DIAGNOSTIC MICROBIOLOGY

An evaluation of pre-poured selective media for the isolation of *Neisseria gonorrhoeae*

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Fourteen commercial media supplied as pre-poured plates were compared with an 'in-house' selective medium for their ability to support the growth of 105 gonococcal isolates (representing a wide variety of serovars encountered in natural infection), 25 meningococcal and 20 *Neisseria lactamica* isolates, and to inhibit the growth of 71 isolates of non-pathogenic neisseriae and miscellaneous organisms. Only two of the pre-poured plate media and the in-house selective medium yielded growth of duplicate cultures of all 105 gonococcal isolates after incubation for 24 h: one other medium provided growth of all the isolates after incubation for 48 h. The ability of the various media to suppress the growth of the 71 isolates of non-pathogenic neisseriae and miscellaneous organisms ranged from 97.2 to 71.8% of isolates inhibited. Of the four media that enabled growth of all the gonococcal strains, inhibition was 94.4% for the in-house medium, 85.9% and 80.3% for the two media on which all gonococci grew after 24 h and 71.8% for the medium on which all of the gonococci grew after 48 h. Failure of growth of gonococci was associated with: serogroup IA isolates ($p < 0.001$), AHU auxotype ($p < 0.001$) and the presence of vancomycin rather than lincomycin in the selective medium ($p < 0.02$). The use of 10% blood and a highly nutritious medium based on the original New York City (NYC) or modified New York City (MNYC) formulation were also important in supporting growth of gonococci. One of the main problems in lack of selectivity was a failure to inhibit the growth of yeasts. As effective inhibition of yeasts was obtained with other media containing the same concentration of amphotericin, failure may be due to batch variation of supplement, media preparation, or reduced shelf life of the media. None of the commercially available pre-poured media performed as well as the in-house medium despite the fact that some of the media were prepared to a very similar formula.

Introduction

The definitive diagnosis of gonococcal infection relies on culture, usually on selective media, and identification of *Neisseria gonorrhoeae* [1]. Most selective media contain a rich nutrient base supplemented with blood, partially lysed by heat (chocolate agar) or completely lysed by saponin; an antimicrobial cocktail is added to inhibit micro-organisms other than pathogenic neisseriae. Details of the main selective media, Thayer Martin (TM) medium and New York City (NYC) medium and their modifications have been reviewed elsewhere [2].

In this laboratory, primary culture for gonococci is performed on modified New York City (MNYC)

medium containing lysed horse blood 10% and lincomycin 1 mg/L [3]. Various pre-poured selective media for culture of gonococci are available commercially but surprisingly few comparative data have been published. Fourteen pre-poured selective media available commercially were evaluated. Their ability to support growth of gonococci, meningococci and *N. lactamica* isolates, and to inhibit growth of 71 isolates of non-pathogenic neisseriae and other miscellaneous organisms was compared with 'in-house' selective and non-selective media.

Materials and methods

Media

Fourteen gonococcal selective media were obtained from four manufacturers of pre-poured media (Table 1). All plates were within their expiry date at the time of evaluation. For comparison, an in-house selective

medium based on the formula for MNYC medium was prepared as described previously [3]; the same medium without antibiotics was included as a control. Each medium was labelled with an identification code to help reduce observer bias (Table 1).

Organisms

N. gonorrhoeae (105) isolates tested included the five WHO reference strains recommended for antibiotic susceptibility testing and 100 clinical isolates (30 penicillinase-producing) representing a wide variety of serovars encountered in natural infection (Table 2). Other organisms tested included *N. meningitidis* (25, mainly clinical isolates including some of known serogroup); *N. lactamica* (20, all clinical isolates); *Moraxella catarrhalis* (20, all clinical isolates including 19 penicillinase-producing strains); *Gemella haemolysans* NCTC 10243; *N. animalis* NCTC 10212; *N. canis* NCTC 10296; *N. caviae* NCTC 10293; *N. cinerea* NCTC 10294; *N. cuniculi* NCTC 10297; *N. denitrificans* NCTC 10295; *N. elongata* NCTC 10660; *N. elongata* subsp. *glycolytica* NCTC 11050; *N. flavescens* NCTC 8263; *N. ovis* NCTC 11018; *N. pharyngis* NCTC 4590; *N. mucosa* NCTC 10777; *N. mucosa* var *mucosa* NCTC 10774; *Neisseria* sp. NCTC 11049; *Escherichia coli* (6, including NCTC 11560 and 10418); *Proteus mirabilis* (6); *Pseudomonas aeruginosa* (6, including NCTC 10662); *Staphylococcus aureus*

