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An adhesive surface sampling technique for airborne viruses

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SUMMARY

A mixture of sucrose, glycerol and bovine serum albumin produces a stable coating in a Petri dish which remains adhesive for up to an hour when exposed in a slit sampler. Virus aerosols can be collected on this surface followed by the direct addition of cell cultures to demonstrate the presence of viable virus. The technique is applicable to the Andersen sampler. A modified version of this sampler has been produced with the same particle collection efficiency as the standard Andersen sampler. The plaque counts obtained by the adhesive surface sampling technique are believed to give an indication of the number of particles collected bearing viable virus.

INTRODUCTION

Recovery of viable virus from aerosols of natural origin by several types of aerobiological sampler has proved difficult (Artenstein & Cadigan, 1964; Downie *et al.* 1965; Westwood, Boulter, Bowen & Maber, 1966; Maclean, Bannatyne & Givan, 1967). The use of large volume sampling (10,000–20,000 l./min.) has shown that more frequent recoveries of viable virus could be obtained than had been previously possible (Gerone *et al.* 1966; Artenstein, Miller, Lamson & Brandt, 1968; Winkler, 1968).

The total volume of air sampled by most instruments is mainly limited by loss of water from the sampling medium. Control of this loss was considered most likely where sampling took place onto a surface, e.g. slit sampler (Bourdillon, Lidwell & Thomas, 1941) and the Andersen Sampler (Andersen, 1958), rather than into a liquid. Both of these samplers have been used previously to collect airborne virus. Samples were collected on agar followed by washing (Vlodavets, Gaidamovich & Obukhova, 1960; Jensen, 1964) and on gelatin followed by liquefaction (Dahlgren, Decker & Harstad, 1961; Kuehne & Gochenour, 1961; Couch *et al.* 1965; Gerone *et al.* 1966; Wolfe, Greisemer & Farrell, 1968; Rechsteiner, 1968). It was suspected that loss of viable virus could take place due to inefficient washing. May (1966) showed that bacteria could not be effectively washed off agar. Vlodavets *et al.* (1960) concluded that the apparent low concentration of virus collected by a slit sampler (followed by washing) was explained by partial retention of particles on the agar surface. Jensen (1964) sampled on agar and agar coated with skim milk followed by washing to recover the virus. He found that plain agar alone was only half as efficient as skim milk on agar. It is likely that the skim milk

coating prevented adhesion of virus to the agar, and also limited its diffusion into the agar.

To determine the extent of the loss of viable virus due to washing and diffusion, the gelatin liquefaction and washing methods were examined. The losses were eliminated by the development of an alternative sampling technique which in addition enabled the sampling period to be considerably prolonged.

MATERIALS AND METHODS

Organisms. Bacillus subtilis var *niger*, spores (A.T.C.C. 9372); vaccinia virus, Lister Institute vaccine; polio virus, Sabin attenuated type 1 strain L Sc 2ab; Semliki Forest virus (Smithburn & Haddow, 1944).

Cell lines. Mainly a HeLa cell line (Appleyard & Westwood, 1964) was used but other lines were also tested including eight animal kidney cell lines, a guinea pig heart line, chick embryo cells and a Wistar 38 cell line.

Methods. Suspensions of spores ($7{\cdot}5 \times 10^3$/ml.) in phosphate buffer manucol agar with 0·1% antifoam, or viruses (2×10^5–1×10^8 pfu/ml.) in 199 medium with antifoam were sprayed in an aerosol apparatus (Henderson, 1952) at 55% R.H., 23° C. The aerosol could be suspended in a drum (Goldberg, Watkins, Boerke & Chatingny, 1958) or led into an Andersen or slit sampler, both sampling at 1 ft.^{3}/min. (28·3 l.). Control samples were taken from the spray tube with Porton impingers, operating at 0·406 ft.^{3}/min. (11·5 l.), containing phosphate buffer or 199 medium mixtures as appropriate. Vaccinia virus was assayed on monolayers and the polio virus and Semliki Forest virus in suspended cell cultures in Noble agar.

