Comparison of two laboratory techniques for detecting genital mycoplasmas in clinical specimens
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Introduction
Commercial kits designed for isolation and identification of Mycoplasma hominis and Ureaplasma urealyticum have now become available for routine diagnostic use (Taylor-Robinson 1996, Waites et al. 1996). High rates of genital mycoplasma infections have been determined both by culture and by a commercially available kit, the Mycoplasma IST kit (bioMerieux), for the detection of M. hominis and U. urealyticum (Clegg et al. 1997). The aim of this prospective study was to compare the current culture system with a commercially available kit, the Mycoplasma IST kit (bioMerieux), and to develop a diagnostic method for the detection of the genital mycoplasmas; in particular M. hominis and U. urealyticum from genital tract specimens of both male and female patients attending a District Hospital General-Genito-Urinary Medicine Clinic.

Material and Methods
Random clinical specimens of either method used were collected from 100 patients in each system. Swabs were placed into transport media (3ml of 10B broth) and (R1, medium), for culture and the Mycoplasma IST kit, respectively. For conventional culture Shepard's growth medium (Shepard 1983; Waites & Cassell 1996) were used for recovery of M. hominis and U. urealyticum. Both broth (10B) and agar (A8) were prepared with modification. The mycoplasma broth containing one swab was vortexed for 30s, serial 10-fold dilutions of inoculated broth were made in 10B broth with urea for culture of U. urealyticum and with arginine for M. hominis as well. The urea and arginine broths were incubated aerobically at 37°C. Broths showing a pH shift (pH change indicated by phenol red; yellow to amber for ureaplasma 10B and amber to red for arginine 10B broth) were subcultured onto A8 agar plates which were then incubated anaerobically in a candle jar at 37°C for up to 7 days. The agar plates were examined with stereomicroscope every 24-72 hrs for the characteristic colonies of ureaplasma which appear in the presence of calcium chloride indicator as tiny brownish granular clumps (15 to 60 μm diameter) and for M. hominis colonies (30 to 300 μm diameter) and have a characteristic "fried-egg" appearance. The number of presumptive positives for mycoplasmas or ureaplasmas isolated were recorded. For commercial system, the swab in R1 transport liquid medium was processed according to the manufacturer's instructions. The inoculated transport medium was also used to reconstitute a lyophilised urea-arginine broth, R2 (provided with the Mycoplasma IST kit) which was then used to inoculate a cupule strip; 16 cupules: 1 control, 2 identification, 2 enumeration (M. hominis and U. urealyticum) and 11 for testing 6 major antibiotics including doxycycline, erythromycin and tetracycline). The strip and broth were both incubated for 48 hrs at 37°C and observed for colour changes.

Discussion
The number of genital mycoplasmas detected from each method were similar. Both methods were suitable for clinical laboratory use and they were easy to perform. Mycoplasma IST could be used for identification as well as providing information on antimicrobial susceptibilities (Duggan 1994, Vazquez et al. 1995 and Clegg et al. 1997). The test is however expensive for routine use but did not require prior media preparation. The reagents and the test kit have a shelf-life of up to 12 months. Results of the Mycoplasma kit were within 24-48 hrs. The conventional broth-agar culture method, on the other hand, requires prior media preparation. The media can only be stored for up to 3 months at 4°C. Although reading the results in this method require an additional incubation time due to inoculation of serially diluted specimens followed by subculturing of broth-to-agar, we found the conventional to be the most sensitive method for the isolation of both genital mycoplasma and ureaplasmas. This in agreement with other workers (Taylor-Robinson 1996, Waites and Cassell 1996).

Results
100 patients specimens screened for M. hominis, 17 specimens yielded positive results by conventional culture and 26 specimens by Mycoplasma IST. U. urealyticum was isolated on A8 agar plates from 58 by culture method and detected in 55 specimens by Mycoplasma IST. Isolates were identified as M. hominis or U. urealyticum by growth inhibition test (disc method) as described by Clyde (1983).

References
Duggan G E, (1994). MSc Thesis, Manchester Metropolitan University, UK

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