(6, including NCTC 6571 and S113); *Streptococcus agalactiae* (6); *Candida albicans* (4); *C. glabrata* (1) and *C. kefyr* (1).

Test protocol

Saline suspensions equivalent to a McFarland 0.5 standard, c. 10^8 cfu/ml were prepared from an overnight culture on in-house non-selective medium. Various dilutions of stock suspensions were used to inoculate in-house selective and non-selective media and the 14 commercial pre-poured media. The entire series of plates was tested with the same suspension dilutions of each organism, inoculated in alphabetical order (Table 1), the in-house non-selective medium being inoculated last and acting as a control. A multi-point inoculator with a pin volume of 1 μ l was used for inoculation. Cultures were incubated at 37°C in a CO₂-enriched atmosphere and examined after incubation for 16–24 h and 40–48 h: the number of colonies was calculated from the appropriate dilution and counts were expressed as cfu/ml. All counts were performed in duplicate. Although some spreading of *P. mirabilis* was evident on certain media, the number of discrete colonies could be determined from plates that had only a few colonies present. For organisms expected to grow on selective media (gonococci, meningococci and *N. lactamica*), test inocula were 10^5 , 10^4 and 10^3 cfu/ml; thus 'no growth' represented <100 cfu. For organisms expected

Table 1. Gonococcal selective media included in the evaluation

Code	Medium	Manufacturer/ code no.	Antibiotics*						Blood/supplements
			V	L	C	A	T	N	
A	Modified New York City	In-house	–	1	6.6	1	5	–	Lysed horse blood 10%; yeast dialysate 2.5%; glucose 0.1%
B	GC Selective VCNT (chocolate)	Unipath PO136A	3	–	7.5	–	5	36500	Heated horse blood 10%; yeast dialysate 4%
C	GC Selective VCNT (lysed)	Unipath PO135A	3	–	7.5	–	5	36500	Lysed horse blood 5%; yeast dialysate 4%
D	GC Selective VCAT (lysed)	Unipath PO137A	2	–	7.5	1	3	–	Lysed horse blood 5%; yeast dialysate 4%
E	GC Selective LCAT (lysed)	Unipath PO226A	–	4	7.5	1	5	–	Lysed horse blood 5%; yeast dialysate 4%
F	NYC plus LCAT	bioMérieux	–	1	6	1	6.5	–	Lysed horse blood 10%; polyvitex
G	Selective chocolate agar	bioMérieux 43241	3	–	7.5	1	5	–	Haemoglobin 10 g/L; polyvitex; glucose 100 g/L
H	NYC plus VCAT	bioMérieux	2	–	7.5	1	3	–	Lysed horse blood 10%; polyvitex
I	GC selective medium with lysed blood	Difco 9224-30	3	–	7.5	0.9	5	–	Lysed horse blood 5%; VX supplement; glucose 0.21%
J	Neisseria isolation medium	Difco 9188-30	3	–	7.5	0.9	5	–	VX supplement
K	GC selective medium with haemoglobin	Difco 9160-30	3	–	7.5	0.9	5	–	Haemoglobin 2.5 g/L; Supplement B
L	GC selective medium (chocolate)	Difco 9223-30	3	–	7.5	0.9	5	–	Heated horse blood; VX supplement
M	New York City	Difco 9387-30	2	–	7.5	1	3	–	Lysed horse blood 5%, serum 20%, yeast dialysate 2.5%; glucose 0.5%
N	Modified New York City	Becton Dickinson	2	–	7.5	1	3	–	Lysed horse blood 8%; suplex
O	GC Lect	Becton Dickinson	1	2	7.5	1.5	3	–	Haemoglobin 10 g/L; Isovitalex
P	Non-selective modified New York City	In-house				None			Lysed horse blood 10%; yeast dialysate 2.5%; glucose 0.1%

V, vancomycin; L, lincomycin; C, colistin; A, amphotericin; T, trimethoprim; N, nystatin.

