Evaluation of Mycotrim-GU for Isolation of *Mycoplasma* Species and *Ureaplasma urealyticum*

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The Mycotrim-GU (Hana Biologics, Berkeley, Calif.) biphasic culture system and a conventional system were compared for their ability to detect *Ureaplasma urealyticum* and *Mycoplasma* species in 100 clinical specimens. Both systems detected 18 *Mycoplasma* spp. isolates. The average colony detection time was 1.9 days with the Mycotrim-GU and 2.3 days with the conventional system. The Mycotrim-GU agar detected all 33 *U. urealyticum* isolates recovered in the study, and the conventional agar detected 31. In addition to the *U. urealyticum* isolates recovered from the agar, there were several specimens that, although they did not grow colonies on the agar, gave an alkaline broth change. Of these specimens, two were found with the conventional system and seven were found with the Mycotrim-GU. The average detection time of *U. urealyticum* colonies was 2.0 days for the conventional agar and 1.7 days for the Mycotrim-GU. The Mycotrim-GU offers several advantages over the conventional system: it is commercially available, consists of a one-flask system which is ready to use, has a significantly longer shelf life, and is cost competitive. This study showed the Mycotrim-GU to be an effective system for detecting the genital mycoplasmas.

Mycoplasma spp. and *Ureaplasma urealyticum* are considered to be important human pathogens (11, 12). However, the lack of commercially prepared media has discouraged most laboratories from culturing for these organisms. The only media previously available have been dry or in component form. The final medium is prepared by the user, and the shelf life is short due to antibiotic deterioration.

A variety of specialized agars and broths have been developed to enhance isolation and detection of both *U. urealyticum* and *Mycoplasma* spp. (1–3, 5, 7–9, 13, 14). The detection principle in common among the broths is the occurrence of a shift to an alkaline pH, indicated by a color change when the organisms are present. Mycoplasma colonies on agar show a characteristic "fried-egg" morphology. A number of components have been added to the agar to enhance detection. Especially helpful was the introduction of differential agar medium containing manganous sulfate ($MnSO_4$) or calcium chloride ($CaCl_2$) to facilitate visualization of *U. urealyticum* colonies (6–8, 10).

A system for genital mycoplasma isolation, the Mycotrim-GU (Hana Biologics, Inc., Berkeley, Calif.), is now available in a ready-to-use form. Broth and differential agar capable of detecting both *U. urealyticum* and *Mycoplasma* spp. are provided in a single culture flask. The system is attractive to the clinical laboratory because it eliminates media making, has a 6-month shelf life, and simplifies specimen culturing by reducing the number of broth vials and agar plates to be examined.

In the present study we evaluated the efficacy of the Mycotrim-GU system in isolating mycoplasmas by comparing it with a conventional isolation system in terms of isolation rates, time required for detection, contamination rate, and relative cost.

MATERIALS AND METHODS

Specimens. A total of 100 patient specimens submitted to the Medical Microbiology Division at the University of California Irvine Medical Center were cultured in the study. These included cervical, endometrial, fallopian tube, male urethral, and semen specimens. Also included in the 100 specimens were five joint fluids from one immunodeficient patient with septic arthritis. Specimens were collected on wooden, plastic, or wire swabs with cotton or calcium alginate tips. Swabs were placed in 1 ml of transport medium (13), held at 4°C, and cultured within 24 h of collection. If necessary, transport medium was added to specimens to bring them to 1 ml, and the swabs were stirred on a Vortex apparatus before inoculation. Semen and joint fluids were inoculated directly onto the media.

