# Evaluation of the Mycoplasma Duo kit for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* from urogenital and placental specimens

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**ABSTRACT**

This study compares the Mycoplasma Duo kit for the detection of genital mycoplasmas with conventional culture using A7 differential agar for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in clinical samples. Detection of the mycoplasmas is based on the specific metabolic properties of each organism to hydrolyse either arginine or urea. The Mycoplasma Duo test showed a significantly higher detection rate than did culture, although many of the culture-negative results may have been due to the presence of bacterial overgrowth.

KEY WORDS: Culture.
Mycoplasma hominis.
Ureaplasma urealyticum.

## Introduction

*Mycoplasma hominis* and *Ureaplasma urealyticum* are commensal organisms that have been associated with post-partum fever and endometritis, post-operative gynaecological infection, pelvic inflammatory disease (PID), neonatal infection, non-gonococcal urethritis (NGU), infertility, early spontaneous abortion and septic arthritis.[1-4]

Traditionally, detection and identification of *M. hominis* and *U. urealyticum* have relied on culture techniques performed in specialist or reference laboratories. Culture is labour-intensive and the organisms have fastidious growth requirements and are easily overgrown by other commensal microorganisms.

To improve the detection and identification of *M. hominis* and *U. urealyticum*, other techniques such as polymerase chain reaction (PCR) methods have been used.[5-7] However, no commercial or standardised PCR assays are available, and the use of PCR may not be cost-effective and has been limited to specialist laboratories.

As an alternative, culture-based assays have been developed that are based on a selective medium and enzymatic colour changes, and these include some commercial kits.[3,7-10] However, these assays have not been used widely or have limited availability.

More recently, the Mycoplasma Duo assay (Bio-Rad, Marnes la Coquette, France) has become available, but, as yet, has not been evaluated rigorously.[11] This assay incorporates specific substrates that are altered enzymatically by the presence of genital mycoplasmas, resulting in a characteristic colour change within 24-48 hours.

The aim of this study is to compare the performance of the Mycoplasma Duo kit with culture for the detection of genital mycoplasmas in clinical specimens.

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## Materials and methods

One hundred and ninety-one consecutive clinical specimens received in the authors' laboratory for *M. hominis* and *U. urealyticum* testing were included in the study. All were tested by culture and the Mycoplasma Duo test. The specimens included 164 swabs (genital and placental sites) received in Amies transport medium, 18 placental tissue specimens and nine first-catch urines (FCU; 0.2 mL). Each sample was inoculated in the Mycoplasma Duo suspension medium, and all testing was performed from this inoculated medium, in order to achieve test sample consistency.

The inoculated suspension medium was seeded in the Mycoplasma Duo microplate (Bio-Rad 62740), following the manufacturer's instructions, and incubated at 37°C without $CO_2$ for up to 48 h. The microplate consists of six microwells containing dehydrated substrates used for identification, growth factors for mycoplasmas, and agents for inhibiting the growth of the concomitant polymorphic flora.

*M. hominis* hydrolysed arginine present in the test kit, while *U. urealyticum* hydrolysed the urea, with the subsequent release of ammonia and alteration to the pH of the medium. The reaction was visualised by a change in colour of the phenol red indicator from yellow to red (Fig. 1).

Differential quantitation of the mycoplasmal species was accomplished by titration of the inoculum, with detection of $\geq 10^4$ colour changing units (CCU)/mL considered to be evidence of disease.[12] After initial characterisation, the manufacturer's instructions were followed.

Quantitative culture was performed by inoculating 20 µL

Mycoplasma Duo suspension medium on A7 differential agar (penicillin G: one million units/L, amphotericin B: 2.5 g/L),[13] thus setting the minimum detection level at $10^4$ colony-forming units (cfu)/mL.[14,15] Plates were incubated at 37°C in 5% $CO_2$ for two to 10 days. Both *M. hominis* and *U. urealyticum* grow well and easily on A7 differential agar and are differentiated from one another by colonial morphology and by direct detection of urease formation.

*M. hominis* grow as 50-300 µm characteristic 'fried egg' colonies (Fig. 2), which were confirmed using Dienes' stain (Fig. 3).[16] *U. urealyticum* grow as 15-50 µm dark brown colonies, due to the accumulation of manganese oxide in the colony (Fig. 2). As growth characteristics are unique, further testing was not undertaken.[17] Culture on A7 differential agar was controlled using *M. hominis* ATCC 23114 and *U. urealyticum* ATCC 27618 (Remel, USA).