*Antibiotic concentration in mg/L except nystatin which is given in units/L.

Table 2. Serotype and auxotype of 100 clinical gonococcal isolates included in the evaluation

Serovar	Number of isolates	Number of isolates of auxotype*							
		A	P	PA	AHU	PAU	PHU	PAHU	NR
IA-2	6	0	0	0	5	0	0	0	1
IA-4	3	0	0	0	0	0	0	0	3
IA-5	2 [†]	0	0	0	1	0	0	0	0
IA-6	5	2	0	0	0	0	0	0	3
IA-7	1 [†]	0	0	0	0	0	0	0	0
IA-16	4	0	0	0	4	0	0	0	0
IA-21	3	0	0	0	3	0	0	0	0
IA-25	2	0	0	0	2	0	0	0	0
IB-1	10	0	0	0	0	0	1	1	8
IB-2	7	0	0	0	0	0	0	1	6
IB-3	7	1	4	0	0	0	0	0	2
IB-5	2	1	1	0	0	0	0	0	0
IB-6	8	0	5	0	0	0	0	0	3
IB-7	5	0	3	0	0	0	0	0	2
IB-8	4	2	1	0	0	0	0	0	1
IB-15	3	0	0	3	0	0	0	0	0
IB-17	7	0	0	0	0	0	0	0	7
IB-19	2	0	2	0	0	0	0	0	0
IB-25	2	0	2	0	0	0	0	0	0
IB-26	1	0	0	0	0	0	1	0	0
IB-29	2	0	0	0	0	2	0	0	0
IB-31	1	0	0	0	0	0	0	0	1
IB-00 [‡]	13	0	0	0	0	0	0	0	13
Total	100	6	18	3	15	2	2	2	50

*Requirement for: A, arginine; P, proline; H, hypoxanthine; U, uracil; NR, non-requiring.

[†]One IA-5 isolate and the IA-7 isolate failed to grow on any of the auxotyping media.

[‡]Non-typable strains.

to be inhibited by selective media (non-pathogenic neisseriae and non-neisseriae), test inocula ranged from 10^8 to 10^3 cfu/ml; thus 'no growth' represented complete inhibition of 10^5 cfu.

Statistical analysis was by the χ^2 test with the Minitab PC software package.

Results

Gonococci

The performance of media in culturing 105 gonococcal isolates is summarised in Table 3. The only media to grow all 105 isolates in duplicate after incubation for 24 h were the in-house selective medium A, commercial media F and N, and the in-house non-selective control medium, P; medium L supported growth of all isolates but only after incubation for 48 h. After incubation for 24 h the vast majority of duplicate cultures gave identical results while after incubation for 48 h there was total concordance. Overall performance ranged from growth of 78.1% of strains (medium J) to 100% (media A, F and N) after incubation for 24 h and 88.6% (medium J) to 100% (media A, F, N and L) after incubation for 48 h. Mean counts after 48-h culture on selective media ranged from 1.28×10^8 cfu/ml (medium B) to 2.02×10^8 cfu/ml (medium N). The in-house selective medium (A) gave a count of 1.91×10^8 cfu/ml compared to 2.21×10^8 cfu/ml on non-selective control medium (P) indicating that the cut-off for failure to grow on the selective media was *c.* 200 cfu.

Table 3. Sensitivity of gonococcal selective media*

Medium	Number of isolates (%) cultured at	
	24 h	48 h
A	210 (100)	210 (100)
B	200 (95.2)	206 (98.1)
C	176 (83.8)	188 (89.5)
D	194 (92.4)	202 (96.2)
E	193 (91.9)	200 (95.2)
F	210 (100)	210 (100)
G	194 (92.4)	202 (96.2)
H	202 (96.2)	204 (97.1)
I	174 (82.9)	196 (93.3)
J	164 (78.1)	186 (88.6)
K	176 (83.8)	194 (92.4)
L	201 (95.7)	210 (100)
M	178 (84.8)	196 (93.3)
N	210 (100)	210 (100)
O	200 (95.2)	204 (97.1)
P	210 (100)	210 (100)

*One hundred and five isolates tested in duplicate (n = 210).

