NOTES

USE OF CRYSTAL VIOLET OR BRILLIANT GREEN DYES FOR THE DETERMINATION OF SALMONELLAE IN DRIED FOOD PRODUCTS

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The greater efficiency in the recovery of small numbers of salmonellae from dried foods, particularly dried egg albumen, by enrichment of the food in lactose broth as contrasted to direct inoculation of selective enrichment broths has been shown (*unpublished data*).

Another type of enrichment has been found applicable for the examination of other desiccated foods such as dried milk, baby foods, etc. Experiments have demonstrated that 0.002 per cent brilliant green or 0.004 per cent crystal violet in reconstituted nonfat dried milk will permit unrestricted growth of salmonellae and coliform bacteria when small numbers of the organisms are artificially introduced. At the same time the growth of *Streptococcus lactis* is inhibited, as shown by the lack of significant lowering of the pH for 48 hr or more, depending upon the numbers of cells of *S. lactis* added.

Sterile reconstituted nonfat dried milk (100 g/liter) with 0.04 per cent brilliant green and artificially inoculated with *Salmonella tennessee* in low numbers (approximately 1/ml) was incubated for 24 hr and streaked on brilliant green agar. Large numbers of salmonellae were observed on the plates. Higher concentrations of the dye (up to 0.07 per cent) restricted growth for 24 hr but not for 48 hr. No multiplication occurred in milk with 0.1 per cent dye even when incubated for 72 hr.

At the recommended concentration of 0.002 per cent brilliant green or 0.004 per cent crystal violet there is negligible inhibition of salmonellae and small numbers can be detected. But, direct streaking on brilliant green agar should be supplemented by loop inoculation into selenite-cystine broth (North and Bartram, Appl. Microbiol., **1,** 130, 1953) which is incubated 6 to 24 hr before streaking on selective agar media.

Dried egg albumen has been examined for salmonellae by enrichment in sterile reconstituted nonfat dried milk with dyes at these concentrations. After incubation periods of 24, 48, and 72 hr and at much longer periods up to 8 days, excellent recovery was obtained without "skips" in the most probable number determinations both by direct streak, and by loop inoculation into selenite-cystine enrichment broth. The longer enrichment periods, up to 72 hr, may be necessary for detection of salmonellae in the highest dilutions.

DIFFERENTIATION OF VARIOLA FROM OTHER MEMBERS OF THE POXVIRUS GROUP BY THE PLAQUE TECHNIQUE

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Several investigators (Noyes, Proc. Soc. Exptl. Biol. Med., **83,** 426, 1953; Youngner, J. Immunol., **76,** 288, 1956; Porterfield and Allison, Virology, **10,** 233, 1960) report that vaccinia virus and other poxviruses are capable of forming plaques on monolayers of susceptible tissue cells. This note describes a method of differentiating variola and alastrim from other mem-

TABLE 1

Comparative titers of representative poxviruses determined by egg and plaque assay techniques

Poxvirus	Strain	Titer per ml	
		CAM*	Plaque
Variola	Yamada	5.2×10^7	0
	New Delhi	2.4×10^7	0
	Harper	4.4×10^7	0
	Horn	2.4×10^7	0
	Minnesota	2.9×10^7	0
	Stillwell	8.5×10^3	0
	Lee	6.6×10^4	0
	Indian	4.6×10^4	0
	Kali Muthu	6.6×10^7	0
	7MB-YM	6.0×10^5	0
	10MB-YM	4.0×10^4	0
	1 CHL-YM	1.2×10^5	0
	10 CHL-KM	6.2×10^2	0
Alastrim	Rochdale	3.5×10^5	0
Vaccinia	IHD	8.7×10^6	1.7×10^6
	D-Vac	2.2×10^7	1.8×10^6
	Minnesota	1.7×10^8	4.4×10^7
Cowpox	White pock variant	2.6×10^5	3.4×10^4
	Red pock variant	6.0×10^5	5.2×10^5
Rabbitpox	Utrecht	1.1×10^7	9.6×10^6
Monkeypox		8.6×10^3	8.0×10^3

* Pock counts on chorioallantoic membranes.

bers of the poxvirus group based on the inability of the first two viruses to form plaques on monolayers of chick embryo fibroblasts. Furthermore, certain poxviruses may be distinguished from others by differences in their respective plaque morphology.

A protocol suggested by Youngner (*personal communication*), modified in minor detail, was employed. Cells were prepared from 9-day-old chick embryos by treatment with trypsin in phosphate buffered saline. Cells were washed once in 0.5 per cent lactalbumin hydrolyzate and 5 per cent calf serum in Hanks' balanced salt containing twice the usual concentration of bicarbonate. The resuspended cells were planted in petri plates (55 mm) in 4-ml samples (1×10^7 cells/ml) and then incubated at 36 C in an atmosphere of 5 per cent CO_2. A medium change was made after 24 hr to remove debris and unattached cells. After an additional 24 hr the cells were washed with phosphate buffered saline and appropriate virus dilutions were added in a volume of 0.5 ml of the same solution. A 5-ml overlay containing 1.1 per cent agar in medium 199 supplemented with 5 per cent calf serum and twice the normal concentration of bicarbonate was added after allowing 4 hr incubation for virus adsorption. To develop the plaques, 0.5 ml neutral red (1:1000 aqueous solution) was added on the 4th day.

