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Rapid Presumptive Identification of Enteropathogenic *Escherichia coli* in Faecal Smears by Means of Fluorescent Antibody

2. Use of Various Types of Swabs for Collection and Preservation of Faecal Specimens

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Faecal specimens that cannot be cultured immediately for enteric pathogens are usually collected in buffered glycerol-saline solution for preservation and transport to the laboratory. Specimens collected in this way are not suitable for fluorescent antibody studies since the glycerol prevents fixation of faecal smears. Transport of specimens frozen with solid carbon dioxide is a satisfactory but expensive method of preservation. The purpose of the study reported in this paper was to explore the possibility of using various types of swabs for preserving and transporting faecal specimens to be examined for enteropathogenic Escherichia coli. *Both cotton and Dacron fibres were used and freezing and desiccation were investigated as methods of preservation. The results showed that freezing of faecal swabs was contra-indicated, whereas drying at room temperature enhanced the recovery of enteropathogenic* E. coli. *Cotton and Dacron swabs gave equivalent results regardless of whether the organisms were detected by culture or by immunofluorescence.*

The fluorescent antibody (FA) technique for the detection of enteropathogenic *Escherichia coli* (EEC) has been used successfully by several workers (Whitaker et al., 1958 ; Thomason, Cherry & Ewing, 1959 ; Nelson & Whitaker, 1960 ; Nelson et al., 1961 ; Cohen, Page & Stulberg, 1961). In the studies by the first two groups of authors mentioned, fluorescent antibody examinations were made on frozen stool specimens. For routine examinations of children, it is usually easier to obtain rectal swab specimens than passed specimens. However, our data indicated that preservation of the former by freezing was detrimental to viability, since we observed that rectal swab specimens positive by cultural examination became negative after being frozen and thawed. It was determined that considerable loss of viable organisms occurred during freezing regardless of the method used. Furthermore, preservation by freezing is unpractical and expensive when it is necessary to ship small numbers of specimens from outlying districts to a central laboratory for examination.

The usual transport medium for faecal specimens is buffered glycerol-saline. Specimens preserved in this mixture are unsatisfactory for FA examination unless the glycerol is removed (Thomason, Cherry & Edwards, 1959). This washing procedure is time-consuming and results in a mechanical loss of organisms. It was thought that swabs might provide a simple and inexpensive yet efficient method for preserving faecal specimens for both FA and cultural detection of EEC. Stuart et al. (1954) and Stuart (1956, 1959) successfully used cotton swabs that were boiled in phosphate buffer (pH 7.4) and then impregnated with charcoal for the transport of bacteriological specimens. Hollinger & Lindberg (1958) reported that Dacron swabs appeared superior to cotton swabs for collecting throat

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cultures for group A streptococci. The present report is an evaluation of some methods of preserving faecal specimens for transport to a central laboratory for FA and cultural examination. It also includes data on the relative efficiency of Dacron and cotton for preparing swabs.

MATERIALS AND METHODS

Strains

Three strains of *E. coli*, consisting of OB groups O55:B5, O111.B4 and O126:B16, were studied.

Specimens

One hundred and twelve faecal swab specimens were processed.

Media

Heart infusion broth (Difco) was used for preparing broth cultures of *E. coli*. MacConkey agar (Difco) and blood agar base (Baltimore Biological Laboratories) were used for all other cultural studies.

Antisera

OB antisera were prepared for nine enteropathogenic groups of *E. coli* as described by Ewing (1956a, 1956b).

Preparation and labelling of globulins

A globulin fraction of each antiserum was prepared and labelled by the methods described by Thomason et al.[1]

Staining smears with fluorescent antibody

Swab specimens were washed in 0.5 ml of sterile physiological saline. Smears of the washings were made on glass slides, air-dried, heat-fixed, and treated with the appropriate FA reagents according to the procedure described by Thomason et al.[1]

Isolation of Escherichia coli

Fresh faecal swab specimens were streaked on MacConkey and blood agar base plates immediately after collection. After 18-24 hours' incubation at 37°C, representative colonies were selected for slide agglutination tests with OB antisera of the EEC. Dried faecal swabs were soaked and washed in 0.5 ml of sterile physiological saline, and a measured amount of the suspension was cultured as described above.