The failure of media to support the growth of certain serovars is summarised in Table 4. The overall failure rate after incubation for 48 h of 100 clinical isolates tested on 15 selective media was 4.2% (64 of 1500). The failure rate of 14.1% (55 of 390) with serovar IA isolates was significantly higher than the rate of 0.8% (9 of 1110) found with serovar IB isolates ($\chi^2 = 124.8$; $p < 0.001$). Generally, the same isolates of gonococci failed to grow on certain media. For example: one IA-5/AHU and one or more IA-21/AHU isolates were the only failures associated with media B, D, G, H and O (all vancomycin-containing media); in addition to failures with isolates IA-5/AHU and IA-21/AHU, medium

E (lincomycin) and medium M (vancomycin) failed to support growth of one IA-16/AHU and one IA-4/NR isolate, respectively; medium I (vancomycin) and medium K (vancomycin) both failed with one non-typable IB/NR isolate and with two IA-4/NR and three IA-2/AHU isolates; medium C (vancomycin) failed with three IA-2/AHU isolates, two IA-4/NR 2 isolates, one IA-5/AHU isolate, three IA-21/AHU isolates and one non-typable IB/NR isolate; medium J (vancomycin) failed with one IA-2/AHU isolate, one IA-4/NR isolate, one IA-5/AHU, three IA-21/AHU isolates, one IB-1/NR isolate and five non-typable IB/NR isolates.

Vancomycin-containing media accounted for 93.2% (59 of 64) of all growth failures and the overall failure rate in relation to the total number of challenges was 1.7% (5 of 300) for media containing lincomycin and 4.9% (59 of 1200) for media containing vancomycin ($\chi^2 = 6.2$; $p < 0.02$). All five failures on lincomycin-containing medium occurred with the medium containing 4 mg/L (F) and none on the two media containing 1 mg/L (A and F).

The majority of growth failures, 76.6% (49 of 64), were with strains of the AHU auxotype and the remainder were with non-requiring strains (Table 4). Failure rates were 21.8% (49 of 225) for AHU strains, 2% (15 of 750) for non-requiring strains and 0% (0 of 525) for strains of other auxotypes (AHU strains *versus* non-requiring strains, $\chi^2 = 110.4$; $p < 0.001$; non-requiring strains *versus* isolates of other auxotypes, $\chi^2 = 10.63$; $p < 0.01$).

Table 4. Serotype of gonococci failing to grow on selective media after incubation for 48 h

Serovar	Number of isolates tested	Total challenge from 15 selective media	Number (%) of cultures not growing	Number of media with growth failure
IA-2	6	90	7*(8)	3
IA-4	3	45	6†(13.3)	4
IA-5	2	30	11*(36.7)	11
IA-6	5	75	0	0
IA-7	1	15	0	0
IA-16	4	60	1*(1.7)	1
IA-21	3	45	29*(64.5)	11
IA-25	2	30	1*(3.3)	1
IB-1	10	150	1†(0.7)	1
IB-2	7	105	0	0
IB-3	7	105	0	0
IB-5	2	30	0	0
IB-6	8	120	0	0
IB-7	5	75	0	0
IB-8	4	60	0	0
IB-15	3	45	0	0
IB-17	7	105	0	0
IB-19	2	30	0	0
IB-25	2	30	0	0
IB-26	1	15	0	0
IB-29	2	30	0	0
IB-31	1	15	0	0
IB-00	13	195	8†(4.1)	4
Total	100	1500	64(4.2)	

* AHU auxotype strains.

†Non-requiring strains.