PRELIMINARY INVESTIGATIONS

Liquefaction of 12% gelatin, to recover vaccinia virus, followed by addition to HeLa cells resulted in disturbed monolayers. This was probably a toxic effect of the gelatin used and has been observed by other workers (Artenstein *et al.* 1968).

Washing methods were used to recover vaccinia and polio viruses from agar surfaces. Equal volumes of virus suspension were spread on agar and added to HeLa cell suspensions as controls. The agar surfaces were irrigated thoroughly with nutrient medium to recover virus and the washings mixed with HeLa cells.

Plaque counts obtained with vaccinia and polio virus are given in Table 1. Recovery of virus, despite thorough washing, was erratic and, on average, less than 50% could be removed from the agar surface. With polio virus, HeLa cell suspensions were added to the agar surfaces both after and before washing. The resulting plaque counts (Table 1; Polio 1*c*) showed that after washing a good deal of virus was still present on the surface of the agar, while addition of HeLa cells 10 min. after spreading the virus suspension showed that much of the virus appeared to have diffused into the agar (Table 1; Polio 2*c*). This was confirmed by spreading polio virus on the surface of agar containing suspended HeLa cells with subsequent plaque formation. The ability of the virus to diffuse is of course a prerequisite of plaque formation in the agar suspended tissue cell technique.

When direct sampling on prepared cell cultures was attempted it was found that monolayers, under liquid medium, were quickly disrupted when exposed in a slit sampler. Exposure of suspended cell cultures in agar resulted in the death of the cells near the surface after 15 min. A Semliki Forest virus aerosol was sampled for periods up to 30 min. on suspended cell cultures with a slit sampler. Plaque formation was not visible after more than 7 min. sampling.

Gelatin liquefaction, washing methods and direct sampling on cell cultures were found therefore to have severe limitations. Prolongation of the sampling period

Table 1. *Recovery of vaccinia and polio viruses from agar surfaces*

Virus	Samples	Plaque counts per sample					Mean counts
Vaccinia	(*a*) Controls	22	30	27	20		25
	(*b*) Agar surface washings	4 8	5 15	16 12	19 6		11
Polio 1	(*a*) Controls	34 52	32 41	36 62	33 51	32 48	42
	(*b*) Agar surface washings	23 11	12 15	26 10	25 11	25 5	17
	(*c*) Cells added to washed agar	9 11	10 11	5 16	5 31	7 35	14
Polio 2	(*a*) Controls	90 54	88 60	60 44	67 42	68 60	63
	(*b*) Agar surface washings	29 10	20 10	6 14	3 20	5 17	13
	(*c*) Cells added without washing	7 8	9 16	8 9	10 26	17 —	12

— = No sample.

to sample larger volumes of air required control of evaporation. This necessitated the development of a suitable medium to produce a sampling surface resistant to water loss on which to collect airborne virus particles. If tissue culture cells could then be added directly to this surface, to demonstrate viable virus without further manipulation of the sample, the losses occurring in the recovery methods described above would be avoided.

Development of an adhesive sampling surface

The use of an adhesive surface to collect airborne virus was designed to serve two purposes. First, by reducing water loss it would enable much larger volumes of air to be sampled using long sampling periods (30–60 min.), and second by eliminating losses inherent in other sampling methods it would enhance the recovery of small amounts of viable virus.

The main requirements of such a sampling surface were that it should remain adhesive for the whole of the sampling period, retaining particles produced by

shattering on impact (Davies, Aylward & Lacey, 1951), and it should be compatible with viruses and cell cultures.

Twenty-one substances and mixtures were examined for (*a*) their ability to remain adhesive for 1 hr. on a slit sampler, (*b*) water solubility at 37° C. on completion of the sampling period and (*c*) compatibility with the viruses and cell lines given in Materials and Methods. The substances tested included calf serum, bovine serum albumin, gelatin, egg albumin, carboxymethyl cellulose, polyethylene glycol, dextran, glycerol, hydrolysed starch, sucrose, skim milk and various mixtures of

Table 2. *Effect of the S.G.B. surface on plaque formation by vaccinia and polio virus*

