Technical methods

A simple method for quantitating salivary levels of virus using calcium alginate swabs

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Some body fluids, because of their inaccessibility and/or small volume, are often difficult to collect for microbiological quantitation. Oropharyngeal and nasopharyngeal secretions, of both humans and laboratory animals, are examples of fluids the collection of which in accurate volumes can be tedious and time-consuming. The task is even more difficult when large numbers of subjects are involved. In our studies of feline leukaemia virus (FeLV) we utilised a simple, inexpensive method for saliva collection using calcium alginate swabs. Because this method could be applicable to many situations in clinical pathology and microbiology we report it here in detail.

Method

Calcium alginate swabs (Calgiswabs, Inolex, Glenwood, Ill, USA) were used in all experiments. For saliva collection, swabs were rotated for 5 to 10 seconds in a buccal crease. To determine the mean amount of fluid absorbed by the swabs, saliva specimens were obtained from human volunteers. To determine the absorption of virus-containing saliva, specimens were obtained from a cat known to be excreting FeLV. After narcotising the animal, drooled saliva was obtained in an iced petri dish. Immediately after collection of the drooled saliva, paired collected specimens were obtained by rotating one swab in the drooled saliva and another in the cat's buccal crease.

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For extraction of virus from the swabs the tips were cut and placed in sterile vials containing 1.8 ml McCoy's 5A medium with 15% fetal bovine serum, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin (Grand Island Biological Co, Grand Island, New York). The tubes were frozen rapidly at -90°C until tested. At the time of thawing, the tubes were mechanically agitated and the supernatant was either overlaid directly on the indicator cells or diluted and subsequently overlaid on the indicator cells.

To determine the accuracy of swab-collected specimens in fluids other than saliva, aliquots of cell culture supernatant from a standard FeLV-producer lymphoid cell line (F422) (Noronha *et al.*, 1974) were tested. Virus titres of aliquots of the same supernatant were compared after collection by pipette or swab. Titres of FeLV were determined by titration on an indicator cell line (Fischinger *et al.*, 1974; Francis *et al.*, 1977). Briefly, triplicate solutions of virus were made in cell culture medium and placed over 1-day-old, DEAE dextran-treated CCC81 monolayers in six-well cluster plates (Falcon Plastics, Oxnard, Calif, USA). After 13 days foci were counted and virus concentrations were reported in focus forming units (ffu).

Results

The calcium alginate swabs were remarkably consistent in construction, and, as a result, the amount of fluid absorbed by them was predictable. Thirty-eight swabs taken from four different lots purchased over a two-year period were tested. They absorbed a relatively constant amount of saliva ($0.0097 \text{ g} \pm 0.003 \text{ g}$ for one standard deviation).

The comparison of virus concentrations in swab-collected supernatant medium and pipette-collected supernatant revealed similar results (Fig. 1). Each of the nine values for swab samples or of the 15 values for pipette samples represents a mean figure from triplicate plating of each sample. Although the mean titre of virus in the supernatant collected by swab was less than that of the pipette-collected samples, the range of both groups prevented any significant differentiation of the values.

The last experiment compared virus concentrations in drooled saliva and saliva collected by swabbing the oral mucosa (Fig. 2). For this experiment virus

Fig. 1 Comparison of levels of infectious FeLV obtained from tissue culture supernatant titred directly with virus levels obtained with the use of calcium alginate swabs previously soaked in the same supernatant fluid.

Fig. 2 Comparison of levels of infectious FeLV obtained using calcium alginate swabs dipped in drooled saliva with virus levels obtained using swabs moistened directly in the buccal crease of the same cat.

titres of swabs dipped in drooled saliva were compared with virus titres of simultaneously collected oral swabs. Each value represents a mean figure from the triplicate plating of each swab sample. The correlation was good, but virus titres from oral swab specimens were consistently five- to 10-fold higher than paired titres from specimens of drooled saliva (Fig. 2). Approximately half of this difference disappeared when cellular debris was removed before testing. The remainder was apparently due to different concentrations of virus in drooled versus mucous membrane associated saliva.

Calcium alginate swabs are useful for the quantitative collection of body fluids. Their remarkably constant construction, resulting in predictable fluid absorption, allows good quantitation without tedious weighing of swabs. Concentrations of cell-associated agents, however, can be spuriously elevated because of removal of cells by the swabbing procedure. These swabs should have multiple applications in microbiology, especially when large numbers of specimens are collected for quantitative analysis.

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