

Evaluation of PPLO, A7B, E, and NYC Agar Media for the Isolation of *Ureaplasma urealyticum* and *Mycoplasma* Species from the Genital Tract

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Four agar media (PPLO, NYC, A7B, and E) which are commonly used for the isolation of urogenital tract *Mycoplasma* species and *Ureaplasma urealyticum* were compared by culturing swabs of the endocervix of 334 pregnant women on all four media. To permit growth of both *Mycoplasma* and *U. urealyticum*, selective ingredients were omitted from the media tested. A7B and E agar were both satisfactory for the isolation of *Mycoplasma* species, recovering 92 and 82%, respectively, of all *Mycoplasma* species isolated. Only A7B agar was satisfactory for *U. urealyticum* isolation, recovering 96% of all isolates. Several modifications of PPLO, NYC, and E agar failed to significantly improve recovery of *U. urealyticum* on these media. A7B agar was clearly superior to all other media tested in terms of recovery rate, typical appearance of colonies, and ease of reading. A7B can be used for the isolation of both *U. urealyticum* and *Mycoplasma* species from urogenital sites.

There is growing interest in the isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* from the urogenital tract because of numerous reports on the possible role of these organisms in nongonococcal urethritis (16, 18), infertility (3, 8, 23, 24), abortion and stillbirth (4, 11), premature birth and low birth weight of infants (2, 4, 11), and pelvic inflammatory disease (14). Several agar and broth media have been described for the isolation of *M. hominis* and *U. urealyticum*, but there is little information on comparative isolation rates with different media. Because of the special growth requirements of *Ureaplasma* species (i.e., lower pH optimum and a requirement for urea), separate media are generally employed for the isolation of *U. urealyticum* and *M. hominis*. Recently, however, NYC agar was shown to allow the simultaneous isolation of *Neisseria gonorrhoeae*, large-colony mycoplasmas, and T mycoplasmas (*U. urealyticum*) from the genital tract (5-7). Shepard (19, 21) has devised a differential medium, A7B agar, which also supports the growth of both *U. urealyticum* and *Mycoplasma* species. This medium was designed primarily as a medium to differentiate *Ureaplasma* from *Mycoplasma* species. A comparison of A7B with other media for the isolation of *Mycoplasma* species has not been done.

Several other media which have been used for the isolation of *Ureaplasma* or *Mycoplasma* species are selective for either *Ureaplasma* or *Mycoplasma* species. For example, E agar is recommended in the *Manual of Clinical Microbiology* for the isolation of mycoplasmas (9). This medium contains thallium acetate, an agent which is inhibitory to *U. urealyticum* (22). Another medium, MES agar, is recommended for the isolation of *U. urealyticum* (9). Similarly, the Centers for Disease Control manual *Laboratory Diagnosis of Mycoplasma Infections* recommends PPLO broth and agar supplemented with erythromycin (inhibitory to *U. urealyticum*) or lincomycin (inhibitory to *M. hominis*) for the isolation of *M. hominis* and *U. urealyticum*, respectively, from the urogenital tract (25).

The use of a number of different media and culturing conditions may partly explain the wide variation in the reported isolation rates of *Ureaplasma* and *Mycoplasma* species in various human populations. For instance, recovery of *M. hominis* from pregnant women has been reported to be as low as 7.5% (8) and as high as 39% (1) in different studies, whereas T mycoplasma recovery ranged from 23 to 71% in the same reports.

The object of the present study was to compare the isolation rates of *U. urealyticum* and *Mycoplasma* species on several of the more commonly used agar media and to determine whether any one medium, used nonselectively, would yield good recovery of both types of organisms from urogenital tract specimens. After some preliminary testing, two of the media formulations were changed in an attempt to increase recovery.