Although serovar IA and the AHU auxotype were associated with failure to grow, there was a considerable variation between serovar IA isolates: serovars IA-2 (5 of 6 AHU), IA-7 (non-auxotypable), and IA-25 (2 of 2 AHU) showing similar failure rates ($p < 0.05$), serovars IA-5 (1 of 2 AHU) and IA-21 (3 of 3 AHU) significantly higher failure rates ($p < 0.05$), and serovars IA-6 (0 of 5 AHU) and IA-16 (4 of 4 AHU) significantly lower failure rates ($p < 0.05$) when compared with all serovar IA isolates.

Meningococci

After incubation for 24 h the performance of media in supporting growth of meningococci was 92% (23 of 25) with media D and J; 96% (24 of 25) with media B, C, E, G, H, I, K, L, M, N and O; and 100% with media A, F and P. After incubation for 48 h results were identical except for media D and E on each of which a further isolate grew. Mean counts after culture for 48 h on selective media ranged from 3.13×10^8 cfu/ml (medium B) to 4.53×10^8 cfu/ml (medium O). The in-house selective medium A gave a count of 4.36×10^8 cfu/ml while the non-selective control medium P gave a count of 4.40×10^8 cfu/ml. These results suggest that the cut-off for failure to grow on the selective media was *c.* 450 cfu. The same meningococcal strain, a non-groupable type 15 strain sensitive to penicillin and sulphadiazine, was responsible for all growth failures.

N. lactamica

All 20 strains of *N. lactamica* grew on all of the media after incubation for 24 h. Mean counts after 48 h ranged from 1.28×10^8 cfu/ml (medium B) to 2.14×10^8 cfu/ml (medium N). The in-house selective medium A gave a count of 1.88×10^8 cfu/ml while the non-selective control medium P gave a count of 2.08×10^8 cfu/ml.

Non-pathogenic neisseriae and miscellaneous organisms

The results of testing the 71 non-pathogenic neisseriae and miscellaneous organisms in duplicate are summarised in Table 5. None of the selective media inhibited all of the organisms after incubation for 48 h although medium G gave 100% inhibition after incubation for 24 h. After incubation for 48 h, inhibition ranged from 72.5% (medium L) to 97.9% (medium G): their performance in supporting the growth of gonococci after 48 h was 100% and 96.2%, respectively. Details of the organisms that grew on the various selective media are summarised in Table 6. Generally, results of duplicate cultures were concordant, apart from *N. cinerea* and one of the strains of *N. mucosa* which often grew on only one of the duplicate plates. Although results in Table 6 are based on 'growth' or 'no growth' the extent of growth was often substantially reduced compared with the non-selective control medium P.

Table 5. Specificity of gonococcal selective media; non-pathogenic neisseriae and miscellaneous organisms tested in duplicate*

Medium	Number of isolates (%) inhibited at	
	24 h	48 h
A	140 (98.6)	135 (95.1)
B	138 (97.2)	124 (87.3)
C	128 (90.1)	126 (88.7)
D	138 (97.2)	132 (93)
E	138 (97.2)	135 (95.1)
F	128 (90.1)	123 (86.6)
G	142 (100)	139 (97.9)
H	134 (94.4)	125 (88)
I	134 (94.4)	131 (92.3)
J	126 (88.7)	123 (86.6)
K	138 (97.2)	135 (95.1)
L	116 (81.7)	103 (72.5)
M	138 (97.2)	136 (95.8)
N	118 (83.1)	115 (81)
O	138 (97.2)	135 (95.1)
P	0	0

*n = 142.

Discussion

Selective media improve the cultural diagnosis of gonorrhoea by preventing the growth of other bacteria which may mask the presence of gonococcal colonies or inhibit the growth of gonococci directly. Many organisms present in the normal endocervical flora inhibit gonococci [4–9]. In assessing the suitability of pre-poured selective media the most important factor is the ability to support the growth of all gonococcal isolates, followed by the effective inhibition of a wide range of other bacteria. As gonococcal infection can occur at levels as low as 10^2 cfu/ml, and other organisms may often be present at levels well in excess of 10^6 cfu/ml [10] cut-off values of *c.* 10^2 cfu/ml for growth of gonococci and 10^4 – 10^6 cfu/ml for inhibition of non-pathogenic neisseriae and miscellaneous organisms were chosen to represent a meaningful test of the selective media in clinical practice. The inclusion of

AHU strains is important in comparing lincomycin and vancomycin as selective agents; Reichart *et al.* [11] included only 2.7% of such isolates in their study comparing GC-Lect and modified Thayer Martin (MTM) medium but stressed that future studies should include more AHU isolates. In the present study 15% of isolates were AHU strains.

