Comparison of Commercially Available Media for Detection and Isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis*

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The Mycotrim Triphasic flask system (Irvine Scientific, Irvine, Calif.) was compared with a system composed of Mycotrim GU broth (Irvine Scientific) and A7 or A8 agar (Remel, Lenexa, Kans.) for the ability to detect *Ureaplasma urealyticum* and *Mycoplasma hominis* from 129 genital specimens. Of the 64 specimens positive for *U. urealyticum*, 25, 96, and 100% were detected on Mycotrim Triphasic agar and A7 and A8 agars, respectively. All 18 specimens that grew *M. hominis* were detected by A7 and A8 agars, and 94% grew on Mycotrim Triphasic agar. Mycotrim GU broth detected all of the positive specimens, and Mycotrim Triphasic broth detected all but one. Mycotrim GU broth inoculated simultaneously with either A7 or A8 agar was found to be more sensitive and cost-effective than the Mycotrim Triphasic system.

The genital mycoplasmas *Ureaplasma urealyticum* and *Mycoplasma hominis* have been implicated in a broad range of infections, including urethritis, urinary tract infection, chorioamnionitis, spontaneous abortion, pelvic inflammatory disease, and pneumonia in newborns. There is also evidence that they may play a role in infertility (6, 10). The laboratory identification of these organisms has presented problems, mainly because of their fastidious nature, requiring both special transport and culture media for growth (3). Various formulations of agar and broth have been investigated, but most of these media are difficult to prepare and are not commercially available, thus making them impractical for most clinical laboratories. In addition, several of the recommended media are inhibitory to either *U. urealyticum* or *M. hominis* (8, 12). While many investigators recommend simultaneous broth and agar inoculation (2, 4, 5), only one commercially prepared system combining both, the Mycotrim Triphasic flask system (Irvine Scientific, Irvine, Calif.), is currently available. This system consists of modified A8 agar with an arginine-urea-based broth. In a previous study from our laboratory, this system was found to be more sensitive for the isolation of *U. urealyticum* than A7 agar alone (11). However, in recent years we have noticed that when used on a daily basis this triphasic system has appeared to decrease in sensitivity and that broth changes occur quite often with colonies not detected on the agar surface. Therefore, a comparison of the Mycotrim Triphasic flask system with other commercially available media, A7 and A8 agars (Remel, Lenexa, Kans.) and Mycotrim GU broth (Irvine Scientific), was conducted in order to reassess this system as well as to establish a sensitive and cost-effective scheme for the isolation and identification of genital mycoplasmas.

Specimens in this study were obtained from both inpatients and outpatients seen at the University of California Irvine Medical Center. There were a total of 129 specimens, including 122 cervical specimens, 5 amniotic fluid specimens, 1 urethral specimen, and 1 pelvic fluid specimen. Following collection, cervical specimens were placed in 1 ml of urogenital transport medium (pH 6.0) containing PPLO broth, yeast extract (10%), horse serum (20%), penicillin G (500 U/ml), and amphotericin B (5 µg/ml) and stored at 4°C. All specimens were cultured within 24 h of collection. Prior to inoculation, all media were allowed to come to room temperature for 30 min. An antibiotic disk (Irvine Scientific) containing cephaloridine (100 µg/ml) and nystatin (50 U/ml) was placed in the Mycotrim Triphasic flask and allowed to diffuse into the broth. After vortexing for 10 s, 0.1 ml of each specimen was inoculated to each of the four media being evaluated (A7 and A8 agars, the Mycotrim Triphasic flask system, and Mycotrim GU broth) and incubated aerobically at 35°C. The A7 and A8 agar plates were incubated in a moist chamber to avoid drying of the agar. Cultures were examined daily for 7 days. Growth on A7 and A8 agars was quantitated as follows: 1+, 1 to 10 colonies; 2+, 11 to 100 colonies; and 3+, >100 colonies. Time spent scanning the agar surface was recorded and any bacterial contamination was noted.