Conventional media. Arginine broth, urea broth, and glucose agar were made as outlined by Velleca et al. (13), with the following modifications: the pH of the glucose agar was lowered from pH 7.8 to 7.4, and 0.2% arginine was added. PPLO broth and agar bases (Difco Laboratories, Detroit, Mich.), horse serum, and 25% fresh yeast extract (GIBCO Diagnostics, Madison, Wis.) were the basic components used. Dry-form A7 agar (GIBCO) was used by the protocol of Shepard and Lunceford (10) with the addition of putrescine to make A7B agar (8). Broths were dispensed in 1-ml amounts, and agar was dispensed in 5-ml amounts. Each patient specimen was inoculated to one each of arginine and urea broths and glucose and A7B agar plates. The broths were inoculated with 0.2 ml of the specimen, and the agar plates were inoculated with 0.1 ml of the specimen. The final dilution of the specimen in the broths was 1:5. Broths were incubated with caps tight at 35 to 37°C (in air). Glucose plates were incubated at 35 to 37°C in 5 to 10% CO_2, and A7B plates were incubated at 35 to 37°C anaerobically in a Biobag (type A; Marion Scientific, Kansas City, Mo.) with a final CO_2 concentration of 5 to 10%. Broths were examined daily for evidence of an alkaline pH change in the absence of turbidity. Plates were examined for typical colonies daily both macroscopically with light passing through the plate and microscopically at 100× magnification. Cultures were held and examined for a total of 7 days.

Mycotrim-GU. The Mycotrim-GU consists of 5 ml of broth

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and CaCl₂ differential agar in a 25-cm² tissue culture flask. The broth and agar both contain 2.1% PPLO broth, 10% horse serum, 10% fresh yeast extract, 0.5% glucose, 0.5% arginine, 0.005% phenol red, and 0.1% urea. In addition, the agar contains 1.0 mM calcium chloride, 20 mM potassium phosphate buffer (pH 6.0), and 1.5% agar. Additional details are available in the manufacturer's package insert. One antibiotic disk containing nystatin and cefoperazone was added to each flask at least 30 min before use to achieve final concentrations in the agar and the broth of 100 μg of cefoperazone per ml and 50 U of nystatin per ml. Flasks were inoculated with 0.3 ml of the specimen according to the manufacturer's instructions (which specified that flasks may be inoculated with 0.3 ml up to 1.0 ml of specimen) and incubated with caps tightened at 35 to 37°C. The final dilution of the specimen in the broth was approximately 1:16. Cultures were first observed at 18 to 24 h and daily thereafter for pH changes. An alkaline shift from the initial medium pH of 6.0 to 6.3 (yellow) to pH 7.0 (orange) was considered typical of *Mycoplasma* spp., and a pH change to 8.0 or more (red) was typical of *U. urealyticum*. The broth in the flask was subcultured at 24 h after inoculation by tilting the flask to bring the broth in contact with half of the agar surface. The agar surface was examined daily macroscopically with light passing through the agar and microscopically with 100× magnification. In this way, the presence of *Mycoplasma* spp. colonies with a fried-egg appearance or the accretion colonies of *U. urealyticum* (or both) was determined. Flasks were held for a total of 7 days.

RESULTS

The overall distribution of *Mycoplasma* spp. and *U. urealyticum* isolates is shown in Table 1. *Mycoplasma* spp., *U. urealyticum*, or both were isolated from 40% (40 of 100) of the specimens tested with the Mycotrim-GU and from 39% (39 of 100) of the specimens with the conventional system. *Mycoplasma* spp. were recovered from 18% (18 to 100) of the specimens. Both the conventional culture system and Mycotrim-GU recovered the same 18 *Mycoplasma* spp. isolates. *Mycoplasma* spp. were recovered in mixed culture with *U. urealyticum* in 11 specimens with the Mycotrim-GU and in 10 specimens with the conventional system. In two instances (2 of 18) the conventional arginine broth failed to turn alkaline, but *Mycoplasma* spp. colonies were detected on both A7B and glucose agars. An alkaline shift in pH from yellow (pH 6.0) to orange (pH 7.0) was observed with the Mycotrim-GU in the seven cases where only *Mycoplasma* spp. was isolated. The average time for *Mycoplasma* spp. colonies to appear on agar was 2.3 days for the conventional system and 1.9 days for the Mycotrim-GU.

