

A Modified Reduced Transport Fluid for the Preservation of *Neisseria gonorrhoeae* during Transport

M. H. FINLAYSON, F. F. KORALEWSKI, R. A. KINDERMANN

SUMMARY

Reduced transport fluid (RTF) was modified by altering its pH and by the addition of a yeast dialysate. This reduced transport yeast-containing fluid (RTYF) was shown to be superior to RTF in maintaining viability of *Neisseria gonorrhoeae* in cultures and in clinical material.

S. Afr. med. J., **49**, 1787 (1975).

In a preliminary communication, Finlayson *et al.*¹ showed that *Neisseria gonorrhoeae* could be grown from urethral swabs 18-24 hours after being transported without the use of ambient CO₂ in reduced transport fluid (RTF). This fluid was described by Loesche *et al.*² for the transportation of oral streptococci in dental plaques. It was shown that gonococci transported on buffered charcoal-impregnated swabs remained viable over longer periods than when transported on buffered plain cottonwool swabs.¹ As the optimum pH for growth of *N. gonorrhoeae* is 7.2-7.6,³ the pH of the RTF was adjusted to pH 6.9. RTF was further modified by the addition of yeast dialysate (RTYF).⁴

MATERIALS AND METHODS

Thayer-Martin plates and Stuart's transport medium were prepared and used as described by Finlayson *et al.*^{1,2} except that the Thayer-Martin plates contained 3 µg trimethoprim lactate and 1 µg of amphotericin B per millilitre of medium as recommended by Faur *et al.*⁵ in place of VCN inhibitor. RTF was modified by changing the pH to 6.9. Yeast dialysate (25 ml), prepared as described by Faur *et al.*⁴ was added to 1 litre of RTF to produce a modified transport fluid (RTYF).

Cottonwool swabs were boiled in Sorenson's phosphate buffer pH 7.4 for 10 minutes, then dried and sterilised. Charcoal-impregnated swabs were made by rolling the swabs, after they had been boiled in Sorenson's buffer,

in activated charcoal, then squeezing out excessive buffer, and drying and sterilising them in hot air.

Strains of *N. gonorrhoeae* were freshly isolated from clinical specimens.

RESULTS

The pH of the RTF as used for transport of oral streptococci was found to be 6.1, which is below the optimal range for growth of *N. gonorrhoeae*. By adjustment of the pH of the RTF to 6.9, cultures of *N. gonorrhoeae* remained viable for a much longer period than when they were kept at pH 6.1. Table I compares the viability of *N. gonorrhoeae* on buffered charcoal swabs in RTF at pH 6.1 and 6.9.

TABLE I. COMPARISON OF VIABILITY OF CULTURES OF *NEISSERIA GONORRHOEAE* IN RTF AT pH 6.1 AND 6.9 ON 20 BUFFERED CHARCOAL SWABS

Transportation time (hours)	Number of swabs with viable <i>N. gonorrhoeae</i>	
	pH 6.1	pH 6.9
4	20	20
8	20	20
24	12	20
28	7	20
32	7	20
34	2	20
36	2	16
38	2	16
40	Nil	16
48	Nil	16
50	Nil	14
52	Nil	14
54	Nil	6
56	Nil	4

Twenty buffered charcoal swabs containing *N. gonorrhoeae* were kept for varying periods in RTF at pH 6.1 and pH 6.9. *N. gonorrhoeae* was grown from 20 swabs after 8 hours in RTF at pH 6.1, whereas the same number of swabs gave a growth of *N. gonorrhoeae* after 36 hours in RTF at pH 6.9. Furthermore, *N. gonorrhoeae* was not

Department of Medical Microbiology, University of Stellenbosch and Tygerberg Hospital, Parowvallei, CP

M. H. FINLAYSON, B.Sc., M.B. CH.B., D.P.H., F.R.C. PATH.
F. F. KORALEWSKI, ARZT (MÜNSTER)
R. A. KINDERMANN, Technologist

Date received: 28 April 1975.

grown from any swabs after 40 hours in RTF at pH 6,1, whereas growth was obtained from 16 of the 20 swabs after this period in RTF at pH 6,9 and from 14 of the 20 swabs after 52 hours in RTF at pH 6,9.

Fifty charcoal-impregnated swabs containing urethral pus from male patients with gonococcal infection were kept in RTF at pH 6,9 and cultured on Thayer-Martin plates after storage for 2-48 hours. The results of this experiment are shown in Table II.

TABLE II. VIABILITY OF *NEISSERIA GONORRHOEAE* ON 50 CHARCOAL-IMPREGNATED URETHRAL SWABS KEPT IN RTF AT pH 6,9 FOR VARYING TIMES

Hours	Number of swabs growing <i>N. gonorrhoeae</i>
2	50
16	44
18	43
20	43
22	38
24	35
26	30
28	30
30	25
34	15
40	5
48	Nil

After 16 hours in RTF *N. gonorrhoeae* was cultured from 44 of the swabs, and after 34 hours growth was obtained from only 15 of the swabs.

An attempt was made to increase the viability of *N. gonorrhoeae* held in RTF pH 6,9 by the addition of yeast dialysate.³ Eighty recently isolated cultures of *N. gonorrhoeae* were held in RTYF and cultured at intervals on Thayer-Martin plates.

TABLE III. VIABILITY OF CULTURES OF *NEISSERIA GONORRHOEAE* ON 80 CHARCOAL-IMPREGNATED SWABS KEPT IN RTYF FOR VARYING TIMES

Hours	Number of swabs growing <i>N. gonorrhoeae</i>
40	80
48	69
50	73
52	73
72	61

These results (Table III) show that all 80 cultures remained viable on charcoal-impregnated swabs for a period of 40 hours. Thereafter the number of viable strains decreased until, after 72 hours, 61 cultures remained viable.

