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The optimal mode of transport for swabs obtained from surfaces examined for organisms causing food-borne disease

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INTRODUCTION

It has been well established that the prevention of food-borne disease outbreaks should preferably be based on constant supervision of food-producing establishments and that, in addition to direct examination of the foods themselves, verification of the sanitary condition of the surfaces in constant contact with food products is a very essential step (Mossel, Kampelmacher & v. Noorle Jansen, 1966). Inspectors in charge of such food-borne disease prevention programmes may not always have facilities to examine such surfaces completely on the spot. In such cases they will have to ship their swabs to a central laboratory. Methods of chilled transport are troublesome and relatively costly, while shipping in selective culture media allows the receiving laboratory to detect one group of organisms only. Hence other ways of transport have quite some interest in this area.

In this study, three such methods have been studied in a strictly quantitative way, viz. the shipping of various types of swabs (i) at various water activities (Scott, 1957); (ii) in various types of saline; (iii) in Stuart type (1959) transport media. Four types of organisms of general interest in public health bacteriology have been chosen, i.e. fermentative Gram negative rods of the Enterobacteriaceae group, such as *Salmonella* and *Escherichia coli*, *Staphylococcus aureus*, Lancefield group A streptococci and spores of *Bacillaceae*. In addition a few tests were carried out with *Pseudomonas aeruginosa*, and with *Vibrio parahaemolyticus*.

MATERIALS AND METHODS

Bacteria

The strains of *Salmonella typhimurium* and *E. coli* had been recently isolated from minced meat. The *Staph. aureus* strain had been recently isolated from a furuncle, the group A streptococcus from a sore throat, the strain of *Ps. aeruginosa* from a urinary tract infection. The strain of *V. parahaemolyticus* was obtained from Dr R. Sakazaki, National Institute of Health, Tokyo, Japan. The strain of *Bacillus cereus* stemmed from an outbreak of food poisoning caused by an Indonesian rice dish.

Freshly prepared 24 hr. cultures in brain heart infusion broth of all strains were used. In the case of studies on spores of *B. cereus*, these cultures were heated for one minute at 80° C. which, in agreement with Knaysi (1951), we found entirely effective in killing the vegetative cells (Mossel, 1967) without unduly reducing the numbers of spores.

Inoculation of swabs

Cotton as well as calcium alginate swabs of approximately 25 mg. weight were applied to the tips of wooden applicators of 12 cm. length. They were sterilized in a pressure cooker for 15 min. at 121° C. in glass cylinders with cork stoppers.

Three swabs of each type were used per strain and per given simulated transport condition. Hence, when the survival of a strain at a given humidity was studied after 0, 3, 7 and 24 hr. exposure twelve swabs were prepared. Inoculation was carried out by evenly distributing over the swabs 0·02 ml. quantities of a suitable dilution of the brain heart infusion broth cultures of the test organisms. This mode of inoculation corresponded with counts of the order 10^3–10^4 per swab, depending on the viable count of the suspension used and the degree of osmotic shock to which the organisms were exposed when being transferred from a medium of a_w of *ca.* 1·00 to the very dry swabs (Mossel & Koopman, 1965).

Exposure of swabs to various transport environments

Some sets of swabs were transferred to sterile culture tubes, containing 1 ml. sterile saturated solutions of potassium nitrate and sodium bromide respectively. The latter solutions could be expected to secure a_w values of 0·95 and 0·59 respectively (O'Brien, 1948; Stokes & Robinson, 1949). The actual a_w figures for every freshly prepared and sterilized, saturated solution were verified by the direct manometric method of Legault, Makower & Talburt (1948).

Other sets of swabs were preserved in 10 ml. quantities of saline, and saline + 0·1 % peptone, according to Straka & Stokes (1957) and in the same volume of Ringer solution, with 1·0 % of sodium hexametaphosphate ('Calgon') added. This is the solution usually applied for the dispersion of calcium alginate plugs (Higgins, 1950) and found non-toxic to various bacteria before (Mossel & Büchli, 1964). Tentatively 0·1 % of peptone was also added to this fluid.