In addition to the in-house selective media only two of the commercial pre-poured selective media (F and N) supported growth of all 105 isolates of gonococci in duplicate after incubation for 24 h (Table 3); although medium L yielded growth of all 105 isolates after incubation for 48 h it failed to support the growth of three isolates in duplicate and three isolates in one of the duplicate cultures after 24 h. When gonococci grew on a particular medium the counts were generally similar to all other media. A non-groupable type 15 isolate of meningococcus failed to grow on media L and N and these media also allowed an unacceptable percentage of non-gonococcal neisseriae or miscellaneous organisms to grow (28.2% and 19.7% respectively). Although medium F was the only commercial selective media to support the growth of all gonococci and meningococci, it allowed 14.1% of non-gonococcal neisseriae or miscellaneous organisms to grow compared with 5.6% for the in-house medium; this difference in inhibition was due to the complete failure of medium F to inhibit growth of yeasts, a serious problem, as certain strains of *Candida albicans* produce a substance inhibitory to gonococci [8, 9]. The other organisms growing on medium F and the in-house selective medium A were identical and included *G. haemolysans*, *N. cinerea*, *N. mucosa* var *mucosa* and a methicillin-resistant staphylococcal strain. Clearly, none of the commercial media in this trial performed as well overall as the in-house selective medium.

In general, media that contained 10% blood (A, B, F and H) gave significantly better results for supporting the growth of gonococci after incubation for 48 h (mean

Table 6. Growth of 71 non-pathogenic neisseriae and miscellaneous organisms cultured in duplicate on gonococcal selective media for 48 h

Organism	Number of isolates	Number (%) of isolates growing on corresponding medium														
		A	B	C	D	E	F	G	H	I	J	K	M	L	N	O
<i>Candida</i> spp.	6*	0	4	5	0	0	6	0	5	0	6	0	5	0	5	0
<i>Neisseria</i> spp.	14†	2‡	2	2‡§	2‡§	2‡	2‡	2‡	2‡	2‡§	1‡	2‡	2‡§	1	1‡§	2‡§
<i>M. catarrhalis</i>	20	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>G. haemolysans</i>	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>E. coli</i>	6	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>P. mirabilis</i>	6	0	3	2	4	1	0	0	2	3	3	2	6	2	3	1
<i>Ps. aeruginosa</i>	6	0	0	0	0	0	0	0	0	1	0	0	5	0	0	0
<i>S. aureus</i>	6	1	0	0	0	1	1	0	0	0	0	0	0	0	2	1
<i>St. pyogenes</i>	6	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0
Total	71	4	9	9	6	4	10	2	9	6	10	4	20	3	14	4
		(5.6)	(12.7)	(12.7)	(8.5)	(5.6)	(14.1)	(2.8)	(12.7)	(8.5)	(14.1)	(5.6)	(28.2)	(4.2)	(19.7)	(5.6)

Note: media were tested in duplicate and in most cases growth occurred on both duplicates (see text).

**C. albicans* (4), *C. glabrata* (1), *C. kefyr* (1).

†*N. animalis* (1), *N. canis* (1), *N. caviae* (1), *N. cinerea*† (1), *N. cuniculi* (1), *N. denitrificans* (1), *N. elongata* (1), *N. elongata* subsp. *glycolytica* (1), *N. flavescens* (1), *N. mucosa* (1), *N. mucosa* var *mucosa*§ (1), *N. ovis* (1), *N. pharyngis* (1), *Neisseria* sp. NCTC 11049 (1).

‡ and § one isolate included as growth was inhibited on one of the duplicate cultures.

