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Isolation of *Mycoplasma* Species and *Ureaplasma urealyticum* from Obstetrical and Gynecological Patients by Using Commercially Available Medium Formulations

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One hundred duplicate endocervical specimens from obstetrical and gynecological patients were cultured for *Mycoplasma* spp. and *Ureaplasma urealyticum*. Rates of recovery of these organisms from commercially prepared A7 medium and the Mycotrim-GU system were compared. We detected 14 (93%) of the total 15 isolates of *Mycoplasma* spp. on A7 plates and 11 (73%) in the Mycotrim-GU system. We detected 34 (89%) of the total 38 isolates of *U. urealyticum* on A7 plates and 32 (84%) in the Mycotrim-GU system. The times of detection for both types of organism were similar in the two systems. We conclude that cultivation on A7 medium as described is a more cost-effective method of recovery of *Mycoplasma* spp. and *U. urealyticum* than the Mycotrim-GU system.

Although several investigators have suggested a pathogenic role for *Mycoplasma* spp. and *Ureaplasma urealyticum* (1, 2, 4–6, 9–12, 14–18), few laboratories have made attempts to isolate these organisms (2, 19). Many microbiologists and clinicians may not recognize the pathogenic potential of these organisms and consider their isolation unusually difficult, expensive, or both.

A differential agar medium (A7) which supports the growth of both *Mycoplasma* spp. and *U. urealyticum* was formulated in 1976 (13) and is now available from several commercial sources (Scott Laboratories, Inc., Fiskeville, R.I.; BBL Microbiology Systems, Cockeysville, Md.; Remel, Lenexa, Kans.). A biphasic medium contained in 50-ml plastic flasks is also now available from Hana Biologics (distributed by Syva Co., Palo Alto, Calif.). The Hana medium allows the cultivation of *Mycoplasma* spp. and *U. urealyticum* and is designated as the Mycotrim-GU system.

Since the Mycotrim-GU system is relatively new and few laboratories engage in cultivation of *Mycoplasma* spp. and *U. urealyticum*, there have been few reports on its performance. We therefore compared the recovery of *Mycoplasma* spp. and *U. urealyticum* from the Mycotrim-GU system and commercially available differential A7 plates in terms of rate of isolation, time of earliest detection, rate of contamination, and cost.

MATERIALS AND METHODS

Patient population. Specimens were collected from two main categories of patients: (i) obstetrical patients who were tested for a battery of sexually transmitted diseases at their first prenatal office visit and (ii) gynecological patients who were seeking medical attention for vaginitis. Sixty-two patients were in the obstetrical group and 35 were in the gynecological group. In addition, two patients were seen for a follow-up examination for postpartum endomyoparametritis, and one patient was seen for an infertility work-up. Specimens were collected from consecutive patients who were visited in a private outpatient obstetrics and gynecology clinic from April to August 1985.

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Processing of specimens. Duplicate endocervical specimens were collected with cotton-tipped swabs after the portio of the cervix had been wiped clean with a large, sterile cotton applicator. One swab was placed in the biphasic Mycotrans *Mycoplasma* transport medium (Hana Biologics) containing a cefoperazone-sodium-nystatin disk supplied with the transport system. The other swab was placed in sucrose-phosphate transport medium (Calscott 2SP; Scott Laboratories). Specimens were refrigerated (2 to 8°C) for 2 to 24 h before processing. The Mycotrim GU biphasic medium containing a cefoperazone-sodium-nystatin disk was inoculated directly as recommended by the manufacturer (Hana Mycotrim-GU package insert, December 1985). Forceps were used to manipulate the swab inside the narrow-mouthed bottle. Excess liquid from the swab was inoculated into the liquid phase above the agar by pressing the swab against the side of the flask. Swabs received in Calscott tubes were inoculated onto differential A7 plates by streaking the surfaces of the plates in two directions, perpendicular to each other. Descriptions of both the Mycotrim-GU biphasic medium (19) and A7 plates (13) have been presented elsewhere.

Incubation and interpretation. The screw-on caps of the Mycotrim-GU flasks were tightly closed, and the flasks were incubated agar-side-up in ambient air at 35°C. In the absence of colony growth after 24 h in the Mycotrim-GU system, the agar was reinoculated by tilting the flask to allow the liquid phase to cover approximately one-half of the agar surface (Hana Mycotrim-GU package insert, December 1985). A7 plates were incubated in an anerobic glove box (Forma Scientific, Div. Mallinckrodt, Inc., St. Louis, Mo.) at 35°C.