Finally, sets of swabs were kept in 12 ml. Stuart's transport medium. Because in tentative experiments we had observed some growth, particularly of Enterobacteriaceae, it was also attempted to modify Stuart's medium so that it might be a maintenance medium only. Rather than trying to use antibiotics, as Stuart himself did, for our purpose a less selective mode of inhibition of bacterial growth was investigated. Reduction of the pH of the medium to values in the range 5·0–6·4 could be considered promising (Dernby, 1921). Hence, in addition to Stuart's original medium of pH = 7·4, the same medium adjusted to pH 5·0, 5·5, 6·0, 6·7 and 7·1 by adding sufficient quantities of sterile 10 % tartaric acid solution was also used.

Conditions existing during actual transport were imitated by exposing the swabs to a temperature of 19–22° C. for up to 24 hr.

Counts of organisms after various periods of simulated transport

After 3, 7 and 24 hr. of storage at *ca.* 21° C. the tubes containing swabs in saline or Ringer-calgon were shaken for 2 min. Thereupon 0·1 ml. quantities were plated in duplicate on the surface of blood agar, incubated for about 24 hr. at 37° C. and the numbers of colonies obtained counted.

The swabs stored over the various salt solutions and those kept in Stuart's medium and its modifications were transferred to tubes containing 10 ml. saline, or Ringer-calgon. After shaking for 2 min., surface plate counts on blood agar of the dispersions thus obtained were again made.

All results were calculated as numbers of viable organisms per one swab.

RESULTS

The results obtained have been brought together in Tables 1–3. Those of Table 3 have been summarized in Table 4.

In Table 1 the fate of various types of bacteria on cotton swabs at various water activities is presented. In Table 2 similar tests on alginate swabs are reported.

Only in the case of *Staph. aureus* at $a_w = 0{\cdot}95$, could differences between survival on cotton and alginate swabs be detected during 'conditioned' dry transport.

'Conditioned' dry transport both at $a_w = 0{\cdot}58$ and $a_w = 0{\cdot}95$ lead to considerable losses in viable cells of *Salmonella typhimurium*. All liquid transport media lead to growth of *S. typhimurium*, particularly those containing peptone, as could be anticipated. In the transport medium of Stuart, generally, growth of the salmonellas tested occurred.

As could be expected *Staph. aureus* showed a higher a_w resistance than *S. typhimurium*. Particularly on alginate at $a_w = 0{\cdot}58$ almost no decrease occurred under 'conditioned' transport; although there was some decrease at $a_w = 0{\cdot}95$, especially on cotton. In Stuart's transport medium neither growth nor decrease was noticed, but some growth occurred in the presence of peptone. These observations are in agreement with the higher nutrient requirements of *Staph. aureus*.

Str. pyogenes showed rapid losses during 'conditioned' transport, at any r.h. Decreases in numbers were observed during simulated transport in saline and to a somewhat lesser extent in Ringer-calgon and peptone. There was no growth and also virtually no decrease in Stuart's medium.

Spores of *B. cereus* showed only slight losses under 'conditioned' transport on cotton. No growth in Stuart's transport medium was observed. This behaviour is typical for spores, which confirms the correctness of the procedure used for preparing the spores.

Because some organisms showed a tendency to grow in Stuart's transport medium, modifications of this fluid of various pH values were tried. The results are presented in Tables 3 and 4.

With *S. typhimurium* once more, growth in Stuart's medium occurred at pH = 7·4–7·1. When the pH was reduced, virtually no decrease in numbers of viable cells was observed.

Table 1. *Survival of various bacteria during simulated transport on cotton swabs*

The figures are logarithms to base 10 of the counts per swab, after 0, 3, 7 and 24 hr. storage at about 21° C.