[1] See the article on page 137 of this issue.

EXPERIMENTAL RESULTS

Survival of EEC when frozen or dried on untreated Dacron and cotton swabs

Tenfold dilutions of broth cultures of *E. coli* O55:B5 and O111:B4 were prepared in infusion broth and plate counts were determined. Duplicate sets of sterile Dacron[2] and cotton swabs were dipped into various dilutions of each of these cultures and placed in sterile screw-capped tubes. One set of each type of swab was held at room temperature while the other set was placed in the freezer at −20°C.

The swabs were sampled immediately, after 24 and 72 hours' storage, and thereafter at weekly intervals. Counts were made by washing a representative swab of each type in 1 ml of 0.85% saline and plating 0.1 ml of the suspension on MacConkey agar plates. Smears were made by applying a standard loopful of suspension to a circular area 1.5 cm in diameter on a slide. Smears then were stained with the appropriate FA reagents.

The results showed that the plate counts decreased after freezing, whereas counts made on unfrozen swabs either increased or did not show significant decreases (Fig. 1 and 2). This was true also for the FA counts. Colony counts, as well as the number of fluorescing organisms, increased during the first 24 hours that the swabs were held at room temperature. No difference was observed in the recovery of EEC from Dacron in comparison to cotton swabs, regardless of the method used to preserve them. EEC were viable and stained well from both types of dried swabs during the eight-week period in which they were sampled, although their morphology was often atypical following prolonged dehydration and prior to beginning of multiplication.

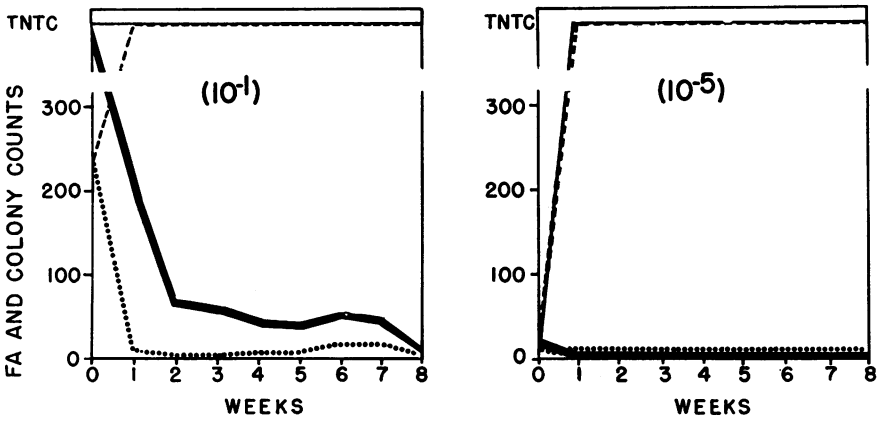
Survival of EEC in faecal specimens dried on buffered and non-buffered Dacron and cotton swabs

One set each of Dacron and cotton swabs were boiled in Sorensen's phosphate buffer (M/15) at pH 7.4 for 5 minutes. This procedure was recommended by Stuart (1959) to neutralize acids from the wood of the swab sticks. The colour and resinous odour of the boiling solution indicated substantial removal of such materials. The swabs were shaken free of excess solution, placed in screw-capped tubes, autoclaved and dried in the

[2] Dacron batting was obtained from the Pacific Felt Co., San Francisco, Calif., USA.

FIG. 1

COMPARISON OF DETECTION OF *ESCHERICHIA COLI* O55 : B5 ON DRIED AND FROZEN DACRON SWABS MADE FROM 10^{-1} AND 10^{-5} DILUTIONS OF A BROTH CULTURE [a]



[a] FA and colony counts are relative. FA counts represent the average number of organisms per field multiplied by 6.