In the Mycotrim-GU system, a color change of the liquid phase of the medium from yellow to orange due to hydrolysis of arginine suggested the presence of *Mycoplasma* spp. A bright pink color due to hydrolysis of urea suggested the presence of *U. urealyticum* alone or in combination with *Mycoplasma* spp. since the intensity of the pink color could mask the lighter color which developed owing to arginine hydrolysis. Both the Mycotrim-GU system and A7 plates, however, were examined microscopically (magnification, ×10) to detect typical large colonies (resembling fried eggs) of *Mycoplasma* spp. or small, dark accretion colonies typical

TABLE 1. Isolates of *M. hominis* and *U. urealyticum* recovered by A7 medium and the Hana Mycotrim-GU system

System used	No. (% of total) of positive specimens containing:			Total no. (% of overall total) of organisms identified as:	
	M. hominis only	*U. urealyticum* only	Both organisms	*M. hominis*	*U. urealyticum*
A7 medium	3 (100)	23 (88)	11 (92)	14 (93)	34 (89)
Mycotrim-GU	2 (67)	23 (88)	9 (75)	11 (73)	32 (84)
Both	3	26	12	15	38

of *U. urealyticum*. Both systems were examined daily for 7 days. Each system was interpreted by a different technologist who did not know the results of the other test system.

Identification of isolates. *U. urealyticum* is the only member of the family *Mycoplasmataceae* known to hydrolyze urea. Consequently, isolates with colony morphology consistent with that of *U. urealyticum* which demonstrated the ability to hydrolyze urea were identified as *U. urealyticum*. Although several species of *Mycoplasma* can hydrolyze arginine, grow under anaerobic conditions, and be recovered within the time span utilized, *M. hominis* is the species most frequently isolated from the genital tracts of patients (2). Thus, organisms which exhibited colony morphology typical of *Mycoplasma* spp. were presumptively identified as *M. hominis* owing to the source of the specimen.

RESULTS

The recovery of each organism from either system is presented in Table 1. Of 100 specimens cultured, 3 were positive for *M. hominis*, 26 were positive for *U. urealyticum*, and 12 were positive for both organisms. Thus, of the 100 specimens cultured, 15 isolates of *M. hominis* were recovered either alone or in combination with *U. urealyticum*. Thirty-eight isolates of *U. urealyticum* were recovered either alone or in combination with *M. hominis*.

Among the 62 obstetrical patients, 2 (3%) specimens were positive for *M. hominis*, 16 (26%) were positive for *U. urealyticum*, and 7 (11%) were positive for both organisms. Among the 35 gynecological patients, one (3%) specimen was positive for *M. hominis*, 10 (29%) were positive for *U. urealyticum*, and 4 (11%) contained both organisms. A specimen from one patient, who was seen as a follow-up for postpartum endomyoparametritis, yielded both organisms.

Fourteen (93%) of the 15 isolates of *M. hominis* recovered either alone or in combination with *U. urealyticum* were detected on A7 medium (Table 1). Eleven (73%) of the 15 *M. hominis* isolates were detected in the Mycotrim-GU system. Thirty-four (89%) of the 38 isolates of *U. urealyticum* were recovered in A7 medium. Thirty-two (84%) of the isolates of *U. urealyticum* were recovered in the Mycotrim-GU system.

The average times of detection of *M. hominis* were 3.0 days on A7 plates and 3.4 days in the Mycotrim-GU system. The average times of detection of *U. urealyticum* were 2.5 days on A7 plates and 2.4 days in the Mycotrim-GU system.

The rates of contamination in the two systems were compared. Nine specimens inoculated into the Mycotrim-GU system were contaminated with yeast cells, and one of them was also contaminated with bacteria. Of the nine specimens contaminated with yeast cells in the Mycotrim-GU system, eight demonstrated growth of yeast cells on A7 plates. One of those was also contaminated with bacteria. One of the specimens contaminated with yeast cells was positive for *U. urealyticum* in both systems.

In addition to the clear-cut color changes described for the Mycotrim-GU system, a questionable color change which was neither orange nor pink developed in 11 cases. Colonies were not observed in the solid-phase medium, and the cultures were considered negative by the Mycotrim-GU system. Colonies of *U. urealyticum* were observed on A7 plates in three cases. Neither organism was observed in eight cases.

DISCUSSION

The Mycotrim-GU system and A7 plates were both relatively simple to use for cultivation of *M. hominis* and *U. urealyticum*. The rates of recovery and average times of detection of either organism were similar in the two sytems as were the low incidences of contamination. In the few cases in which contaminating organisms were detected, they did not appear to affect interpretation of results in either system. The effect of contamination, however, may need to be studied further, especially in specimens containing small numbers of *M. hominis* or *U. urealyticum*.