98.8%) than media that contained 5% blood (C, D, E, I and M; mean 93.5%; $p < 0.001$) or haemoglobin (G, K and O; mean 95.2%, $p < 0.01$). Media containing lincomycin performed significantly better than those containing vancomycin; both the media (A and F) that enabled the growth of all gonococcal and meningococcal strains contained lincomycin (1 mg/L), whilst all of the gonococci grew on only two (L and N) of 11 vancomycin-containing media. All growth failures with lincomycin occurred with AHU auxotype strains on the medium containing 4 mg/L but not with the media containing 1 mg/L suggesting that a concentration of 4 mg/L is inhibitory to certain strains of gonococci. All the media containing vancomycin failed to support the growth of the same non-groupable type 15 meningococcal isolate which was known to be sensitive to penicillin and sulphadiazine and may also be sensitive to vancomycin. Vancomycin in selective media is known to have an inhibitory effect on gonococci [12, 13] and is associated with the AHU auxotype [14, 15] which in turn shows a correlation with serogroup IA isolates [16–18]. In 1978 the antibiotic formulation of the original NYC medium [19] was modified by reducing the vancomycin from 3 to 2 mg/L [20] because 3 mg/L was inhibitory to the growth of as many as 10% of gonococcal strains [13, 21]. However, these results suggest that the four media containing vancomycin 2 mg/L performed significantly less well than the two media containing lincomycin 1 mg/L: after incubation for 48 h the percentages of gonococci growing were 96.2, 97.1, 93.3 and 100% for the vancomycin media compared with 100% for the two lincomycin-containing media ($p < 0.01$). Interestingly, the medium containing vancomycin 2 mg/L that yielded growth of all gonococcal isolates was the second least selective medium allowing growth of 19.7% of non-pathogenic neisseriae and miscellaneous organisms. Although an increased growth of unwanted organisms on rectal and pharyngeal cultures has been associated with media containing lincomycin 1 mg/L [22] this can be reduced by decreasing the level of glucose in the media [23]. Furthermore, as most contaminants appear after incubation for >24 h this is not a major problem when plates are examined on day 1. The NYC and MNYC formulation supports luxuriant growth which means that gonococcal colonies are clearly visible by 24 h in the majority of primary cultures [3, 24]. GC-Lect medium (medium O) contains both vancomycin 2 mg/L and lincomycin 1 mg/L but performed no better than media containing lincomycin or vancomycin alone. Reichert *et al.* found GC-Lect medium performed better than MTM medium containing vancomycin 3 mg/L; however, the isolates missed by the MTM medium were not susceptible to vancomycin, all having vancomycin MICs ≥ 4 [11]. This supports earlier findings comparing MNYC medium containing lincomycin 1 mg/L with Thayer Martin medium containing vancomycin 4 mg/L [3]; none of the strains isolated only on MNYC medium was sensitive to vancomycin at the concentration present in the Thayer Martin medium. The improved perfor-

mance with lincomycin-containing media may be related to the greater safety margin with lincomycin (MIC usually 16 times the concentration present in the medium) than with vancomycin (MIC usually 2–4-fold greater than the corresponding medium concentration). This increased safety margin combined with the better nutritional value of MNYC medium may be particularly important in the case of small inocula [3]. Although Martin-Lewis medium was not included in this evaluation it was shown recently that NYC medium containing vancomycin 2 mg/L markedly enhanced the recovery of gonococci from clinical specimens when compared with Martin-Lewis medium which contains vancomycin 4 mg/L [24].

Overall, the 15 selective media were significantly poorer ($p < 0.001$) at supporting the growth of the 26 serogroup IA clinical isolates (86%) than the 74 IB clinical isolates (99.2%), supporting the view that the failure of certain selective media to support the growth of gonococci is due to a combination of nutritional inadequacy – possibly related to a low concentration of blood – and an inhibitory effect of vancomycin. Of the IA clinical isolates included in this evaluation 57.7% (15 of 26) were of the AHU auxotype. AHU strains may be even more prevalent in certain non-selected populations; serovar IA-2 has been shown to account for the majority of serovar IA infections and also to account for as much as 96.8% [18] and 98% [16] of AHU strains. In contrast there is a correlation between serogroup IB isolates and the non-requiring (NR) auxotype which accounted for 58.1% (43 of 74) of IB isolates in the test strains and 64.5% [18] and 63.2% of IB isolates in non-selected populations [25]. Although isolates of serovar IA and the AHU auxotype were associated with failure to grow there was a considerable serovar variation, with isolates of certain minor serovars such as IA-5 and IA-21 showing the strongest correlation. This has important implications for the detection and control of these minor serovars within the community and could be significant in the overall epidemiology of gonococcal infection.