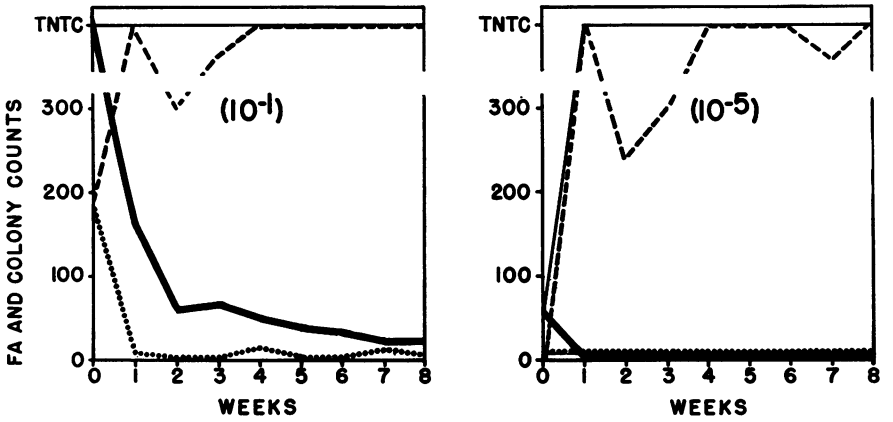
—— Dried swab plate count. ▬▬ Frozen swab plate count.

----- Dried swab FA count. ••••••• Frozen swab FA count.

TNTC = Too numerous to count.

FIG. 2

COMPARISON OF DETECTION OF *ESCHERICHIA COLI* O111 : B4 ON DRIED AND FROZEN DACRON SWABS MADE FROM 10^{-1} AND 10^{-5} DILUTIONS OF A BROTH CULTURE [a]



[a] FA and colony counts are relative. FA counts represent the average number of organisms per field multiplied by 6.

—— Dried swab plate count. ▬▬ Frozen swab plate count.

----- Dried swab FA count. ••••••• Frozen swab FA count.

TNTC = Too numerous to count.

oven. The four sets of swabs, buffered and non-buffered cotton and buffered and non-buffered Dacron, were rotated in pooled sterile human faecal specimens which were seeded with varying numbers of the O126:B16 strain of *E. coli*. Colony counts of the swabs were done immediately, after 24 and 72 hours, and thereafter at weekly intervals for a period of eight weeks.

A significant increase in numbers of *E. coli* occurred on the buffer-treated swabs during the first 24 hours. These high counts were maintained throughout the period of study. There was little or no increase on untreated swabs during this period, and after the first week no *E. coli* could be cultured. No difference between the Dacron and cotton swabs was apparent. The results of this experiment are summarized in Fig. 3.

Since a difference was shown between the treated and untreated swabs with respect to detection of EEC in faeces, it was decided that buffer-treated swabs should be used in subsequent studies.

FA and cultural studies of fresh and stored faecal swab specimens

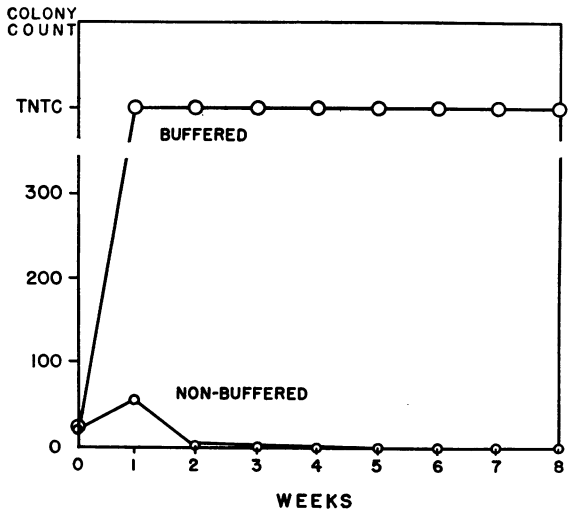
Five rectal swabs were taken from each of 291 children with diarrhoea, as reported in our field evaluation study.[1] These consisted of one buffer-treated Dacron swab and four buffer-treated cotton swabs. Two of the latter were used for intensive cultural studies; a third was examined by FA techniques immediately after collection. The treated Dacron swab and the remaining cotton swab, in 15 × 150 mm tubes with tight-fitting screw-caps, were held at room temperature four weeks or longer before cultural and FA examinations were performed.

