A Note on the Use of Reduced Transport Fluid (RTF) for Isolation of *Neisseria gonorrhoeae*

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SUMMARY

Swabs containing pus from urethral discharges obtained from 55 male patients attending venereal disease clinics were transported in Stuart's transport medium or RTF (reduced transport fluid) and then cultured on Thayer-Martin plates. Forty-eight swabs showed the presence of *Neisseria* in smears and 45 gave good growth of *N. gonorrhoeae* when cultured 18 - 24 hours after the swabs were taken from the patient.

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The use of a transport medium for the culture of sensitive micro-organisms has, to a large extent, been motivated by attempts to improve the isolation and growth of *Neisseria gonorrhoeae*.[1,2] The first really successful medium for transport of this organism was that of Stuart.[3] Gastrin *et al.*[4] examined a number of different media and recommended a modified Stuart's medium for transport of most bacteria on cottonwool swabs.

Particular difficulty was encountered by workers in the transportation of specimens of streptococci from oral infections. Moller[5] described modified transport media for storage of endodontic specimens. Loesche *et al.*[6] described a new medium named RTF and recommended its use for transportation of dental plaque specimens. Their work was confirmed by Rundell *et al.*[7] This medium contains EDTA, which enhances cell dispersion and eliminates possible toxic effects of trace heavy metal ions.[8,9] It also contains dithiothreitol as a reducing agent.[10] In view of the successful use of RTF in the transportation of specimens containing delicate oral streptococci, we carried out tests on the transportation of pus specimens containing gonococci.

MATERIALS AND METHODS

Swabs containing pus from urethral discharges were obtained from 55 male patients attending the Cape Town municipal VD clinics.

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Pus specimens were taken from the same patient, in some cases on charcoal-impregnated swabs, and placed in tubes containing either Stuart's medium or RTF. In other cases, an additional plain sterile cottonwool swab was placed in RTF. After varying intervals of time during which the swabs stood at room temperature (± 20 - 25°C), Petri dishes containing Thayer-Martin medium were inoculated and incubated for 48 hours in a CO_2 atmosphere (candle-flame) and then examined. Suspicious colonies were tested for oxidase activity, smears prepared and stained and, when positive, further identification and antibiotic sensitivity tests were carried out.

The charcoal-impregnated swabs, Stuart's transport medium and Thayer-Martin medium were prepared as described by Finlayson and Gibbs.[11] RTF was prepared as follows:[7]

Solution 1: 0,6% K_2HPO_4
Solution 2: 1,2% NaCl
1,2% $(NH_4)_2SO_4$
0,6% KH_2PO_4
0,25% $MgSO_4.7H_2O$
Solution 3: 0,1M Na EDTA (formula weight 416)
Solution 4: 1% dithiothreitol—store at 10°C.

Combine 75 ml of solution 1, 75 ml of solution 2, 10 ml of solution 3, and 20 ml of solution 4. Dilute to 1 litre with distilled water. Filter-sterilise using a membrane filter (0,22-μm pore size) and store at 10°C until needed.

RESULTS AND COMMENT

Table I indicates the results obtained. Although the number of specimens was limited, it will be noted that under our conditions, transport on charcoal swabs in RTF gave a large number of cultures of *N. gonorrhoeae*—45 positive cultures from 55 swabs, of which 48 swabs gave positive smears. The shortest period between the taking of the specimen and the inoculation of culture medium was ± 18 hours. In some cases 24 hours elapsed.

TABLE I. ISOLATION OF *N. GONORRHOEAE* FROM SWABS TRANSPORTED IN RTF

Batch	Total number of swabs	Smears positive for *N. gonorrhoeae*	Positive cultures from RTF
1	16	7	11
2	9	1	9
3	19	19	16
4	11	11	9
	55	48	45

A preliminary comparison of these results with the results obtained after transport on Stuart's medium showed that growth of *N. gonorrhoeae* was much heavier and colonies larger than on culture media inoculated after transport in Stuart's medium. Furthermore, the growth of contaminating organisms was much reduced, as can be seen in Fig. 1.

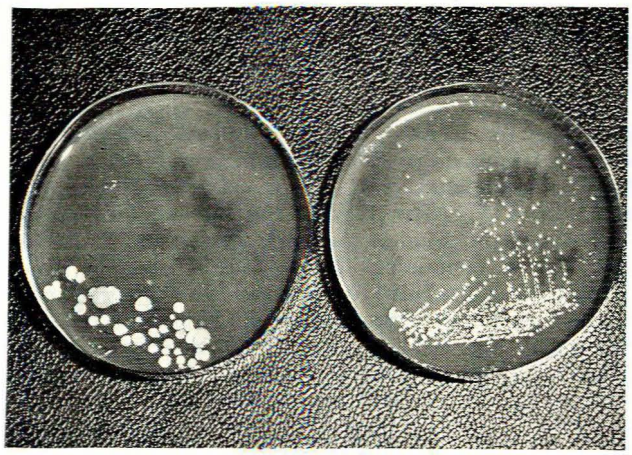

Fig. 1. Thayer-Martin plates. Left: **inoculated with charcoal swab from urethral discharge after transport in Stuart's transport medium at 25°C for 24 hours. Note large colonies of contaminating organisms and scanty small colonies of** *N. gonorrhoeae*. Right: **inoculated with charcoal swab from urethral discharge from same patient at same time, after transport in RTF at 25°C for 24 hours. Note numerous small colonies of** *N. gonorrhoeae* **and scanty growth of contaminating organisms.**

It would appear that RTF is very suitable for the transport of specimens containing *N. gonorrhoeae*. It not only preserves the organisms during a period of 18 - 24 hours, but also provides heavy growth and little contamination of the culture medium.

A number of positive cultures, 17 from 30 swabs, were obtained by using plain cottonwool swabs in the RTF and inoculating culture medium 18 - 24 hours later. Should these results be borne out by further investigation, the use of plain cottonwool swabs could prove most useful. This swab is easier to prepare and can be used to make smears for microscopical examination without introducing carbon particles, which sometimes make identification of gonococci extremely difficult.

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