Failure to inhibit yeasts was a significant factor in the lack of selectivity of several media. Only two media (B and C) contained nystatin, which showed extremely poor inhibition of yeasts. Amphotericin has been shown to be superior to nystatin in suppressing yeast contaminants in selective media [26]. In the present study, most media containing amphotericin ≥ 0.9 mg/L inhibited yeasts completely. However, media F, H, J, L and N which contain similar concentrations of amphotericin failed to inhibit yeasts; this may be due to batch variation of supplement, media preparation, or reduced shelf-life of the media. Interestingly, manufacturers of media that failed to inhibit yeasts also produced media containing the same concentration of amphotericin that gave complete inhibition.

Colistin was generally effective in inhibiting non-

gonococcal neisseriae and gram-negative bacilli, although medium L (colistin 7.5 mg/L) allowed the growth of two *Moraxella*, five *Pseudomonas* and six *Proteus* strains. All the media, apart from medium N, allowed the growth of *N. cinerea* in at least one of the duplicate cultures and most of the media also allowed *N. mucosa* var *mucosa* to grow. In practice the latter organism is rarely isolated [27] and can be distinguished readily from *N. gonorrhoeae* by sugar utilisation tests. *N. cinerea* is also a relatively rare isolate but is of much greater significance as, not only is it able to grow on gonococcal selective media [28], but it may cause problems in identification [29, 30] and result in the misdiagnosis of gonorrhoea. Medium N, the only medium to inhibit *N. cinerea*, was poor at inhibiting *P. mirabilis* gram-positive bacteria and yeasts.

Only three media (A, F and G) inhibited all six strains of *P. mirabilis*, suggesting that the failure of the other media, which contained similar concentrations of trimethoprim, must be due to problems in media preparation or stability. The same reasons may explain the failure of media N and O to inhibit certain strains of streptococci and staphylococci. Media (A, E and F) containing lincomycin failed to inhibit the MRSA isolate, in keeping with the poorer inhibition of gram-positive organisms by lincomycin in comparison to vancomycin [21] but this is much less important than the inhibitory effect of vancomycin on gonococci.

These results support previous findings that the improved nutritional status of media based on the original NYC medium [19, 26] or the much simpler MNYC formulation [3] provides superior performance both in terms of the number of positive clinical cultures and the rapidity and quality of growth when compared with conventional media [3, 22–24, 31]. In addition, the results support earlier reports of the superior performance of media containing lincomycin in place of vancomycin in both chocolate agar [21] and MNYC medium [22, 23].

In conclusion, none of the commercially available pre-poured media performed as well as the in-house medium in spite of the fact that some of the media were prepared to a very similar formula. We consider that manufacturers are providing too great a selection of media, some of which perform inadequately, probably due to a combination of factors including the level of nutrition, inappropriate choice of antibiotic supplements, and too long a shelf-life. The results of this evaluation and a review of the literature suggest that the use of 10% blood, a highly nutritious medium (based on the original NYC or MNYC formulation), and lincomycin in place of vancomycin, are crucial to the production of an effective selective medium. We urge manufacturers to take note of these points. A rational restriction in the variety of selective media is likely to decrease production costs, allow a shorter shelf-life and improve the overall performance.

We thank the following companies who supplied plates: Becton Dickinson UK Ltd, Cowley, Oxford OX4 3LY; bioMérieux UK Ltd, Basingstoke, Hampshire RG22 6HY; Difco Laboratories Ltd, West Molesey, Surrey KT8 2SE and Unipath Ltd, Basingstoke, Hampshire, RG24 8PW.

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