In a total of 112 swab specimens which gave presumptive positive tests either by FA or culture, 93% were FA positive and 56% culturally positive when first examined. After storage for 4-8 weeks, 42% of the Dacron and 37% of the cotton swabs were culturally positive. The latter figures may be compared with the 56% which were originally positive by culture. Thirty-two of the specimens were suspected of containing shigellae or salmonellae in addition to EEC. These specimens were examined for EEC 6-8 weeks after collection, and the percentage recovery of EEC was somewhat less than from the specimens examined within 4-5 weeks. The results are summarized in the table opposite.

Included in the total of 112 specimens was a group of 33 specimens taken by a paediatrician and one of the authors. In this series an attempt was made to equalize sampling variation between specimens to be examined immediately and those to be stored. This was done by obtaining approximately the same amount of faecal material on each swab and alternating the order in which the swabs were taken. These specimens were examined immediately and after 4 weeks of storage. Inspection of the table shows that 55% of these were positive by culture when examined immediately and that 51% of the Dacron and 48% of the cotton swabs yielded EEC when the dried swabs from the same specimens were examined one month later.

FIG. 3

COMPARISON OF SURVIVAL OF *ESCHERICHIA COLI* O126: B16 (SEEDED FAECAL SPECIMENS) ON BUFFERED AND NON-BUFFERED DACRON OR COTTON SWABS



TNTC = Too numerous to count.

DISCUSSION

The purpose of the present study was to explore the relative value of Dacron or cotton swabs treated in various ways for preserving faecal specimens for both FA and cultural examinations for enteropathogenic *E. coli*.

It was determined that faecal swabs dried at room temperature were superior to swabs that were kept frozen. There was an increase in numbers of EEC during the slow drying of the swabs held at room

[1] See the article by Cherry et al. on page 159 of this issue.

RECOVERY OF ENTEROPATHOGENIC *E. COLI* FROM FRESH AND STORED FAECAL SWAB SPECIMENS

No. of specimens	Fresh untreated cotton swabs		Stored swabs				
	FA positive	Culture positive	Weeks of storage	Treated Dacron		Treated cotton	
				FA positive	Culture positive	FA positive	Culture positive
112	104 (93 %)	63 (56 %)	4-8	85 (76 %)	47 (42 %)	86 (77 %)	41 (37 %)
32 [a]	30 (94 %)	17 (53 %)	6-8	26 (81 %)	9 (28 %)	23 (72 %)	11 (34 %)
33 [b]	30 (91 %)	18 (55 %)	4-4½	27 (82 %)	17 (52 %)	25 (76 %)	16 (48 %)

[a] Contained, or were thought to contain, shigellae or salmonellae in addition to EEC.
[b] A special group contained in the total of 112 specimens that were positive for EEC only.

temperature. Apparently, enough nutrient was available in faecal material to support the growth of these organisms for a short time. Cohen, Page & Stulberg (1961) observed the multiplication of EEC in broth enrichment cultures inoculated with frozen stool specimens. This device permitted the detection of additional positive specimens when the immunofluorescent technique was applied.

Swabs that were boiled in phosphate buffer before use appeared to support the viability of the EEC in faecal specimens better than swabs that were not pre-treated with buffer. In the field study treated faecal swabs were held for one month or more before examination, with only a moderate decrease in the number of cultural and FA positives. Most of the reduction in numbers of FA positive specimens is due to the dehydration of the organisms, resulting in atypical morphology and confusion in reading the smears. If the faecal suspensions from the swabs are allowed to stand for an hour or two, the bacteria are rehydrated and the percentage of FA positive specimens increases.