To date there has been one other published report, by Wood et al. (19), which compared the Mycotrim-GU system with conventional methodology. In spite of several technical differences between the earlier study and the present study the results were very similar. Wood et al. (19) reported an 18% positivity rate for *Mycoplasma* spp. and a 33% positivity rate for *U. urealyticum*. We observed a 15% positivity rate for *M. hominis* and a 38% positivity rate for *U. urealyticum*. *U. urealyticum* was detected by the conventional system of the earlier study in an average of 2.0 days. We detected *U. urealyticum* on A7 plates in an average of 2.5 days. *U. urealyticum* was detected in an average of 1.7 days in the Mycotrim-GU system in the earlier study, whereas we detected the organism in an average of 2.4 days in that system. Wood et al. (19) reported average detection times of *Mycoplasma* spp. of 2.3 days in the conventional system and 1.9 days in the Mycotrim-GU system. Our average detection times for *M. hominis* were 3.1 days on A7 plates and 3.4 days in the Mycotrim-GU system. Thus, our detection times for *M. hominis* were longer both on A7 plates and in the Mycotrim-GU system than in the previous report (19). Cultures which became positive on a Monday following a weekend during which the systems were not interpreted were excluded from the calculation of detection time. The reason for the discrepancy in the present work and the earlier investigation is not apparent.

The earlier report (19) suggests that it is necessary to purchase the individual components of the medium necessary for isolation of *Mycoplasma* spp. and *U. urealyticum* and prepare it from the components. The original A7 medium, however, is available from several commercial sources as mentioned earlier. The cost of A7 medium was $0.58 per plate and the cost of the 2SP transport medium was $0.84. Thus, the total cost of that system was $1.42. The cost of the Mycotrim-GU system was $8.00 per test; $6.00 for each flask and $2.00 for the transport medium. Since A7 plates performed as well as the Mycotrim-GU system, A7 plates are a cost-effective means of culturing for *M. hominis* and *U. urealyticum*. Perhaps a reduction in the sizes of the culture flask and transport vials would reduce the cost of the Mycotrim-GU system.

Some investigators suggest initial inoculation of specimens into broth medium as well as onto a solid medium to

increase the isolation rate and allow the most rapid detection of these organisms (3, 7, 8). However, the increase in isolation of either organism has been minimal in some studies (8, 19). In addition, a color change in a broth medium without colony growth on plated media may not be considered conclusive evidence of the presence of *Mycoplasma* spp. or *U. urealyticum* (7, 8). Consequently, a color change in the absence of growth on a solid medium necessitates subculturing from the broth to a plated medium and its incubation to allow colony growth. Subculturing is a tedious process and timing is of utmost importance to maintain the viability of the organisms, particularly that of *U. urealyticum*. Prolonged growth in the alkaline pH after a color change has occurred in the broth may be lethal (8). One of the most attractive features of the Mycotrim-GU system was that it combines the broth and solid media within one flask. The observation that the Mycotrim-GU system did not allow a more rapid detection time or recovery of a greater number of isolates than A7 medium alone suggests some deficiency in the broth medium of the system. The difficulties encountered with the use of broth media for recovery of these organisms have been recognized by other investigators (7, 20).

M. hominis has been implicated as an etiologic agent in intraamniotic infection (1, 4), postpartum fever (10, 11), postpartum endometritis (18), and pelvic inflammatory disease (12, 16). *U. urealyticum* is a potential causative agent of nongonococcal urethritis in men (15). An association between placental infection with *U. urealyticum* and low birth weight of neonates has also been suggested (5, 6). In addition, some investigators suggest an etiologic role for *U. urealyticum* in acute urethral syndrome in women (14).

As the roles of *M. hominis* and *U. urealyticum* as agents of disease become more clearly defined, the importance of cultivation of these organisms will increase. The appropriate antibiotic therapy for these organisms is quite different from that used for other bacterial agents associated with the same disease states that are caused by *M. hominis* and *U. urealyticum*.

In conclusion, we found that a single, commercially prepared, differential A7 plate performed as well as the biphasic Hana Mycotrim-GU system in the recovery of *M. hominis* and *U. urealyticum*. A7 medium is also more cost effective than the Mycotrium-GU system since the cost of materials is less and the time of detection of the organisms was not decreased by use of the Mycotrim-GU system.

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