Trimethoprim as an additional selective agent in media for the isolation of *N. gonorrhoeae*

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SYNOPSIS The incorporation of trimethoprim into existing selective media using either saponin-lysed blood agar or chocolate agar as the base is suggested for the routine isolation of the gonococcus. The principal advantage over other selective media is the inhibition of *Proteus mirabilis* and other commensal organisms as a consequence of which multiple swabs can be plated out onto a single selective plate irrespective of source, while the recovery rate of the gonococcus from these specimens is increased. The relative merits of both basic media are briefly discussed.

The isolation of *N. gonorrhoeae* on selective media is still hampered by their failure to suppress the growth of some commensal organisms, notably *Proteus mirabilis*, spreading members of which overgrow other organisms and render isolation of the gonococcus impossible. Also, if multiple swabs are inoculated onto a single plate, one colony of this organism will spread and invalidate all results. The incorporation of first ristocetin and polymyxin (Thayer and Martin, 1964), and, more recently, vancomycin, sodium colistimethate, and nystatin (Martin, Billings, Hackney, and Thayer, 1967) into chocolate agar (VCN medium) inhibited many commensal organisms (Wende, Forshner, and Knox, 1964; Wilkinson, 1965) but did not suppress the growth of *Pr. mirabilis*. This paper describes how this problem can be overcome by the incorporation of trimethoprim into the more recent Thayer and Martin medium. A further modification is the replacement of nystatin by pimarcin, a more stable anti-fungal agent.

Methods and Materials

First, the amount of trimethoprim was determined by using varying amounts of trimethoprim

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incorporated into the VCN medium of Thayer and Martin. The lowest amount of trimethoprim which would inhibit the growth of 10 spreading strains of *Pr. mirabilis* was 1.5 µg/ml. Six times this amount did not affect the recovery of *N. gonorrhoeae* and 3 µg/ml was selected as the amount which would safely inhibit *Pr. mirabilis* while not affecting the gonococcus. Vancomycin was then omitted from the above mixture but had to be reintroduced as certain commensals, notably staphylococci, were insufficiently suppressed.

A comparison was made in the first part of the study of the isolation rates of *N. gonorrhoeae* on plain chocolate agar, the VCN medium and the VCN medium with trimethoprim 3 µg/ml. In the latter medium the vancomycin was increased from 3 µg/ml to 5 µg/ml, a previous experiment showing this concentration to be more effective against Gram-positive cocci without reducing the yield of gonococci. A similar study was made in the second part but using saponin-lysed blood agar as the basal medium. Vancomycin was used at a concentration of 3 µg/ml as in the original Thayer and Martin medium, but in the trimethoprim-containing medium nystatin was replaced by an equivalent amount of pimarcin (20 µg/ml) as the nystatin was found to be unstable in the poured media. The chocolate agar was made by adding 5% defibrinated whole horse blood (Oxoid) to

Selective Agents	Gonococcal Strain No.										Mean Percentage	Selective Agents	Gonococcal Strain No.										Mean Percentage		
	1	2	3	4	5	6	7	8	9	10			11	12	13	14	15	16	17	18	19	20			
Chocolate agar base													Saponin-lysed blood agar base												
VCN	94	39	49	118	74	75	102	105	71	72	80	VCN	65	46	35	88	72	105	122	53	97	73	76		
VCN+T	97	25	12	80	76	55	96	100	32	67	64	VC+PT	35	46	27	43	60	93	122	60	126	73	69		

Table I Miles and Misra gonococcal counts on selective media expressed as a percentage of the colony counts on basal media

V = vancomycin 3 units/ml (¹ = 5 units/ml)
 C = sodium colistimethate 7.5 µg/ml
 N = nystatin 12.5 units/ml
 T = trimethoprim 3 µg/ml
 P = pimarcin 20 µg/ml

blood agar base (Oxoid no. 2) and heating to 85°C in a water bath until the medium became chocolate. The saponin-lysed blood agar plates were made by incorporating 10% defibrinated whole horse blood lysed with saponin into blood agar base, 8 ml of 3% saponin (B.D.H.) being used for each 100 ml defibrinated horse blood (Ridley, personal communication). All selective agents were added at 48°C.