MATERIALS AND METHODS

NYC agar. NYC agar contained the following ingredients per liter of medium (5-7): Bacto-Agar (Difco Laboratories), 10 g; GC medium base (Difco), 36 g; horse erythrocytes (3% solution in water), 200 ml; 50% glucose solution, 10 ml; yeast dialysate (prepared by dialysis of 454 g of brewer's yeast [Sigma Chemical Co.] versus 1,250 ml of cold distilled water for 48 h), 25 ml; agamma horse serum (GIBCO Diagnostics, Madison, Wis.), 120 ml; and VCAT antibiotics (vancomycin, 2 µg/ml; colistin, 5.5 µg/ml; amphotericin B, 1.2 µg/ml; and trimethoprim lactate, 3 µg/ml). The pH was 7.1 ± 0.1. In this study, agamma horse serum was used instead of citrated horse plasma (5-7) because of difficulty in finding a reliable source of the latter.

A7B agar. Differential medium A7B contained per liter of medium (19, 21): Trypticase soy broth (BBL Microbiology Systems), 24 g; MnSO₄ · H₂O, 0.155 g; agar (GIBCO), 10.5 g; agamma horse serum (GIBCO), 200 ml; CVA enrichment (GIBCO) or supplement VX (Difco), 5 ml; fresh 25% yeast extract (GIBCO), 10 ml; 10% ultrapure urea (Schwarz/Mann, Orangeburg, N.Y.), 10 ml; 4% L-cysteine hydrochloride (Sigma Chemical Co.), 2.5 ml; penicillin G potassium

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(200,000 U/ml), 5.0 ml; and putrescine dihydrochloride (Sigma), 1.65 g. The pH was adjusted to 6.0.

PPLO agar. PPLO agar was prepared by adding the following ingredients per liter of medium (25): heart infusion (Difco), 35 g; Bacto-Peptone (Difco), 7 g; NaCl, 3.5 g; Bacto-Agar (Difco), 9.8 g; agamma horse serum (GIBCO), 200 ml; 25% fresh yeast extract (GIBCO), 100 ml; penicillin, 500 U/ml; polymyxin B, 25 µg/ml; and amphotericin B, 5 µg/ml. The antibiotic supplements used here are the ones recommended for the urea broth medium described in the Centers for Disease Control manual *Laboratory Diagnosis of Mycoplasma Infections* (25). They differ from those employed in the medium recommended for *M. hominis* ("Arginine Broth Medium for *M. hominis*" [25]) only in the concentration of polymyxin employed (*M. hominis* broth medium uses 50 µg of polymyxin per ml). PPLO agar was adjusted to pH 7.0.

E agar. E agar contained the following ingredients per liter of medium (9): peptone no. 110 (GIBCO), 13 g; NaCl, 3.25 g; purified agar (Difco), 6.5 g; yeast dialysate (prepared as for NYC agar), 100 ml; agamma horse serum (GIBCO), 250 ml; penicillin G (100,000 U/ml), 2 ml; and 650 ml of deionized water. The pH was 7.4.

U9B broth. Urease color test medium (U9B) contained per liter of medium (21): Trypticase soy broth (BBL), 7.5 g; NaCl, 5.0 g; monobasic potassium phosphate, 0.2 g; agamma horse serum (GIBCO), 40 ml; 10% urea solution, 5 ml; 1% phenol red solution, 1.0 ml; and potassium penicillin G (100,000 U/ml), 10 ml. The pH was adjusted to 6.0 ± 0.2 .

Culture techniques. Specimens were endocervical swabs taken from pregnant women at the time of their first visit to the obstetrician. The four agar media compared in this study were prepared in two bi-plates. Specimens were collected with two Dacron swabs on plastic sticks, one swab for each bi-plate. The media were inoculated in random order. One of the swabs was placed in a tube of U9B broth. The U9B broth was included to allow detection of small inocula of *U. urealyticum* and to permit the growth of fastidious *U. urealyticum* strains which require reduced oxygen tension for growth (12). Plates were placed in a candle jar during transport to the laboratory where they were incubated at 35°C in an incubator containing 5% CO₂ (22). The U9B broth tube was incubated in air at ca. 35°C. Plates were examined after 2, 3, or 4 days of incubation and again after 7 days. A drop of urea reagent (17) was added to a different portion of the NYC, PPLO, and E agar plates each time the plates were examined to detect *U. urealyticum* colonies. Plates were evaluated for the relative number and size of colonies, amount of bacterial or yeast contamination, amount of interfering background debris, typical appearance of colonies, and ease of reading.