Virus	Samples	Plaque counts per sample					Mean counts
Vaccinia	Controls	43	40	43	39	36	40
	S.G.B.	38	40	34	39	37	38
Polio 1	Controls	14	8	16	11	13	12
	S.G.B.	15	23	17	11	12	16
Polio 2	Controls	37	38	39	39	34	37
	S.G.B.	29	33	38	40	50	38
Polio 3	Controls	140	134	162	132	154	144
	S.G.B.	155	146	140	174	151	153

Table 3. *Effect of exposure in a slit sampler on plaque formation with the S.G.B. mixture*

Virus	Sample	Length of exposure (min.)	Plaque counts per sample					Mean counts
Vaccinia	Control	0	43	40	43	39	36	40
	Slit sampler	6	36	33	39	40	32	36
Polio 1	Control	0	28	26	36	32	30	30
	Slit sampler	5	20	33	34	20	36	29
		10	10	29	47	38	20	29
		20	28	36	—	—	—	32
		30	26	30	—	—	—	28
Polio 2	Control	0	56	64	62	50	62	59
	Slit sampler	5	63	71	77	84	94	78
		10	96	85	98	89	70	88
		20	80	66	—	—	—	73
		30	77	51	—	—	—	64

— = No sample.

the foregoing. A mixture of equal parts of a saturated solution of sucrose and glycerol with 0·1% of 10% bovine serum albumin (S.G.B. mixture) eventually proved satisfactory. An adhesive surface which fulfilled all the requirements was produced by spreading 0·2 ml. of this mixture in a Petri dish. It remained adhesive for up to 1 hr. exposure in a slit sampler. Cell suspensions added to these exposed plates formed satisfactory monolayers.

Quantitative tests with vaccinia and polio virus, to examine the effect of the

S.G.B. surface on plaque formation (Table 2), did not indicate any inhibitory action. Suspensions of both viruses were spread on prepared S.G.B. surfaces and then the plates were exposed for varying periods in a slit sampler. The results (Table 3) did not show any evidence of inhibition of plaque formation. Growth of common airborne bacteria and fungal spores was prevented by the presence of antibiotics and an antifungal agent, amphotericin B (0·0025 mg./ml.) in the nutrient medium.

AEROSOL EXPERIMENTS

Bacterial spore aerosols

In order to establish the comparative sampling efficiency of the slit with the impinger and Andersen samplers preliminary tests were carried out with the bacterial spore in the aerosol apparatus. The results are given in Table 4. Break up of particles in the impinger would of course result in higher colony counts in comparison with the slit sampler. The impinger and the Andersen samplers have higher collection efficiencies than the slit sampler for particles of 1 μ and less.

Table 4. *Comparison of the collection efficiency of the slit sampler with the impinger and Andersen samplers using bacterial spore aerosols and agar plates*

Sampler	Time (min.)	Volume sampled (ft.^{3})	Colony counts per sample					Mean count per ft.^{3}	C.E. (%)
Slit	1	1	209	270	—	—	—	239	60
Imp.		0·406	162	158	—	—	—	397	
Slit	1	1	63	66	57	47	51	56	64
Imp.		0·406	36	32	40	38	34	88	
Slit	1	1	30	30	28	28	38	31	63
And.		1	46	53	52	43	51	49	

Slit, Slit sampler; Imp., Impinger; And., Andersen sampler; C.E., Collection efficiency.

As can be seen from Table 4, very similar percentage collection efficiencies were obtained in the comparisons of the slit sampler with the impinger (60 % and 64 %) and the Andersen sampler (63 %).

Erhlich, Miller & Idoine (1966) compared the collection efficiencies of the slit sampler and the impinger using the same bacterial spore. Particles collected by the slit sampler were recovered by washing. An average figure of 50 % was obtained for the comparative collection efficiency. No account was taken of losses which occur during washing to recover the spores. Each Petri dish in the standard Andersen sampler normally contains 27 ml. of agar to bring the sampling surface to the correct height (0·1 in.) below the orifice plates. In order to use the adhesive surface technique with this sampler, glass disks coated with the S.G.B. mixture were tried initially to replace the agar. However, they proved generally unsuitable for monolayer work. Consequently a modified Andersen sampler was constructed in which the orifice plates were lowered to the requisite distance above the sampling plates coated with the S.G.B. mixture.