The efficiency of the selective agents was judged in two ways. First, Miles and Misra counts were carried out on the media using 10 different strains of *N. gonorrhoeae* in each part of the study and 10 swarming strains of *Pr. mirabilis*. The gonococci were grown overnight on plain chocolate agar and then emulsified and diluted in nutrient broth. The Proteus strains were grown overnight in nutrient broth and dilutions made in quarter strength Ringer's solution. Secondly, clinical evaluation was carried out using swabs taken at the Special Treatment Centre, St Bartholomew's Hospital, and the Lydia Department, St Thomas' Hospital. A few swabs were taken at other hospitals. Rectal swabs formed approximately 4% of the total. All clinical material was taken onto charcoal swabs and transported to the laboratory in Stuart's medium. A single swab was used to inoculate all the plates, those containing no inhibitory agents being inoculated first to avoid carry over of inhibitors. This also tended to prejudice recovery from the selective media. In the second part of the study using lysed blood agar plates each selective plate containing trimethoprim was inoculated with six swabs.

All plates were warmed and dried before inocu-

lation; incubation was carried out at 37°C candle jars and all plates were read after 48 hours. When Gram-negative diplococci were seen on the direct smear organisms recovered were assumed to be gonococci on colonial appearance, oxidase reaction, and microscopic appearance. When the direct smear was negative sugar reactions were used to identify recovered Neisseria.

Results

MILES AND MISRA COUNTS ON PURE CULTURES

Table I shows the mean of triplicate counts of 10 strains of gonococci on both chocolate and lysed blood selective media expressed as a percentage of the recovery on basal media without inhibitors. The colony counts of most strains were reduced by the presence of vancomycin, colomycin, and nystatin and still further reduced by the addition of trimethoprim. Strains varied in their preference for the two basal media, some yielding up to twice as many colonies on one or other medium, but the colony size was always greatest on the lysed blood agar.

When the Miles and Misra counts were repeated using 10 swarming strains of *Pr. mirabilis* no significant difference occurred in the counts between plain chocolate agar, plain lysed blood agar, or either of these incorporating the VCN mixture, but plates containing trimethoprim, 3 µg/ml, caused a reduction in the count by between 10⁵ and 10⁶ organisms/ml. Where the inoculum was sufficiently large to allow the growth of the organism swarming usually occurred within 48 hours.

RESULTS USING CLINICAL MATERIAL

Table II shows the results obtained from the culture of 560 swabs on chocolate agar and 481 swabs on lysed blood based agar. One hundred and seventy-nine were positive on basal media and 201 positive on media incorporating vancomycin, colomycin, and nystatin. The addition of trimethoprim to the selective agents yielded 14 extra

	Chocolate Agar	Lysed Blood Agar	Total
Swabs cultured	560	487	1,047
Patients yielding positive cultures	95	106	201
Positive swabs	99	116	215
Positive Gram films	92	106	198
Isolates on basal media	83	96	179
Isolates on VCN	93	107	200
Isolates on VCN+trimethoprim	98	—	214
Isolates on VC+trimethoprim+pimaricin	—	116	—

Table II Analysis of the isolation of 201 strains of *N. gonorrhoeae*

isolates due to further suppression of commensal organisms, in eight instances, *Proteus*.

Out of 1,047 swabs cultured, 41 yielded a spreading *Pr. mirabilis* on media containing vancomycin, colomycin, and nystatin, but media which also contained trimethoprim completely suppressed this organism.

Four hundred and eighty-seven swabs were inoculated six to a plate on lysed blood agar containing all four selective agents. Only two inoculated segments were unreadable due to overgrowth by commensals; in each instance an enterococcus was responsible.

Discussion

The incorporation of trimethoprim, 3 µg/ml, vancomycin 3 units/ml, sodium colistimethate 7.5 µg/ml, and pimarcin 20 µg/ml into media for gonococcal isolation appears to have several advantages over its predecessors, the most important of which is the inhibitory effect on *Pr. mirabilis* leading to the complete elimination of the numbers found in clinical specimens. The introduction of pimarcin considerably increases the effective life of the media; few contaminants now appear within two weeks of the media being poured. Plates up to two weeks old were used satisfactorily during the course of study, lysed blood agar surviving particularly well. This would be of advantage in smaller centres where media can only be poured at intervals or are obtained commercially.

Incorporation of the four selective agents into both basic media produced sufficient inhibition of contaminants to justify the plating out of several swabs onto a single selective plate, irrespective of

source, six to each plate being used during the second part of the study. These required no spreading and the procedure is therefore easily carried out by untrained assistants.

An advantage of the lysed blood medium was that many of the plates could be read with certainty after 18 to 24 hours' incubation, especially when freshly poured plates were used. Some strains of gonococci were encountered which were only detected after 48 hours' incubation. A good light source, together with a lens or plate microscope, is required to identify oxidase-positive colonies as the medium darkens on incubation. Chocolate agar plates incorporating the selective agents do not have this disadvantage, but require 48 hours' incubation before they can be read.

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