RESULTS

Recovery on media as originally formulated. Table 1 shows recovery of *U. urealyticum* and *Mycoplasma* species on the four agar media from 119 patient specimens. Total recovery of *U. urealyticum* on all agar media was 76/119 (63.9%). A7B yielded the highest recovery rate (60.5%), followed by NYC (54.6%), E (32.8%), and PPLO (18.5%). When the recovery rate on each medium was compared with the total recovery rate, NYC, E, and PPLO agars all gave a significantly lower yield ($P = 0.017$, < 0.001 , and < 0.001 , respectively) of *U. urealyticum*. Only A7B agar gave a recovery rate which approached the maximum yield obtained using all four media (no significant difference at the 95% confidence level).

The total recovery rate of *Mycoplasma* species was 22/119 (18.5%) (Table 1). A7B, NYC, and E agars all gave recovery

rates which were close to the maximum recovery obtained by using all four media (no significant difference at the 95% confidence level). PPLO agar, however, gave only an 8.4% recovery rate ($P = 0.002$).

Recovery on modified agar media. It became evident early in the study that PPLO agar and E agar, as originally prepared, were seriously deficient in the isolation of *U. urealyticum* as compared with A7B. E agar was also often highly contaminated with vaginal flora. On E agar containing penicillin, 119/193 (65%) of the plates had moderate to heavy bacterial or fungal contamination. In addition, the low-agar concentration (0.65%) made inoculation of plates difficult. The agar concentration was, therefore, increased to 1.0%, and to reduce contamination, penicillin was replaced with the VCAT antibiotic combination used in NYC. This formulation is referred to here as modified E agar.

PPLO agar was rarely contaminated with vaginal flora, probably due to the high levels of amphotericin B and polymyxin B used. It has been reported that amphotericin B is inhibitory to *T. mycoplasmas* at a concentration of 5 µg/ml (15). There is no information on the effect of polymyxin on *T. mycoplasmas*, but like amphotericin B, it acts upon the cell membrane (10). To test the possibility that the high antibiotic concentrations used in the original formulation were inhibitory to *Mycoplasma* species and *U. urealyticum*, the VCAT antibiotic combination with lower amphotericin B and colistin (polymyxin E) concentrations was used in PPLO agar. This formulation is referred to as modified PPLO agar.

An additional 215 specimens were plated on A7B, NYC, modified E, and modified PPLO agars. The modified E agar was easier to inoculate, and the contamination rate fell from 65 to 12%, a level commonly found with the other media used. The recovery rates of *U. urealyticum* and *Mycoplasma* species, however, did not change (Table 2). Lowering the antibiotic concentration in PPLO agar caused an increase in the contamination rate from 3 to 15.7% but also allowed for an increase in recovery of *U. urealyticum* from 18.5% (Table 1) to 28.4%. This level of recovery, however, is still far below that obtained with A7B agar (63.8% recovery on A7B from 334 specimens). The recovery rate of *Mycoplasma* species on modified PPLO agar was unchanged. An apparent anomaly, when comparing the results in Table 1 and Table 2, is that NYC showed a reduced recovery rate for *Mycoplasma* species in Table 2 as compared with Table 1, in spite of the fact that there was no change in the formulation of NYC. This is probably due to the use of a new lot number of NYC medium in Table 2. When the data for NYC in the two tables are combined, NYC shows a recovery for *Mycoplasma* species of 47/334 (14.1%). This value is significantly lower ($P < 0.01$) than the maximal recovery rate obtained (66/334 = 19.8%) using all four media. Of the media tested, only A7B agar yielded recovery of both *U. urealyticum* and *Mycoplasma* isolates at levels which approached the maximum recovery obtained by using all four media (no significant difference at the 95% confidence level). From the 334 specimens tested, A7B recovered 213/221 (96.4%) of all *U. urealyticum* isolates and 61/66 (92.4%) of all *Mycoplasma* isolates which were recovered on agar media. Table 3 shows the percent of isolates recovered on each medium. PPLO agar was clearly the poorest medium for both *U. urealyticum* and *Mycoplasma* species. NYC and E agars yielded intermediate recovery levels for *U. urealyticum*, whereas E agar was the only medium besides A7B which gave good recovery of *Mycoplasma* species (no significant difference at the 95% confidence level).