Organism	Time stored (hr.)	Stored at a_w 0·58 Shaken with Saline	Stored at a_w 0·58 Shaken with Ringer calgon	Stored at a_w 0·95 Shaken with Saline	Stored at a_w 0·95 Shaken with Ringer calgon	Stored in Saline	Stored in Saline + 0·1% peptone	Stored in Ringer calgon	Stored in Ringer calgon + 0·1% peptone	Stuart normal shaken with Saline	Stuart normal shaken with Ringer calgon
S. typhimurium	0	4·19	3·93	4·19	3·93	2·33	2·11	2·45	2·58	3·55	3·59
	3	< 2·00	< 2·00	3·83	3·98	2·40	2·40	2·18	2·75	3·26	3·50
	7	< 2·00	< 2·00	3·95	4·05	2·85	4·00	2·75	3·54	3·91	4·18
	24	< 2·00	< 2·00	2·23	2·45	5·30	8·00	2·75	8·00	5·00	5·70
Staph. aureus	0	4·19	4·48	4·18	4·48	2·48	2·70	2·78	2·48	4·78	4·48
	3	4·32	4·08	3·95	4·08	2·48	2·70	2·60	2·60	4·36	—
	7	3·36	3·23	3·60	4·60	2·70	2·70	2·70	2·90	4·30	—
	24	3·89	4·00	3·60	< 2·00	2·00	3·00	2·60	4·60	4·30	—
Str. pyogenes	0	4·30	4·48	4·30	4·30	3·34	2·95	3·36	3·32	4·30	4·48
	3	< 2·00	< 2·00	3·85	4·00	< 2·00	2·30	3·45	3·43	4·30	4·48
	7	< 2·00	< 2·00	2·90	3·58	< 2·00	2·00	3·36	3·04	4·48	4·48
	24	< 2·00	2·48	< 2·00	< 2·00	< 2·00	< 2·00	3·15	2·00	4·30	4·48
B. cereus spores	0	3·65	3·83	3·91	3·91	—	—	—	—	3·87	3·85
	3	3·53	3·48	3·57	3·52	—	—	—	—	3·81	3·74
	7	3·11	2·85	3·48	3·86	—	—	—	—	3·72	3·91
	24	3·40	3·30	3·64	3·28	—	—	—	—	3·88	3·98

Table 2. *Survival of various bacteria during simulated transport on alginate swabs*

The figures are logarithms to base 10 of the counts per swab, after 0, 3, 7 and 24 hr. storage at about 21° C.

Organism	Time stored (hr.)	Stored at a_w 0·58 Shaken with Saline	Stored at a_w 0·58 Shaken with Ringer calgon	Stored at a_w 0·95 Shaken with Saline	Stored at a_w 0·95 Shaken with Ringer calgon	Stored in Saline	Stored in Saline + peptone	Stored in Ringer calgon	Stored in Ringer calgon + peptone	Stuart normal shaken with Saline	Stuart normal shaken with Ringer calgon
S. typhimurium	0	4·30	4·25	4·30	4·25	2·85	2·83	2·88	2·68	4·23	4·18
	3	3·57	3·25	4·30	4·15	2·88	3·20	2·68	2·76	3·82	4·08
	7	< 2·00	< 2·00	4·21	< 2·00	2·92	4·15	3·00	3·81	4·16	4·48
	24	< 2·00	< 2·00	< 2·00	< 2·00	4·18	8·00	4·93	8·00	4·84	5·00
E. coli	0	—	—	—	—	—	—	4·90	—	—	—
	7	—	—	—	—	—	—	4·85	—	—	—
	24	—	—	—	—	—	—	8·00	—	—	—
Ps. aeruginosa	0	—	—	—	—	—	—	4·30	—	—	—
	7	—	—	—	—	—	—	4·78	—	—	—
	28	—	—	—	—	—	—	8·00	—	—	—
Staph. aureus	0	4·70	4·85	4·70	4·85	2·70	2·90	2·85	2·70	—	4·60
	3	4·48	4·60	4·70	4·70	2·90	2·84	2·85	2·78	—	4·30
	7	3·48	4·48	4·70	4·30	2·00	3·40	2·90	3·11	—	4·48
	24	4·60	4·48	4·00	4·00	< 2·00	4·60	2·70	4·90	—	4·48
Str. pyogenes	0	4·48	4·30	4·85	4·85	3·90	3·85	3·60	3·60	4·48	4·30
	3	3·48	2·85	3·60	3·70	2·85	3·70	3·48	3·30	4·00	3·70
	7	2·60	3·00	2·70	3·48	< 2·00	3·48	3·11	2·78	4·30	4·30
	24	< 2·00	< 2·00	2·30	< 2 ·00	< 2·00	3·00	2·95	2·30	4·00	4·00
B. cereus spores	0	3·85	3·85	3·78	3·85	—	—	—	—	3·78	3·70
	3	3·70	3·70	3·95	3·60	—	—	—	—	3·60	3·70
	7	3·85	3·78	3·78	3·85	—	—	—	—	3·70	3·78
	24	3·90	3·78	3·85	3·70	—	—	—	—	3·85	3·78