The Mycotrim Triphasic flask system consists of modified A8 agar containing phenol red as an indicator and Mycotrim Triphasic broth, which contains arginine, urea, and phenol red. Once inoculated, flasks were incubated with the agar side up. According to the manufacturer's instructions, growth should be first indicated by a color change of the broth from yellow to orange or red (corresponding to a pH change from 6.0 to ≥7.0), and broth changes should be confirmed by observation of the colonies on the agar surface. *M. hominis* colonies had a typical "fried egg" appearance. However, *U. urealyticum* accretion colonies were difficult to identify, often appearing small and lacking in precipitate formation. Negative cultures at 24 h were reincubated by inverting the flask and allowing the broth to contact the agar. Gross turbidity of the broth could be seen with bacterial or fungal contamination.

The Mycotrim GU broth used in this evaluation was the same as that used in the Triphasic flask system except that it contained penicillin G (500 U/ml) and amphotericin B (5 µg/ml). This broth, when used alone outside the Triphasic flask system, will be referred to as GU broth to avoid confusion with the broth in the Mycotrim Triphasic flask system. GU broth aliquots of 3 ml were stored at −70°C. As

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with the Mycotrim Triphasic flask system, a color change from yellow to orange or red in the absence of obvious bacterial growth was considered a positive result.

A7 and A8 agars (Remel) differ in their formulations as follows: A7 contains penicillin (1,000 U/ml), amphotericin (2.5 μg/ml), and manganous sulfate as a precipitating agent, while A8 contains colistin (7.5 μg/ml), ampicillin (1.0 μg/ml), amphotericin (2.5 μg/ml), putrescine dihydrochloride (10 mM), and calcium chloride (1 mM) as a precipitating agent. When viewed under low-power (×10) magnification, *M. hominis* colonies were lacy, with a characteristic “fried egg” appearance on both A7 and A8 agars. *U. urealyticum* accretion colonies differed in morphology on the two agar types. On A7 agar, *U. urealyticum* colonies were surrounded by a heavy dark brown precipitate, and on A8 agar, the surrounding precipitate was finer and gold to light brown. Although A8 agar contains putrescine dihydrochloride, which is a polyanine that has been reported to enhance *U. urealyticum* growth by both increasing the numbers of colonies and intensifying ammonia diffusion in the agar (9), we found the *U. urealyticum* colonies to be slightly easier to identify on A7 agar.

Of the 129 specimens included in this evaluation, 54% (70 of 129) were culture positive (67 cervical and 3 amniotic fluid specimens). As can be seen in Table 1, of the positive specimens, 91% (64 of 70) grew *M. hominis*, *U. urealyticum* and *M. hominis* were found together in 17% (12 of 70) of the positive specimens. Of the 64 specimens positive for *U. urealyticum*, 25% (16 of 64), 98% (63 of 64), and 100% (64 of 64) were detected on Mycotrim Triphasic agar and A7 and A8 agars, respectively. All 64 of these cultures gave a characteristic color change in GU broth, and all but one were detected in the broth of the Mycotrim Triphasic flask system. All 18 specimens that grew *M. hominis* were detected by A7 and A8 agars, and 94% (17 of 18) grew in the agar in the Mycotrim Triphasic flask system. All 18 *M. hominis*-positive cultures were accompanied by a corresponding color change in both the Mycotrim Triphasic broth and the GU broth. All specimens with a color change in either the broth of the Mycotrim Triphasic system or the GU broth alone were confirmed to contain either *U. urealyticum* or *M. hominis* by growth of the organism on one or more of the solid media. One specimen with a GU broth change did not exhibit colonies on A7 agar; however, this specimen did exhibit *U. urealyticum* colonies on A8 agar.

*U. urealyticum* and *M. hominis* colony counts on A7 and A8 agars are shown in Fig. 1. There was no statistical difference by the chi-square test between the agars as to the number of organisms seen in each positive specimen (P > 0.1). In general, cultures growing *M. hominis* had high colony counts, whereas lower colony counts predominated in the cultures positive for *U. urealyticum*. The sizes of *U. urealyticum* and *M. hominis* on the two agar types were comparable. The average time to detection of colonies was 2 days with all media. Technologist time examining agar for colonies averaged 3 min for the Mycotrim Triphasic flask system and 0.5 min for A7 and A8 agars. Rates of bacterial contamination were 11% in the Mycotrim Triphasic flask system, 2% in GU broth, 9% on A7 agar, and 4% on A8 agar.