In view of the increased viability of cultures of *N. gonorrhoeae* kept in RTYF, 72 charcoal-impregnated swabs containing urethral pus from male patients with gonorrhoea were held in RTYF and cultured on Thayer-

Martin plates at varying intervals. Duplicate swabs were kept in Stuart's transport medium and cultured on Thayer-Martin plates at the same times.

TABLE IV. VIABILITY OF *NEISSERIA GONORRHOEAE* ON 72 CHARCOAL-IMPREGNATED URETHRAL SWABS KEPT IN RTYF AT pH 6,9 FOR VARYING TIMES

Hours	Number of swabs growing <i>N. gonorrhoeae</i> in	
	RTYF	Stuart's medium
2	67	64
18	67	59
24	57	48
40	31	9
48	14	9

The results shown in Table IV indicate that *N. gonorrhoeae* remained viable in RTYF for 18 hours in 67 of the 72 urethral swabs. Thereafter, the number of strains which survived decreased steadily, until after 48 hours, only 14 strains remained viable. Using Stuart's transport medium, 9 of 72 strains remained viable after 40 hours whereas 31 strains survived for the same period in RTYF.

DISCUSSION

The search for a medium which will enable bacterial growth to be obtained from clinical material after transportation over a period of time has led to a number of formulations, notably by Stuart,⁷ Möller⁸ and Amies.⁹

These transport media have proved satisfactory to a variable degree, depending on the metabolic requirements of the bacteria present. When investigating the bacterial flora of human dental plaques, many of which on first isolation are anaerobic, Gastrin *et al.*¹⁰ and Rundell *et al.*¹¹ found the existing transport media to be unsatisfactory. The latter authors¹¹ devised a transport fluid which they designated RTF (reduced transport fluid). This contained a balanced mineral salt solution, also dithiothreitol (DTT) and sodium ethylene-diaminetetraacetate (EDTA). It proved very satisfactory as a transport fluid for dental plaque streptococci under aerobic conditions.

In view of the fastidious growth requirements of *N. gonorrhoeae* and the CO₂ atmosphere required for continued viability of the transported organism, we carried out experiments with RTF as a transport medium for gonococci in clinical material.³

As is the case with other transport media, the importance of correct pH was observed and it was found necessary to adjust the pH of the RTF to 6,9. Cultures of *N. gonorrhoeae* grew well on Thayer-Martin plates after transportation in RTF at pH 6,9. After 48 hours at 20-25°C in RTF at this pH, 16 of 20 cultures were still viable.

When, however, charcoal-impregnated swabs carrying pus from urethral discharges were kept in RTF at room temperature (20-25°C) for varying periods of time, the

viability of *N. gonorrhoeae* was found to be less than that of pure cultures kept in RTF. Strains of *N. gonorrhoeae* grown in primary isolation from pus appear to be more fastidious than established cultures after transportation in RTF and remain viable for a shorter period.

The addition of a yeast supplement has been recommended by Amies and Garabedian¹² and by Faur *et al.*⁴ to promote the growth of *N. gonorrhoeae*. RTF to which yeast dialysate was added (RTYF) was used by us for the transportation of cultures of *N. gonorrhoeae*. Our experiments showed that all the cultures of *N. gonorrhoeae* tested remained viable 40 hours after being kept in RTYF on charcoal-impregnated swabs. The majority of these cultures were still viable after being kept in RTYF for 72 hours. All cultures were held at room temperature, 18 - 24°C.

When specimens of pus from urethral discharges were kept on charcoal-impregnated swabs in RTYF for varying periods of time, it was observed that the majority of specimens grew *N. gonorrhoeae* after 18 hours, and that nearly half the number of specimens were viable after 40 hours. While the viability of *N. gonorrhoeae* from pus was much greater in RTYF or Stuart's transport medium,

it was less than the viability of pure cultures of *N. gonorrhoeae*. RTYF, however, showed a marked superiority to RTF or Stuart's transport medium for transport of *N. gonorrhoeae* in clinical material.

We thank Dr A. J. Wilson, Director of the City of Cape Town Venereal Disease Clinics, for providing access to clinical material.

REFERENCES

1. Finlayson, M. H., Willey, K. F. D., Brede, H. D. and Wilson, A. J. (1974): *S. Afr. med. J.*, **48**, 1195.
2. Loesche, W. J., Hockett, R. N. and Syed, S. (1972): *Arch. oral Biol.*, **17**, 1311.
3. Joklik, W. K. and Smith, D. T. (1972): In *Zinsser Microbiology*, 15th ed., p. 410. New York: Meredith.
4. Faur, V. C., Weisburd, M. H., Wilson, M. E. and May, P. S. (1973): *Health Lab. Science*, **10**, 44.
5. Finlayson, M. H., Gibbs, B. and Brede, H. D. (1974): *Ibid.*, **48**, 259.
6. Faur, V. C., Weisburd, M. H. and Wilson, M. E. (1973): *Ibid.*, **10**, 55.
7. Stuart, R. D. (1946): *Glasg. med. J.*, **27**, 131.
8. Möller, J. R. A. (1966): *Microbiological Examination of Root Canals and Periapical Tissues of Human Teeth*. Goteberg, Sweden: Akademiforlaget.
9. Amies, C. R. (1967): *Canad. J. publ. Hlth*, **58**, 296.
10. Gastrin, B., Kallings, L. O. and Marcetic, A. (1968): *Acta path. microbiol. scand.*, **74**, 371.
11. Rundell, B. B., Thomson, L. A., Loesche, W. J. and Stiles, H. M. (1973): *Arch. oral Biol.*, **18**, 871.
12. Amies, C. R. and Garabedian, M. (1967): *Brit. J. vener. Dis.*, **43**, 137.