E. coli behaved similarly. Its profuse growth in Stuart's medium at the original pH confirms Stuart's own observations.

The behaviour of *Ps. aeruginosa* was generally somewhat erratic: taking into account the accuracy of surface counts of this organism it may be concluded that Stuart's medium used at a pH range 5·5–7·4 will not lead to considerable losses.

Results obtained with *Vibrio parahaemolyticus* are somewhat different in that the pH range in which no losses occur during transport is only > 5·9 to 7·4.

Table 3. *Survival of various bacteria during simulated transport in Stuart's medium at various pH values*

The figures are the logarithms to the base 10 of the counts per alginate swab after 0, 7 and 24 hr. storage at about 21° C.

Organism	Hours of storage	pH of Stuart's medium 7·4	7·1	6·7	6·2	5·9	5·5	5·0
S. typhimurium	0	4·00	4·00	4·30	4·00	4·30	4·30	4·00
	7	—	4·60	—	—	—	—	3·90
	24	5·30	4·90	3·90	4·00	4·00	4·00	3·90
E. coli	0	4·70	4·70	—	4·60	3·95	3·95	3·85
	7	4·60	4·30	—	4·30	—	—	3·85
	24	8·00	6·00	—	4·78	3·78	3·78	3·70
Ps. aeruginosa	0	5·00	4·00	5·00	4·30	4·30	4·30	—
	7	—	—	—	—	—	—	—
	24	4·60	5·90	4·48	3·90	3·95	3·70	—
V. para-haemolyticus	0	3·87	3·85	3·48	3·81	3·85	—	3·99
	7	3·53	3·59	3·51	3·54	2·00	—	< 2·00
	24	3·52	3·59	3·36	3·02	< 2·00	—	< 2·00
Staph. aureus	0	4·00	3·78	4·00	4·48	3·90	3·90	3·60
	7	—	3·85	—	—	—	—	2·30
	24	3·90	3·30	3·60	3·60	2·60	2·30	< 2·00
Str. pyogenes	0	3·30	2·95	3·30	3·00	3·30	3·00	3·30
	7	—	3·48	—	—	—	—	3·00
	24	3·30	5·30	2·78	2·30	< 2·00	< 2·00	2·70
B. cereus spores	0	3·48	3·70	3·48	3·00	4·00	4·30	3·90
	7	—	3·78	—	—	—	—	3·85
	24	3·30	3·78	3·00	3·00	4·00	4·00	3·70

Table 4. *Summary of the data of Table* 3. *Derived from Table* 3

The figures indicate the ratio of the count at 24 hr to the count at 0 hr.

Organism	pH of Stuart's medium 7·4	7·1	6·7	6·2	5·9	5·5	5·0
S. typhimurium	20	8	0·4	1	0·5	0·5	0·8
E. coli	2000	20	—	1·5	0·7	0·7	0·7
Ps. aeruginosa	0·4	80	0·3	0·4	0·45	0·25	—
V. parahaemolyticus	0·45	0·55	0·8	0·2	< 0·014	—	< 0·01
Staph. aureus	0·8	0·3	0·4	0·13	0·05	0·025	< 0·025
Str. pyogenes	1	200	0·3	0·2	< 0·05	< 0·1	0·25
B. cereus spores	0·7	1·2	0·3	1	1	0·5	0·6

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Unfortunately *Staph. aureus* showed a quite different behaviour: while in Stuart's medium of pH = 7·4 a tendency to maintenance seemed to exist, the organism did not tolerate a lower pH value; at pH < 6·2 within 24 hr. at 21° C. decreases of an order far over 1 D occurred.