Improved recovery of *U. urealyticum* with broth media. Not

TABLE 1. Recovery of *U. urealyticum* and *Mycoplasma* species from 119 specimens by using original formulations of four agar media

Medium	<i>U. urealyticum</i>			<i>Mycoplasma</i> species		
	No. of positive specimens	% Positive	P ^a	No. of positive specimens	% Positive	P
A7B	72	60.5	N.S. ^b	17	14.3	N.S.
NYC	65	54.6	0.017	18	15.1	N.S.
E	39	32.8	<0.001	19	16.0	N.S.
PPLO	22	18.5	<0.001	10	8.4	0.002
Total positive	76	63.9		22	18.5	

^a P, Value obtained by comparing percent positive on test medium with percent positive on all four media.

^b N.S., Not significant at 95% confidence level.

shown in the data presented is the significance of using U9B broth medium for the isolation of *U. urealyticum*. Among 334 specimens, 19 (5.6%) were positive for *U. urealyticum* in the U9B broth only. These 19 specimens may represent swabs which contained very few organisms, or they may represent strains which grow poorly on agar in 5% CO₂. Total recovery of *U. urealyticum* in broth and agar was 240/334 (72%). Agar media recovered 221/240 (92%). U9B broth recovered 233/240 (97%), as judged by growth of the organism from subculture to A7B or by color change of the broth from yellow to red, or both. None of the broths which changed color showed any evidence of bacterial contamination.

DISCUSSION

Several factors may play a role in the higher recovery rate of *U. urealyticum* on A7B than on NYC, E, or PPLO agars. The most obvious are that only A7B agar has exogenous urea and a pH at the optimum for *Ureaplasma* species (pH 6.0). Factors other than pH must also be involved, however, since NYC (pH 7.1) and E (pH 7.4) yielded a much higher recovery of *U. urealyticum* than did PPLO (pH 7.0). A7B also differs from NYC and PPLO in the antibiotics used. A7B contains only penicillin, whereas PPLO was formulated to contain penicillin, polymyxin, and amphotericin, and NYC contains the last two plus vancomycin and trimethoprim lactate. Antibiotics which act upon the cell membrane may be especially inhibitory to the *Mycoplasmataceae*. E agar, which like A7B agar contains only penicillin, grew 81.8% of all *Mycoplasma* isolates.

Other differences among the media include the type and source of peptones and agars used and the type of yeast supplement (i.e., yeast dialysate versus yeast extract). In an effort to simplify the preparation of NYC, the yeast dialysate was replaced with IsoVitaleX (BBL). With this modified NYC agar, *U. urealyticum* recovery was reduced to a level equivalent to that of PPLO agar (data not shown), indicating

that IsoVitaleX cannot substitute for the yeast dialysate in the NYC formulation.

The superior performance of A7B in recovery of genital *Mycoplasma* species is somewhat surprising since the pH of the medium (6.0) is significantly lower than that which is thought to be optimal for genital mycoplasmas (25). However, Shepard and Lunceford (20) have shown that *M. hominis* grows best at approximately pH 6.5. Thus, the lower pH and the factors mentioned above for the increased recovery of *U. urealyticum* on A7B may play a role in its superior ability to recover *M. hominis* as well.

