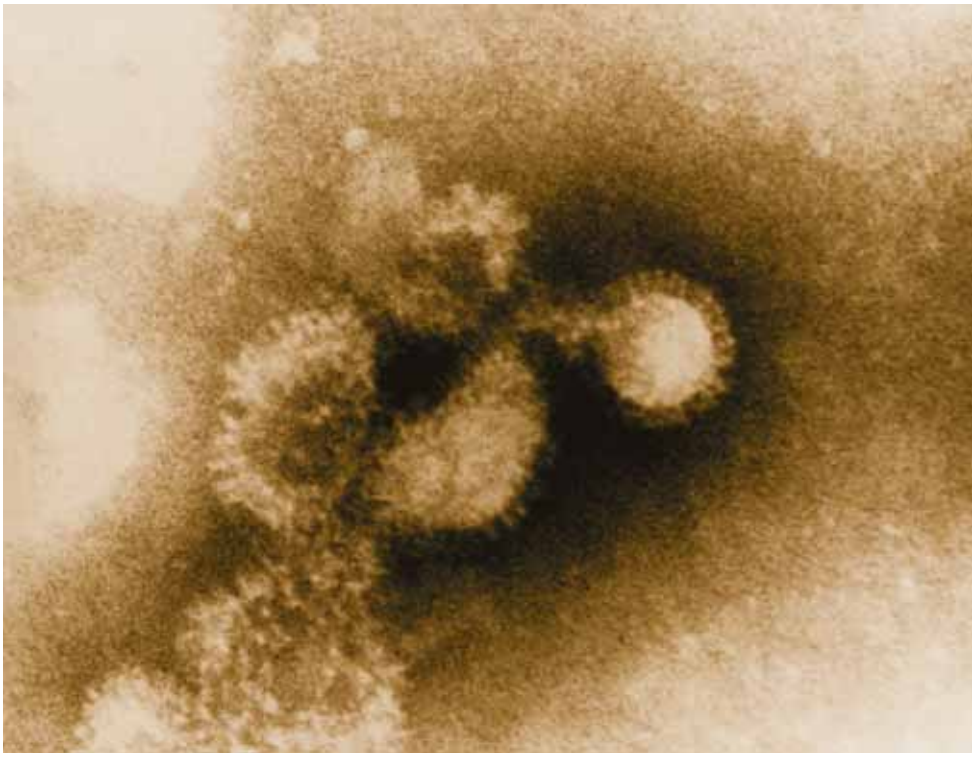
sartorius stedim biotech

Detecting Airborne Influenza Virus A



Application Note #11

Collecting Viruses

#10 #12 #13 #14

turning science into solutions

Detecting Airborne Influenza Virus A (H3N2) in a Children's Polyclinic in Greifswald, Germany, by collecting Virus Using the Water-soluble 12602 Sartorius Stedim Biotech Gelatin Membrane Filter

Introduction

Although the impinger and impaction collectors based on the impaction principle have long ago proved their reliability for the collection of microbial aerosols, filters only recently began to receive increased attention with the development of the water-soluble gelation filter technique and the wide spectrum of applications offered by such filters only for collection of bacterial aerosols; however - after Goettingen-based Sartorius Stedim Biotech GmbH had started commercial-scale manufacture of such gelatin filters.

Until now, the value of filters for detection and collection of virus aerosols has been considered limited, and the filters were thought to be useful for collecting stable, resistant viruses, if at all. There has been very little experimental data about this until recently.

Studies done by the Jaschhof team with experimentally produced static aerosols of Tl and T3 coli phages and influenza viruses, strain A|PR|8|34 (H1N1) using the Sartorius Stedim Biotech Gelatin Membrane Filter (12602) yielded a constant collection efficiency for these viruses, even under conditions involving high inlet velocities (up to 1.6 m/s max.) and an extended collection period (15 minutes max.).

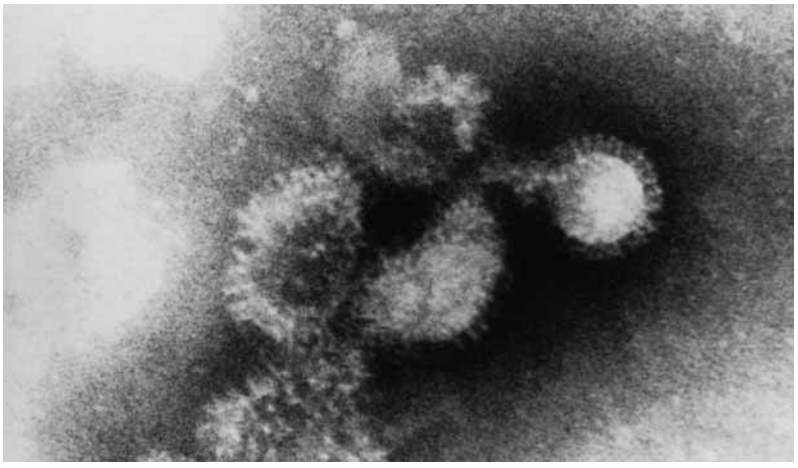
The high resistance of the gelatin filter to mechanical stress and the proven stability of the influenza virus were the reasons for testing the effectiveness of this method in practice during the seasonal rise in morbidity for **a**cute **r**espiratory **d**iseases (ARD) at the beginning of 1990 (3rd – 5th week). This study builds upon results obtained from tests with experimentally produced virus and phage aerosols.

Air samples were taken over a period of 3 days in the waiting room of a Greifswald children's polyclinic and the water-soluble filter material was then used in virus cultivating experiments. Experiments for cultivating the virus isolated from nasal secretions obtained by aspiration from the children in the waiting room, who had a fresh case of ARD, were performed in parallel.