Str. pyogenes showed growth in Stuart's medium of pH = 7·1; a tendency to decrease at lower pH values was observed, particularly at pH ⩽ 5·9.

Finally, spores of *B. cereus* showed virtually neither increase nor decrease in Stuart's medium at any of the pH values tested, which confirms anew the correctness of the mode of spore preparation used.

Table 5. *Fate of various bacteria stored in Ringer calgon and Stuart's medium at* 11–13° C.

The figures are the logarithms to the base 10 of the counts per 1 ml. after 0, 7 and 24 hr. storage.

	Ringer calgon log. of count after (h)			Stuart medium pH = 7·0 log. of count after (h)		
Organism	0	7	24	0	7	24
S. typhimurium	4·13	4·08	4·15	4·19	4·08	3·98
E. coli	3·41	3·45	4·01	3·32	3·30	3·13
	4·95	4·90	4·81	4·60	4·13	4·63
Ps. aeruginosa	3·97	3·87	3·92	3·75	3·59	3·47

DISCUSSION

There was no complete agreement between the numbers of viable cells of a given organism in repeated simulated transport tests, except for the spores of *B. cereus*. This demonstrates that minor differences in the condition of the organisms to be transported from the sampling site to the laboratory may also determine the fraction of surviving cells.

The usual way of unprotected transport of swabs leads to losses in viable cells of some types of bacteria often exceeding two logarithmic cycles. This confirms the observations of Ellner & Ellner (1966). Dry transport has therefore to be abandoned and to be replaced by transport in a suitable aqueous medium.

The extent of growth observed in certain experiments when cells of Enterobacteriaceae were transported in Stuart's medium at pH = 7·1 for 24 hr. showed an average of one logarithmic cycle. The fall in counts of *Str. pyogenes* and *Staph. aureus* when transported in Stuart's medium at decreased pH, for the same period of time, was often more than tenfold. Hence Stuart's medium at pH = 7·1 is to be preferred for the transport of swabs. The increases in counts to be expected when dealing with Enterobacteriaceae—and hence slight shifts in the composition of the original micro flora—can always be limited by shortening the period of transport or by reducing the temperature. We have found that chilling of the medium, to ground water temperature, i.e. 11–13° C., and subsequent transport wrapped in plastic foam ('Tempex') will completely inhibit the growth of Enterobacteriaceae in Stuart's medium of pH *ca.* 7·0 for 10–12 hr. (Table 5).

As is illustrated in Tables 1 and 2 the various bacteria tested behave virtually

in the same way as in Stuart's medium when transported in Ringer-calgon without peptone added. Here again we established that 10–12 hr. transport at 11–13° C. will prevent the proliferation of the only group of bacteria actively growing in Ringer calgon, i.e. Enterobacteriaceae (Table 5). From a purely bacteriological point of view there seems, therefore, little to choose between Ringer calgon and Stuart's medium. However, where swabs are to be transported to the laboratory by lay personnel, Stuart's medium has the obvious advantage of being solid so that it cannot be lost by spilling.

SUMMARY

An investigation was carried out on the influence of the mode of transport at *ca.* 21° C. on the fate of *S. typhimurium*, *E. coli*, *Ps. aeruginosa*, *Staph. aureus*, *Str. pyogenes* and spores of *B. cereus* collected from surfaces by swabbing with cotton and alginate.

Transport at relative humidity 0·58–0·95 leads to losses in viable counts sometimes exceeding two log cycles. In Stuart's medium and Ringer-calgon particularly Enterobacteriaceae showed growth when transported at 21° C. Reduction of the pH value of Stuart's medium led to losses of far over one log cycle in some types of bacteria and could therefore not be recommended.

Slight chilling of the medium, e.g. to 12° C., and insulation during transport appeared to maintain counts of the organisms studied at their original level in both Stuart's medium and Ringer calgon for 10–12 hr.

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