In addition to increased recovery of *U. urealyticum* and *Mycoplasma* species, A7B agar offers other advantages over NYC, PPLO, and E agars. These are as follows. (i) Colonies appear more typical. The spreading surface growth of *Mycoplasma* colonies was nearly always seen on A7B and was often absent on NYC and PPLO agars. (ii) There is no need to add reagent to detect *U. urealyticum* colonies. The urease reaction on NYC and PPLO was quite variable. Often a positive reaction at day 2, 3, or 4 became negative at day 7. This phenomenon was also seen by Shepard (17). Conversely, plates were sometimes negative at day 2, 3, or 4 and became positive at day 7. (iii) There is less contamination by vaginal flora. By using E agar containing only penicillin, 65% of the plates had moderate to heavy bacterial or yeast contamination. With VCAT antibiotics, the contamination rate was reduced to a level approximately equal to that seen on A7B agar. (iv) There is less distracting background debris. NYC and PPLO agar plates were much more difficult to read because of cellular debris and crystal formation. The clarity of A7B and E agars made these media much easier to read.

The major problem encountered in getting good recovery of both *Mycoplasma* and *U. urealyticum* isolates in this study was in the variability obtained using different lot numbers of horse serum. This variability occurred in spite of the fact that agamma horse serum was employed throughout

TABLE 2. Recovery of *U. urealyticum* and *Mycoplasma* species from 215 specimens by using modified formulations of E agar and PPLO agar

Medium	<i>U. urealyticum</i>			<i>Mycoplasma</i> species		
	No. of positive specimens	% Positive	P ^a	No. of positive specimens	% Positive	P
A7B	141	65.6	N.S. ^b	44	20.5	N.S.
NYC	120	55.8	<0.001	29	13.5	0.005
Modified E	82	38.1	<0.001	35	16.3	N.S.
Modified PPLO	61	28.4	<0.001	18	8.4	<0.001
Total positive	145	67.4		44	20.5	

^a P, Value obtained by comparing percent positive on test medium with percent positive on all four media.

^b N.S., Not significant at 95% confidence level.

TABLE 3. Percent of *U. urealyticum* and *Mycoplasma* isolates recovered on various agar media^a

Medium	No. (%) of positive isolates	
	<i>U. urealyticum</i> (n = 221)	<i>Mycoplasma</i> species (n = 66)
A7B	213 (96.4)	61 (92.4)
NYC	185 (83.7) ^b	47 (71.2) ^b
E ^c	121 (54.8) ^b	54 (81.8)
PPLO ^c	83 (37.6) ^b	28 (42.4) ^b
Total recovered	221	66

^a From a total of 334 specimens tested.

^b Significant difference at 95% confidence level. Obtained by comparing percent positive on test medium with percent positive on all four media.

^c Data from original formula and modified formulas combined.

and all four media always received the same lot number of serum. With certain lots of serum, recovery was poor on all media tested. Since A7B medium contains added urea, lot-to-lot variability in horse serum is probably not due to differences in endogenous urea concentration, and the use of agamma serum indicates that antimycoplasma antibodies are not the cause of the variability. These results emphasize the need for quality control testing of all horse serum used in mycoplasma media.

Leland et al. (13) recently reported that a combination of A7 agar and Boston broth was superior to a combination of urea broth and agar and arginine agar plus arginine biphasic medium (25) for the isolation of *U. urealyticum* and *Mycoplasma* species from urine specimens. Our results with A7B agar and endocervical cultures give further evidence that an A7-based agar medium can be used for the isolation of *Mycoplasma* species as well as *U. urealyticum* from genital tract specimens. While this study was in progress, Shepard devised a modified version of A7B agar called A8 (personal communication). This new medium has not yet been evaluated for its relative ability to isolate urogenital tract *Mycoplasma* species.

We conclude that a single agar medium, A7B, in conjunction with a broth medium like U9B, can be used for the simultaneous isolation of genital *U. urealyticum* and *Mycoplasma* species. A7B and U9B recovered 96.7% of all *U. urealyticum* isolates and 92% of all *Mycoplasma* species isolated. The high recovery rates, typical appearance of colonies, lack of need to add reagent, low contamination, and ease of reading all make A7B the medium of choice.

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