Plaques were routinely obtained with 3 strains of vaccinia and with 1 representative strain each of cowpox, rabbitpox, and monkeypox virus, but repeated attempts to induce plaque formation with variola virus and with 1 strain of alastrim failed (table 1).

Plaques formed by monkeypox (not shown) and by cowpox were considerably smaller than those formed by other species (figure 1). Plaque size appeared to be a property of the strain employed, and is a character which has remained stable during repeated recloning (replating) of the virus population. For example, the cowpox strain depicted, a white pock variant, consistently produced small plaques; a red pock variant yielded large plaques equal in diameter to those of the IHD strain of vaccinia.

The plaque technique for differentiating monkeypox virus from variola virus may be especially useful to investigators studying these viral diseases in the monkey, a host which is susceptible to both.

This procedure may also prove valuable as a means of selecting recombinants from mixtures of strains involving variola virus and plaque-forming pox viruses. In addition, it offers an opportunity for observing and selecting plaque-forming viruses of this group which may be reactivated by living (nonplaqueforming) variola virus.

Two criteria are routinely used to differentiate variola from vaccinia in the laboratory: the character of the pock formed on the chorioallantois of the embryonated egg, and the fact that

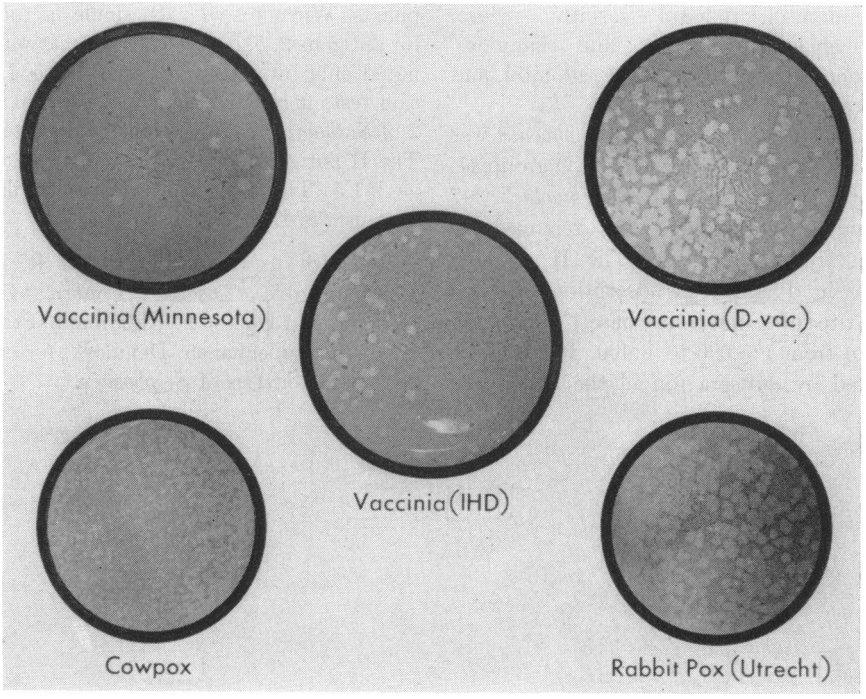

Figure 1. Plaque morphology of representative poxviruses

vaccinia, unlike variola, can be passed serially in the epidermis of rabbits (Downie, cited in *Viral and Rickettsial Infections of Man*, 3rd ed., p. 673. Edited by Rivers and Horsfall, Jr. J. B. Lippincott Co., Philadelphia, 1959). The plaque assay procedure described here offers a third method of differentiation which has been found simple, rapid, and economical in application.

NEW SALMONELLA TYPE: *SALMONELLA PHOENIX*

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Salmonella phoenix was isolated from rectal swabs taken from three infants hospitalized with acute diarrhea. All three cases occurred during the period of 1 month. Clinical data indicated that one of these salmonella infections was acquired in the hospital from one of the other two infants admitted with this organism.

In its cultural characteristics, *S. phoenix* was typical of the salmonella group. The cultures possessed the following biochemical characteristics: Motility, failure to produce indole, methyl-red positive and Voges-Proskauer negative reactions, ability to utilize citrate, and ability to produce hydrogen sulfide. The organism failed to hydrolyze urea and failed to grow in KCN medium. It reduced nitrate to nitrite. The lysine decarboxylase test was positive.

The organism was atypical as it liquefied gelatin and utilized malonate. When tested by the method of Kauffmann and Petersen (Acta Pathol. Microbiol. Scand., **38,** 481, 1956), the organism failed to utilize D-tartrate, although citrate and mucate were rapidly utilized.

Acid and gas were produced promptly from