The sampling characteristics of the modified Andersen sampler were compared with the standard version using spore aerosols. Table 5 gives the results showing that there were no significant differences in collection characteristics of the two versions of the sampler.

Table 5. *Comparison of the standard and modified Andersen samplers using a bacterial spore aerosol*

	Volume sampled (ft.³)	Colony counts for stage number 2	3	4	5	6	Mean total counts
Standard Andersen sampler	1	2	0	3	19	22	50
		0	0	8	15	30	
Modified Andersen sampler	1	0	0	7	12	27	51
		0	0	1	18	42	
		0	0	2	13	31	

The plates in the modified sampler contained a thin layer of agar on which colonies formed satisfactorily. Stage 1 was not used in either sampler.

Table 6. *Comparison of collection efficiency of the slit sampler (using the S.G.B. surface) with the impinger, sampling aerosols of vaccinia and polio viruses*

Sampler	Virus	Time (min.)	Volume sampled (ft.³)	Plaque counts per sample					Mean counts per ft³	C.E. (%)
Slit	Vaccinia	½	0·5	100	112	146	118	142	271	52
				212	94	148	106	178		
Imp.			0·203	11	10	9	14	11	521	
				10	11	9	12	9		
Slit	Vaccinia	½	0·5	160	170	153	158	159	320	65
Imp.			0·203	7	10	10	11	12	492	
Slit	Vaccinia	10	10	117	120	127	—	—	12	57
Imp.			4·06	8	9	8	—	—	21	
Slit	Polio	½	0·5	126	116	120	—	—	240	61
Imp.			0·203	9	8	7	—	—	392	

Slit, Slit sampler; Imp., Impinger sampler; C.E., Collection efficiency.

Note. The plaque counts given for the impinger samples are the results of assay of 1 ml. out of the 10 ml. of sampling fluid in each impinger.

Virus aerosols

Vaccinia virus was sampled with the slit sampler on S.G.B. mixture plates at different aerosol concentrations. Half-minute samples were taken at the higher concentrations and 10 min. samples at the lower. Similar samples were taken with impingers. HeLa cells were poured directly onto the S.G.B. surface after sampling to form monolayers while the impinger samples were assayed by mixing 1 ml. amounts of the sampling liquid with HeLa cells. The results given in Table 6 showed that the comparative collection efficiency varied between 52 and 65%. Polio virus was used in a similar experiment. HeLa cells suspended in agar were added to the adhesive surface on completion of sampling. The plaque counts

obtained are given in Table 6. A comparative collection efficiency of 61 % was obtained.

A vaccinia virus aerosol was sampled with the slit sampler and the modified Andersen sampler with the results given in Table 7. The comparative collection efficiency was 51 %. Impinger samples were also taken during this experiment, giving a comparative collection efficiency of 78 % when compared with the Andersen sampler.

Table 7. *Comparison of the collection efficiency of the slit sampler and the impinger with the modified Andersen sampler using a vaccinia virus aerosol*

Sampler	Volume sampled (ft.^{3})	Plaque counts per sample						Mean counts per ft.^{3}	C.E. (%)
Slit	0·5	160	170	153	158	159		320	51
Impinger	0·203	7	10	11	11	12		492	78
		Plaque counts for stage no.							
		2	3	4	5	6	Totals		
Modified Andersen	0·5	2	9	15	14	238	278	627	
		1	9	15	14	310	349		

Note. The plaque counts given for the impinger samples are the results of assay of 1 ml. out of the 10 ml. of sampling fluid in each impinger. S.G.B. plates were used in the slit and modified Andersen samplers. Stage 1 was not used in the Andersen sampler. C.E. = Collection efficiency.