Materials and Methods

Location Studied:
Waiting room of a Greifswald children's clinic (approx 120 m^3) frequented by a maximum of 57 children accompanied by a parent over a period of 2 hours (Jan. 24, 1990). Temperature: 25°C, 60% relative humidity.

Experimental | Test Materials:
Nasal secretions from children with a fresh case of ARD. Gelatin membrane filter, 12602, after 1.8 m^3 of room air was filtered through it. It was then sealed in a polyethylene bag along with 5 ml of an isotonic buffer for cell cultivation at pH 7.4 with



Scanning electron micograph of the influenza virus A|PR|8|34 (H0N1).
Viruses from a culture of an 11-day old embryonic chicken egg and purified with a saccharose density gradient.
Micrograph: Herrmann | Fischer

0.5% yeast extract (Difco), penicillin G (400 IUs/ml) and streptomycin (400 µg/ml). All samples were processed within 4 hrs (with storage in between in a cooling chamber).

Air sampling:
3 days of air filtration with each sample consisting of 1.8 m^3 of air per filter (120 l/min) during a collection period of 15 min through a Sartorius Stedim Biotech Gelatin Filter (126 02-050).

Virological Analyses:
Nasal secretions: Hank's solution (3 ml per sample) was added and the samples were centrifuged for 30 min (3,000 rpm), each given an additive of 10 µl antibiotic solution/ml of supernatant, and incubated for 1 hr at room temperature. The amnion of 10-day old incubated egges was inoculated with these specimens and incubated for 3 days at 33°C; 3 blind subcultures were prepared; HAT was used to detect the viruses.

Air samples: The filter was liquified for 5 min at 37°C in a water bath; an antibiotic solution as described above was added to the solution which was used to inoculate incubated eggs; FL cell and ascites tumor cells were then cultured; the viruses were detected according to the usual techniques by HAT, CPE, and HAdT.

The influenza virus isolated from the nasal secretions and air samples were typed and identified by cross titration in HAHT with rabbit immune sera against the influenza virus A|Greifswald|1|89 (H1N1) and A|Greifswald|1|90 (H3N2) and antigens from the influenza virus A|Sichuan|2|87 (H3N2) and influenza virus A|Greifswald|2|88 (H3N2).

The v-RNA and virus protein patterns were determined by PAGE.

Results and Discussion
From the experimental material taken on Jan. 24, 1990, three types of viruses isolated from the nasal secretions and an influenza virus A isolated from an air sample were successfully inoculated in the incubated embryonated eggs during the third transfer for subculturinq (influenza virus A|Greifswald|2|90 [H3N2]).

HAT showed that the antigens of the isolated viruses and those of the variants A|Greifswald|2|88 and A|Sichuan|2|87 were largely related and did not exhibit any differences from a strain which was cultivated from a nasal secretion specimen taken from a patient who was in the room at the time of air sampling using filters (influenza virus A|Greifswald|1|90 [H3N2]).

The migration rates of the isolated viruses' proteins (HA, NP, NS and M) and all bands of their v-RNA following PAGE (polyacrylamide gel electrophoresis) matched to a high degree (Fig. 1). Attempts to isolate further viruses from air samples using cell cultures were not successful.

The fact that the air samples were taken in the pre-epidemic phase of the ARD morbidity dynamics demonstrates the effectiveness of the filter collection method used in detecting airborne influenza viruses.

Seventy-five percent of the children (≥ 8 years old) present on the day of the studies had a respiratory infection, but only 40% of these children at most showed acute catarrhal symptoms (coughing, sneezing, runny nose). The detection of the influenza virus A in the air of a children's clinic waiting room, during a period of only a slight increase in seasonal morbidity from acute respiratory diseases in the region, remarkably confirms* the results obtained from using experimentally produced static aerosols of coli phages and influenza virus A.

This verifies, for the first time the suitability of the gelatin filter in practice for the collection of respiratory viruses from the air.

* (see Sartorius Stedim Biotech Application Notes Publication No. SLF4028-e)

Outlook
Considering the uncomplicated method of air filtration involving comparatively less work, this principle using the gelatin membrane filter is bound to receive increased recognition in helping to answer a multitude of questions about the production, stability and spread of infectious virus aerosols (e.g., involved in genetic engineering techniques).

Supplementary issue of the condensed text version of a poster displayed during the Annual Conference of the Society of Virology, Potsdam March 19-23, 1991).

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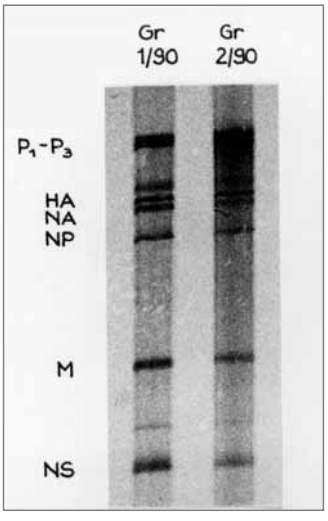

Figure 1:
Separation of the v-RNA in polyacrylamide gel electrophoresis (PAGE) from the influenza virus A|Greifswald|1|90 (H3N2) (isolated from patient specimens) and the influenza virus A|Greifswald|2|90 (collected and isolated from the air.) 3.5% polyacrylamide gel in tris-acetate EDTA buffer.

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Specifications subject to change without notice. Printed in Germany on paper that has been bleached without any use of chlorine. W - G.
Publication No.: SM-4018-e09066 · Order No.: 85030-504-02 · Ver. 06 | 2009