A cost analysis of the Mycotrim Triphasic flask system and A7 or A8 agar used in combination with GU broth was done. The latter system, using an agar medium with a vial of GU broth, was the most cost effective in this evaluation, with its cost being less than half that of the Mycotrim Triphasic flask system. For the Mycotrim Triphasic flask system, the cost of the media was $9.90 and the cost of technical time (3 min) for medium examination was $1.00, on the basis of a salary of $20.00/h; for GU broth with an A7 or A8 plate, the cost of the media was $3.95 and the cost of technical time was $0.17.

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**TABLE 1. Overall distribution of positive cultures**

<table>
<thead>
<tr>
<th>Organism (total)</th>
<th>No. of isolates detected by:</th>
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<tbody>
<tr>
<td></td>
<td>Agar</td>
</tr>
<tr>
<td></td>
<td>Mycotrim&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>M. hominis</em> (18)</td>
<td>17</td>
</tr>
<tr>
<td><em>U. urealyticum</em> (64)</td>
<td>16</td>
</tr>
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</table>

<sup>a</sup> Mycotrim Triphasic flask system.

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**FIG. 1. Distribution of colony count categories for specimens positive for either *U. urealyticum* or *M. hominis*.** The actual colony counts for the categories are as follows: 1+, 1 to 10 colonies; 2+, 11 to 100 colonies; 3+, >100 colonies.
Our laboratory has used the Mycotrim Triphasic flask system for several years, and while it was more sensitive than A7 and A7B agars in previous studies (7, 11), the results of this evaluation confirmed our recent experience with this system. While the manufacturer of this product has changed since our original report (11), according to the present manufacturer of this product the agar formulation has not changed. However, we have noticed a definite decrease in the detection of colonies on the agar surface, especially with *U. urealyticum*, while the broth has remained quite sensitive. The instructions for the inoculation of the Mycotrim Triphasic flask system have recently changed; rather than the previous recommendation of 0.3 to 1 ml, a 1-ml inoculum for specimens in transport media is now advised. In the event that the inoculum volume (0.1 ml) used could have been a factor for our low rate of detection of *U. urealyticum* from the Mycotrim Triphasic agar, we investigated this further. Seven specimens that had grown >100 *U. urealyticum* colonies when originally cultured on A8 medium were chosen. These specimens, which were stored at −70°C in original transport media, were thawed, diluted in transport media, and used to inoculate three Mycotrim Triphasic flasks and three A8 plates. Media were inoculated with 0.1, 0.3, and 1 ml of each specimen and observed for *U. urealyticum* colonies for 7 days. All specimens grew *U. urealyticum* on all A8 plates regardless of inoculum volume, and all the Mycotrim Triphasic flasks exhibited a broth color change. Mycotrim Triphasic flasks inoculated with 0.1, 0.3, and 1 ml of specimen exhibited colonies on the agar surface with 28% (2 of 7), 28% (2 of 7), and 14% (1 of 7) of the specimens, respectively. In these flasks with visible colonies, only 1 to 2 colonies were seen, in contrast to results with the A8 agar, in which the majority of plates had >10 colonies of *U. urealyticum*. Therefore, these results showing the Mycotrim Triphasic agar to be less sensitive than A8 agar were similar to those we obtained with the fresh cultures that had not been frozen. Since the mycoplasmas in general are very fastidious organisms, a number of factors, including the quality of the yeast extract, horse sera, and other components present in the Mycotrim Triphasic flask system, may contribute to an overall decrease in isolation of *U. urealyticum*. A recent study by Cavicchini et al. (1), in which A7 agar was found to be more sensitive than the Mycotrim Triphasic flask system for the isolation of *U. urealyticum*, corroborates our present findings. Either A7 or A8 agar inoculated in parallel with GU broth was a more sensitive and cost-effective agar/broth system than the Mycotrim Triphasic flask system. While *U. urealyticum* colonies were somewhat easier to read on A7 agar, A8 agar was slightly more sensitive for the overall detection of colonies. On the basis of our findings that all specimens positive on agar were also positive in GU broth, we have adopted a system in our laboratory in which A7 or A8 plates are examined only when the GU broth is positive and before a negative culture is finalized. This approach allows for a cost-effective, rapid, and sensitive screening method for the isolation and identification of genital mycoplasmas.

REFERENCES