Specimens showing discordant results between the Mycoplasma Duo test and culture were tested by PCR assays for *M. hominis*[1] and *U. urealyticum*,[2] as described previously. For these assays, DNA was extracted from the Mycoplasma Duo suspension broth (200 µL) using QIAamp DNA minikits (Qiagen, Hilden, Germany). Diagnostic tests were compared using McNemar's test.

## Results

Of the 191 specimens studied, 91 (48%) were negative both by the Mycoplasma Duo test and by A7 culture. The remaining 100 specimens (52%) were positive by Mycoplasma Duo (15 were positive for both organisms), while 68 (36%) were culture-positive (seven were positive for both organisms). The Mycoplasma Duo assay detected all culture-positive specimens. The results for both testing methods are shown in Tables 1 and 2.

Overall, the Mycoplasma Duo test showed a significantly higher detection rate than did culture for both organisms ($P=0.005$ and $P<0.0001$ for *M. hominis* and *U. urealyticum*, respectively).

Of the 40 discordant results obtained, 32 of the bacterial cultures were reported as negative due to bacterial overgrowth, despite the fact that the inoculum (taken from the suspension medium) contained ampicillin.

Testing of the 40 samples with discordant results by a PCR method was positive in six out of eight cases in which *M. hominis* was detected by the Mycoplasma Duo kit, and in 21 of the 32 cases in which *U. urealyticum* was detected by the Mycoplasma Duo kit. No PCR inhibitors were found in any of these specimens using a unique PCR reaction targeting an artificial DNA fragment (unpublished data).

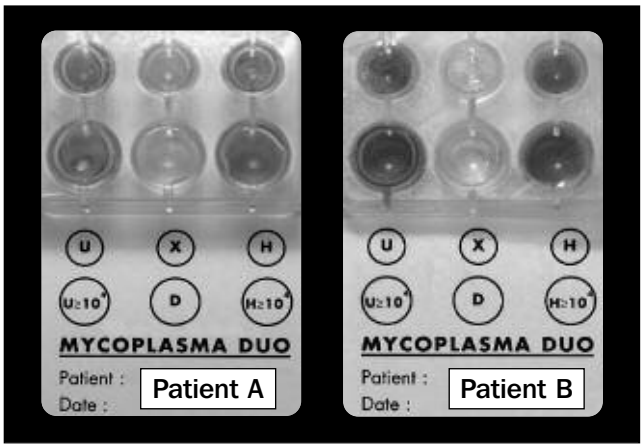


**Fig. 1.** The Mycoplasma Duo assay depicting a negative result for *M. hominis* and *U. urealyticum* in 'Patient A' and a high titre of ≥$10^4$ CCU/mL for detection of *M. hominis* and *U. urealyticum* in 'Patient B'. Cloudiness of the medium can indicate growth of bacteria other than mycoplasmas. Well X provides selective mycoplasma enrichment of a standardised inoculum for subsequent antibiotic susceptibility testing. Well D is used for dilution of the sample.

## Discussion

The findings of this study indicate that the sensitivity of the Mycoplasma Duo kit exceeds that of culture on A7 differential agar. Furthermore, PCR results suggest that most of the Mycoplasma Duo-positive, culture-negative results were true positives, and the authors speculate that the main reason for the discrepancies between the results was bacterial overgrowth on the A7 differential agar, rendering it difficult to detect the mycoplasmas.

The inclusion of A8 testing medium in the study may have addressed this problem as it contains antibiotics that suppress members of the Enterobacteriaceae family, which were the main source of bacterial overgrowth in this study. Broitman *et al*. compared A7 and A8 media when validating Mycotrim GU broth (Irvine Scientific, USA), a modification of A8 agar. They found the detection of *U. urealyticum* to be 98% on A7 agar and 100% on A8, with bacterial overgrowth contamination occurring in 9% and 4% of cases, respectively.[3]

Removing cases where bacterial overgrowth of other microorganisms limited the effectiveness of culture would result in a test group of 154. Of these, 73 (47%) were Mycoplasma Duo-positive and 68 (44%) were culture-positive. Three *M. hominis* and five *U. urealyticum* were confirmed by PCR in two out of three and three out of five cases, respectively.