The recovery of *U. urealyticum* by the conventional system and the Mycotrim-GU is summarized in Table 2. Among the 100 specimens, the conventional system detected 31 isolates; of these, 16 were recovered by both the A7B agar

TABLE 1. Distribution of positive specimens

System	No. of positive specimens				
	Myco-plasma spp. only	*U. urealy-ticum* only	Both	Total *Myco-plasma* spp.	Total *U. urealy-ticum*
Conventional	8	21	10	18	31
Mycotrim-GU	7	22	11	18	33

TABLE 2. Detection of *U. urealyticum*

System	No. of confirmed isolates on agar	Distribution			Avg detection time (days)	
		Agar and broth	Agar only	Broth only	Agar	Broth
Conventional	31	16	15	2	2.0	2.4
Mycotrim-GU	33	33	0	7	1.7	1.4

and urea broth and 15 were recovered by the A7B agar only. The Mycotrim-GU detected 33 isolates of *U. urealyticum* by a broth change and accretion colony formation on the agar. These 33 isolates included the same 31 detected by the conventional system plus two additional isolates detected only by the Mycotrim-GU. There was no significant difference in the number of colonies detected by either system. Unlike the conventional system, colonies were not found in the Mycotrim-GU in the absence of an alkaline broth.

Alkaline broth changes without colony formation were considered false-positive for the purposes of data analysis. In two specimens a broth change occurred in both the conventional urea broth and Mycotrim-GU broth; their respective agar subcultures were negative. These two specimens were joint fluid cultures from one patient from whom *U. urealyticum* was isolated on agar in other cultures of the same site. An alkaline broth change was seen in the absence of colony formation in five additional cases by the Mycotrim-GU alone.

The average time to detection of *U. urealyticum* in broth and on agar for each system is summarized in Table 2. The time for colonies to appear on agar was slightly shorter for Mycotrim-GU (1.7 versus 2.0 days), whereas the time for alkaline color change to appear in the broth was shorter by approximately 1 day (1.4 versus 2.4 days).

Overgrowth by bacteria or yeast occurred at a rate of 4% (4 of 100) with the conventional system. There were three specimens with yeast and one with bacterial overgrowth. All four specimens grew *U. urealyticum* or *Mycoplasma* spp. or both, and the contamination occurred on the agar surface and did not interfere with colony detection. With the Mycotrim-GU, overgrowth occurred in 7% (7 to 100) of the specimens. Four instances of bacterial overgrowth of broth or agar occurred between day 3 and 7, when no mycoplasma was isolated. *U. urealyticum* alone or with *Mycoplasma* spp. was isolated in three other cases; two were contaminated on day 3 with yeast cells or bacteria, and one other was contaminated with yeast cells on day 1. Overgrowth did not interfere with mycoplasma detection.

The cost of each Mycotrim-GU flask to our laboratory was $5.40. The cost of the conventional media can be approximated for our laboratory at $5.50 per specimen, including labor and raw materials to prepare the media.

DISCUSSION

The Mycotrim-GU was found to be equally effective in recovering *Mycoplasma* spp. and better at detecting *U. urealyticum* than the conventional isolation system employed in this investigation. Shepard A7 and A7B agars are commonly used in laboratories that culture for the genital mycoplasmas. In studies by Fiacco et al. (1) and Yajko et al. (14) comparing A7 or A7B agar, respectively, with other conventional media formulations, A7 and A7B were found to be the media of choice. It was most effective for the recovery of *U. urealyticum* and supported the growth of *Mycoplasma* spp. as well (1, 14). Differential agar media A7 and A7B for

culturing *U. urealyticum* contain manganous sulfate (0.015%) for detecting urease activity (8, 10). Robertson and Chen (6) showed that manganese could inhibit some strains of *Ureaplasma*, and Shepard (7) suggested that calcium chloride could be used as a substitute for manganous sulfate in the formation of accretion colonies. The Mycotrim-GU agar is similar to A7 agar in basic composition, differs in the supplements included, and uses calcium chloride as the differential ingredient.