DISCUSSION

The collection efficiency of the slit sampler, using the adhesive surface method for sampling virus aerosols was 52–65 % when compared with the impinger. This result is very similar to that obtained with the tracer spore aerosol (60–64 %). Colony counts obtained with bacteria in a slit sampler indicate the number of particles bearing viable organisms in the aerosol, while the disruptive effects of the sampling process in the impinger give rise to colony counts which relate to the number of viable bacteria in the aerosol. Since the comparative collection efficiency of the slit sampler was very similar for both the spore and the virus aerosols it is possible the plaque counts obtained with the slit sampler give an indication of the number of particles bearing viable virus. A similar conclusion is applicable to the results obtained with the Andersen sampler.

The smallest particles in the bacterial spore aerosol detectable on the last stage of the Andersen sampler could not be smaller than the size of the individual spores, i.e. about 1 μ. Smaller particles are however produced by the Collison spray and through drying of the aerosol. When viruses are used these submicron particles can carry viable virus and hence are detectable when collected on the last stage. This is shown by comparison of the percentage distribution recoveries calculated from the modified Andersen sampler results (Tables 5 and 7) obtained with the spore and virus aerosols. An average of 55 % of the spore aerosol was collected on the last (6th) stage compared with 70 % of the virus aerosol. This accounts for the

apparent fall in the collection efficiency of the slit sampler when compared with the Andersen sampler from 63 % of spore aerosols to 51 % with virus aerosols. The difference is due to the superior collection efficiency of the Andersen sampler for submicron particles compared with the slit sampler. When the impinger was compared with the modified Andersen sampler (Table 7) it had a comparative sampling efficiency of 78 %. Jensen (1964) also found the Andersen sampler was more efficient than glass impingers for sampling virus aerosols.

Guerin & Mitchell (1964) sampling virus aerosols with an Andersen sampler used only 5 ml. of gelatin in each dish instead of the normal 27 ml. This would be likely to alter the sampling characteristics mainly in the direction of loss of smaller particles. The percentage distribution recoveries they obtained for an aerosol agent in a drum were 26, 31 and 31 % for stages 4, 5 and 6 respectively. In contrast, the present author found in a similar drum experiment that 81 % of the virus aerosol collected was retained on the last stage of the sampler.

Couch *et al.* (1965) sampled virus aerosols, from a Collison spray, on gelatin layered on agar (total volume 27 ml.), in an Andersen sampler from aerosol apparatus similar to that used by the author. They observed that 85 % of the particles collected were less than 1 μ in size and that more than 90 % of the virus was retained on the lower three stages. This finding is closely similar to the figures given in Table 7, where 95 % of the virus was collected on the lower three stages. The distribution of virus on these three stages was however different from those reported by Couch *et al.*:

Couch *et al.* (1965)	This report
13 %	4·5 %
68 %	20·6 %
10 %	70·50 %

Couch *et al.* estimated the proportion of aerosolized material retained on each stage and found that 54 % of the total volume collected was in the particle size range 1–2 μ, i.e. on the penultimate stage. After sampling the gelatin was liquefied, serially diluted and inoculated into roller tube cultures. These manipulations tend to cause break up of the collected aerosol particles disseminating the virus. Consequently the plaque counts obtained by this method indicate the amount of viable virus collected on each stage. The larger volume of aerosolized material and hence greater proportion of virus collected on the penultimate stage is shown by the higher *percentage recovery* on this stage calculated from the plaque counts. In contrast the adhesive surface sampling method, since it causes little disturbance of the sample, gives an indication of the number of particles bearing viable virus collected on each stage. As Couch *et al.* found, 85 % of the particles were less than 1 μ, many carrying viable virus, and so, with the method described in this report, would result in a high percentage recovery on the last stage.

The adhesive surface sampling method is a simple technique which enables larger volumes of air to be sampled by the slit and Andersen samplers. Direct addition of cell cultures to the sample without further manipulation aids the

detection of small amounts of viable virus which might be lost by the use of the other methods described. Survival of the viruses used in these investigations on the adhesive surface during prolonged sampling periods was good. But the method has yet to be tried with less robust viruses. The large volume samplers which have been successfully used for the recovery of virus from natural aerosols give information on the amount of viable virus collected. The adhesive surface method indicates the number of particles bearing viable virus and also, with the Andersen sampler, their size distribution.

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