In the group of specimens that were held for 6-8 weeks before re-examination, the number of culturally positive swabs decreased 40% to 50% Apparently this resulted from the prolonged storage period, but the nature of the destructive process is not known. Some of these specimens were positive for *Shigella* and *Salmonella* organisms.

The results obtained with the special group of specimens in which attempts were made to secure adequate and uniform amounts of faecal material on each of the swabs indicated that dried swab specimens were the most nearly comparable to fresh specimens if they were examined within one month of collection. This method of preservation cannot be recommended for salmonellae or shigellae, since the survival of these enteric pathogens has not been studied under these conditions.

Our experience with faecal swabs preserved at room temperature for a few weeks, and with passed specimens maintained in the frozen state for similar periods of time prior to examination, indicates the superiority of the latter both for cultural and for FA studies of *E. coli*. This knowledge must be balanced against the greater difficulty of obtaining stool specimens. For short-term storage, swabs held at room temperature may increase the chances of obtaining positive results by both methods. Our experience indicates that in no case should swab specimens be frozen either in the presence or in the absence of carbon dioxide.

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RÉSUMÉ

Le transport d'échantillons de fèces à un laboratoire central, en vue de la recherche d'*Escherichia coli* par la méthode des anticorps fluorescents exige certaines précautions. Des recherches antérieures ont montré que la congélation altère les échantillons, en supprimant partiellement ou totalement les bacilles viables. Le soluté salin glycériné n'est pas un milieu convenable pour des frottis, à moins que l'on élimine la glycérine par lavage, ce qui complique le travail. La technique des écouvillons, la composition de ces derniers, et leur transport sont étudiés dans cet article. Le coton a été comparé au Dacron, sans que l'un se soit montré supérieur à l'autre dans le maintien de la viabilité des bacilles. Le traitement des écouvillons, de quelque type qu'ils soient, par une solution tampon facilite le développement des organismes au cours de la conservation à la température du laboratoire. Des échantillons récoltés sur le terrain et desséchés sur écouvillons imbibés de solution tampon, transportés de Porto Rico à Atlanta, Ga. (Etats-Unis d'Amérique), maintenus à la température du laboratoire pendant 4 semaines, ont donné, tant à la culture qu'à l'examen par fluorescence, des résultats presque aussi bons que le matériel frais. Toutefois, les résultats sont moins satisfaisants si la période de conservation est plus longue. La réhydratation des bacilles provenant des écouvillons desséchés, une ou deux heures avant l'examen, améliore les résultats.

REFERENCES

Cohen, F., Page, R. H. & Stulberg, C. S. (1961) *A. M. A. Amer. J. Dis. Child.*, **102**, 82

Ewing, W. H. (1956a) *Ann. N. Y. Acad. Sci.*, **66**, 61

Ewing, W. H. (1956b) *Publ. Hlth Lab.*, **14**, 138

Holliger, N. F. & Lindberg, L. H. (1958) *Amer. J. publ. Hlth*, **48**, 1162

Nelson, J. D. & Whitaker, J. (1960) *J. Pediat.*, **57**, 684

Nelson, J. D., Whitaker, J., Hempstead, B. A. & Harris, M. (1961) *J. Amer. med. Ass.*, **176**, 110

Stuart, R. D. (1956) *Canad. J. publ. Hlth*, **47**, 114

Stuart, R. D. (1959) *Publ. Hlth Rep. (Wash.)*, **74**, 431

Stuart, R. D., Toshach, S. R. & Patsula, T. M. (1954) *Canad. J. publ. Hlth*, **45**, 73

Thomason, B. M., Cherry, W. B. & Edwards, P. R. (1959) *J. Bact.*, **77**, 478

Thomason, B. M., Cherry, W. B. & Ewing, W. H. (1959) *Bact. Proc.*, p. 90

Whitaker, J., Page, R. H., Stulberg, C. S. & Zuelzer, W. W. (1958) *A.M.A. Amer. J. Dis. Child.*, **95**, 1