In comparing the Mycotrim-GU with the conventional A7B agar we found that the accretion colonies formed with calcium were smaller and appeared somewhat lacier than the heavy and darker precipitate of manganese accretion colonies. The colony numbers of *U. urealyticum* on Mycotrim-GU agar did not differ significantly from those on the A7B plate, but when colony numbers were low they were often limited to one area of the agar surface. Since the agar surface area is larger than the area of the conventional A7B petri plate (50 by 9 mm), more time and great care is required in examining the Mycotrim-GU agar microscopically. The Mycotrim-GU agar does not contain the polyamine putrescine, which has been reported by Shepard and Combs (8) and Razin et al. (4) to enhance both colony size and number of colonies of *U. urealyticum*. Whether smaller *U. urealyticum* colonies on the Mycotrim-GU agar are due to use of $CaCl_2$, lack of putrescine, or a combination of these and other factors is a question which requires further study.

The observation was made that the tilt subculture of the Mycotrim-GU broth onto the agar surface did not increase the number of *U. urealyticum* colonies over that seen in the original inoculum area, especially compared with the enhancement seen with the *Mycoplasma* spp. subculture. A possible explanation for this lies partially in the sensitivity of *U. urealyticum* to an alkaline pH. Often, when the tilt subculture was performed at 24 h after inoculation, the Mycotrim-GU broth was already pH 7.5 or higher, and many of the organisms responsible for the positive broth probably were nonviable. The sensitivity of *U. urealyticum* to an alkaline pH may also be responsible for the seven instances in this study where the Mycotrim-GU broth, including two conventional urea broths, had alkaline color changes typical of *U. urealyticum* within the first day and the organism could not be detected on agar. Kundsin et al. (2), Leland et al. (3), and Shepard (7) all warn that failure to subculture broths early when pH change is minimal may lead to false-negative subcultures. These seven cases could be subculture failures, but the possibility of a false-positive broth change must also be considered. Since none of the seven cultures was obviously contaminated, it is likely that urea hydrolysis by *U. urealyticum* led to the typical alkaline pH change, although this cannot be proven without a positive subculture. Two of the seven apparent false-positive broth cultures had positive conventional urea broths and were repeat cultures on the joint fluid of one patient who was already documented to have *U. urealyticum* by previous cultures. Although the manufacturer recommends subculture of the Mycotrim-GU at 24 h after inoculation, it was our experience that for many cultures 24 h is not early enough. Therefore, we recommend more frequent observation of the flasks, at least twice during the first day after inoculation, so that the flasks may be subcultured when minimal pH changes are seen and the organisms are potentially still viable.

In this study the conventional urea broth used during the study was less sensitive than the Mycotrim-GU broth. It was positive in only 16 of the 33 confirmed *U. urealyticum* isolates. This occurred in spite of the fact that the original dilution of the specimen in the conventional broth (1:5) was less than that in the Mycotrim-GU (1:16). The explanation for this insensitivity could possibly lie in the horse serum used, since, as Yajko et al. point out, certain lots of horse serum do not support *U. urealyticum* growth as well as others (14). However, two different lots of horse serum were used; both supported the growth of quality control strains, and both were also used to supplement the A7B agar used, which showed no problem isolating the organism. The urea broth also contained polymyxin B, which, as discussed by Fiacco et al. (1), may be inhibitory to some strains of *Ureaplasma*.

The Mycotrim-GU overcomes many of the practical problems associated with performing mycoplasma isolation. As a one-flask system with a 6-month shelf life, it eliminates the need to order a large variety of components and to make fresh medium every 2 to 4 weeks. For the smaller laboratory, this system makes mycoplasma isolation practical for the first time, and for larger laboratories with a higher volume of specimens the cost is roughly equivalent to that of a component system.