**Table 1.** Comparison of *M. hominis* detection by the Mycoplasma Duo test and by culture.

| | Mycoplasma Duo detection | |
|---|---|---|
| | **Positive** | **Negative** |
| *M. hominis* culture | | |
| Positive | 7 | 0 |
| Negative | 8 | 176 |
| | 15 | 176 |

**Table 2.** Comparison of *U. urealyticum* detection by the Mycoplasma Duo test and by culture.

| | Mycoplasma Duo detection | |
|---|---|---|
| | **Positive** | **Negative** |
| *U. urealyticum* culture | | |
| Positive | 68 | 0 |
| Negative | 32 | 91 |
| | 100 | 91 |

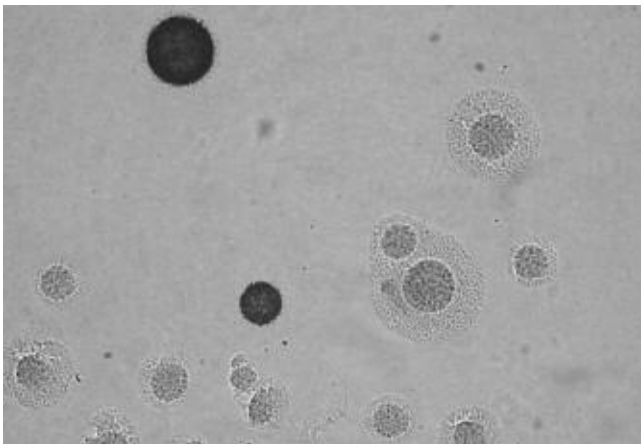

**Fig. 2.** Culture of *M. hominis* and *U. urealyticum* on A7 differential agar. *M. hominis* colonies have a dense central core with a radiating periphery of growth, giving a 'fried egg' appearance. Colonies of *U. urealyticum* are brown, show a 'mulberry' appearance and colour the agar pink due to hydrolysis of urea.

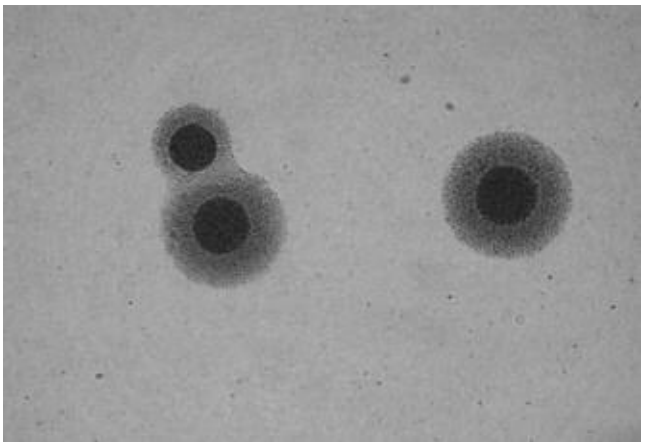

**Fig. 3.** Dienes' stain of A7 differential medium showing *M. hominis* colonies of characteristic appearance.

The findings presented here are similar to those from the evaluation of the Mycoplasma IST 2 assay (bioMérieux, France), in which positive results exceeded those from culture and PCR for the detection of *M. hominis*. The Mycoplasma IST 2 kit utilises the same principle as the Mycoplasma Duo kit and includes additional antibiotic sensitivity testing.[7]

According to the manufacturer's instructions, the Mycoplasma Duo kit is designed for identification and differential titration of genital mycoplasmas (*M. hominis* and *U. urealyticum*); however, *U. parvum* will also be detected by this method as it was originally a biovar of *U. urealyticum* before being reclassified as a distinct species (based on phylogenetic analysis).[18] Thus, the information supplied could be updated to state the ability to detect *Ureaplasma* species (i.e., *U. parvum* [biovar 1] and *U. urealyticum* [biovar 2]).

A limitation of the present study was the fact that positive results were not followed up to determine the clinical relevance of the detection of these organisms, especially as they are known to be also present in commensal flora. Furthermore, the manufacturer's claim that dilution of the specimen permits detection of organisms of clinical relevance has yet to be proved.

The Mycoplasma Duo kit is simple to use and gives results within an acceptable time. It is cost-effective, easy to perform in any routine laboratory, and requires limited expertise. The Mycoplasma Duo assay is a useful alternative to culture for the detection of urogenital and placental mycoplasmas, and can be introduced readily in most diagnostic laboratories. □

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