The Mycotrim-GU performed well in this study, isolating both *U. urealyticum* and *Mycoplasma* spp. as well as or better than the conventional system. It had a slightly higher contamination rate, but this did not interfere with the detection of positive cultures and most often occurred after several days, past the time when the majority of cultures had become positive. The flasks are relatively easy to handle, but care must be taken not to splash the broth onto the agar surface except to subculture or the delicate colonies may be washed off the agar surface. Care should also be taken to frequently examine the flasks macroscopically for broth color changes to subculture at the optimal time. When performing the microscopic examination of the flask, the entire surface of the agar should be thoroughly searched. With these precautions, we recommend the Mycotrim-GU as an effective, convenient culture system for the genital mycoplasmas.

LITERATURE CITED

1. **Fiacco, V., M. J. Miller, E. Carney, and W. J. Martin.** 1984. Comparison of media for isolation of *Ureaplasma urealyticum* and genital *Mycoplasma* species. J. Clin. Microbiol. **20:**862–865.
2. **Kundsin, R. B., A. Parreno, and S. Poulin.** 1978. Significance of appropriate techniques and media for isolation and identification of *Ureaplasma urealyticum* from clinical specimens. J. Clin. Microbiol. **8:**445–453.
3. **Leland, D. S., M. A. Lapworth, R. B. Jones, and M. L. French.** 1982. Comparative evaluation of media for isolation of *Ureaplasma urealyticum* and genital *Mycoplasma* species. J. Clin. Microbiol. **16:**709–714.
4. **Razin, S., G. K. Masover, M. Palant, and L. Hayflick.** 1977. Morphology of *Ureaplasma urealyticum* (T-mycoplasma) organisms and colonies. J. Bacteriol. **130:**464–471.
5. **Robertson, J. A.** 1978. Bromthymol blue broth: improved medium for detection of *Ureaplasma urealyticum* (T-strain mycoplasma). J. Clin. Microbiol. **7:**127–132.
6. **Robertson, J. A., and M. H. Chen.** 1984. Effects of manganese on the growth and morphology of *Ureaplasma urealyticum*. J. Clin. Microbiol. **19:**857–864.
7. **Shepard, M. C.** 1983. Culture media for ureaplasmas, p.137–146. *In* S. Razin and J.G. Tully (ed.), Methods in mycoplasmatology, vol. 1. Academic Press, Inc., New York.
8. **Shepard, M. C., and R. S. Combs.** 1979. Enhancement of

Ureaplasma urealyticum growth on a differential agar medium (A7B) by a polyamine, putrescine. J. Clin. Microbiol. **10:** 931–933.

9. **Shepard, M. C., and C. D. Lunceford.** 1970. Urease color test medium (U-9) for the detection and identification of "T" mycoplasmas in clinical material. Appl. Microbiol. **20:**539–543.

10. **Shepard, M. C., and C. D. Lunceford.** 1976. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T-mycoplasmas) in primary cultures of clinical material. J. Clin. Microbiol. **3:**613–625.

11. **Taylor-Robinson, D., and W. M. McCormack.** 1980. The genital

mycoplasmas. I. N. Engl. J. Med. **302:**1003–1010.

12. **Taylor-Robinson, D., and W. M. McCormack.** 1980. The genital mycoplasmas. II. N. Engl. J. Med. **302:**1063–1067.

13. **Velleca, W. M., B. R. Bird, and F. T. Forrester.** 1975. Laboratory diagnosis of mycoplasma infections, p. A1-A23. *In* Course 8226-C. U.S. Department of Health Education and Welfare, Public Health Service, Centers for Disease Control, Atlanta.

14. **Yajko, D. M., E. Balston, D. Wood, R. L. Sweet, and W. K. Hadley.** 1984. Evaluation of PPLO, A7B, E, and NYC agar media for isolation of *Ureaplasma urealyticum* and *Mycoplasma* species from the genital tract. J. Clin. Microbiol. **19:**73–76.