Mycoplasma genitalium was first isolated from two men with non-gonococcal urethritis (NGU) in 1981. Cultivation of the organism has proved difficult even in the complex media typically used for mycoplasma culture. Axenic primary culture can take up to three months to produce a positive result and is not sensitive diagnostically. The polymerase chain reaction (PCR) has been applied to the detection of M genitalium and has been used to show the presence of the organism in men with NGU, a disease for which an aetiological agent is frequently not identified. Although a proportion of healthy men carry the organism, M genitalium carriage is significantly associated with NGU, independent of the 16S rRNA gene sequence is relatively stable and therefore contains few, if any, polymorphisms in the M genitalium population. In the absence of non-amplification tests it is in any case desirable to be able to detect more than one gene target to provide a sensitive and specific alternative or addition to the available MgPa gene targeting assays.

The unexpected 341 bp product was produced on amplification of material from all M genitalium strains and has recently been associated with NGU, independent of the 16S rRNA gene. The protein encoded by MgPa, the first major adhesin gene encoded by MgPa, the first major adhesin gene encoded by M genitalium has been found in the genital tract of women, many with genitourinary disease, and has recently been associated with both cervicitis and endometritis.

Mycoplasma genitalium carriage is significantly associated with non-gonococcal urethritis, independent of Chlamydia trachomatis

There is currently no commercially available test for M genitalium and most published PCR assays target either the MgPa major adhesin gene or the 16S rRNA gene. The protein encoded by MgPa, the first M genitalium gene to be sequenced, is a virulence determinant and major antigen so that it is an appropriate target for the PCR. However, surface expressed antigens often have unstable gene sequences and intraspecies variation in the MgPa gene has been reported. It is likely that the 16S rRNA gene sequence is relatively stable and therefore contains few, if any, polymorphisms in the M genitalium population. In the absence of non-amplification tests it is in any case desirable to be able to detect more than one gene target to confirm positive results and therefore to confirm the prevalence in a specific population group.

We have designed a sensitive and specific PCR assay targeting the 16S rRNA gene of M genitalium and have validated the assay against previously published methods using urine specimens from men with urethritis attending a genitourinary medicine clinic.

MATERIALS AND METHODS
Polymerase chain reaction
Oligonucleotide primers corresponding to sequences within the M genitalium 16S rRNA gene were synthesised as follows:

- 16SFG2: 5′-CCT TAT CGT TAG TTA CAT TGT TTA A-3′
- 16SRG: 5′-TGA CAT GCG CTT CCA ATA AA-3′

The expected 341 bp product was produced on amplification of material from all M genitalium strains and from none of the other microorganisms tested. The lower limit of detection was 50 genome copies. The new assay detected M genitalium DNA in nine of 54 men with urethritis, in comparison with eight positive specimens detected with the alternative PCR.

Conclusions: This novel PCR targeting the M genitalium 16S rRNA gene has been optimised and now provides a sensitive and specific alternative or addition to the available MgPa gene targeting assays.
extension step of seven minutes at 72°C. Products were visualised by agarose gel electrophoresis alongside a 100 bp ladder (GibcoBRL, Life Technologies, Paisley, UK). The expected product size was 341 bp.

**Assay validation**

**Amplification of target and non-target microorganisms**

Various microorganisms were suspended in water and heated to 100°C for 10 minutes. Cell debris was removed by centrifugation and 5 μl of supernatant added to the PCR assay described above. Table 1 lists the species and strains tested.

**Detection limit of the PCR**

Freeze dried *M genitalium* strain TW48-5G was suspended in SP4 medium and the nucleic acid was extracted by the SP4 medium and the nucleic acid was extracted by the

**Table 1 Organisms tested by 16S based PCR for Mycoplasma genitalium**

<table>
<thead>
<tr>
<th><em>M genitalium</em> strains (ref)</th>
<th>Mollicute species</th>
<th>Other microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>G37</td>
<td><em>Mycoplasma pneumonië</em> (NCTC 10119)</td>
<td>Chlamydia trachomatis serovar L2</td>
</tr>
<tr>
<td>TW10</td>
<td><em>Mycoplasma pirum</em> (NCTC 11702)</td>
<td>Burkholderia cepacia</td>
</tr>
<tr>
<td>R32</td>
<td><em>Mycoplasma gallisepticum</em> (NCTC 10115)</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>UTMB</td>
<td><em>Mycoplasma primatum</em> (NCTC 10163)</td>
<td>Coagulase negative staphylococcus sp</td>
</tr>
<tr>
<td>TW48</td>
<td><em>Mycoplasma hominis</em> (NCTC 10111)</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>M2341</td>
<td><em>Mycoplasma orale</em> (NCTC 10112)</td>
<td>Candida albicans</td>
</tr>
<tr>
<td>M2321</td>
<td><em>Mycoplasma salivarium</em> (NCTC 10113)</td>
<td>Corynebacterium xerosis</td>
</tr>
<tr>
<td>M2288</td>
<td><em>Mycoplasma lipophilum</em> (NCTC 10173)</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>M2300</td>
<td><em>Mycoplasma buccale</em> (NCTC 10177)</td>
<td>Group A streptococcus sp</td>
</tr>
<tr>
<td></td>
<td><em>Acholeplasma laidlawii</em> (NCTC 10116)</td>
<td>Group B streptococcus sp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enterococcus sp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Baciillus sp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brokanella catarrhalis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteroides fragilis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Micrococcus sp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridium ramosum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridium perfringens</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactobacillus casei</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteroides urealyticus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clostridium sp</td>
</tr>
</tbody>
</table>

**Usefulness of the test with clinical specimens**

First catch urine specimens were taken from consecutive male patients with urethritis attending the Bristol Royal Infirmary genitourinary medicine clinic. Patients were diagnosed with NGU if there were more than five polymorphonuclear leucocytes (PMNLs) in each high power (×1000) microscope field (HPF) in five or more fields of a Gram stained urethral smear, or > 10 PMNLs/HPF in five or more fields of a Gram stained thread from 15–20 ml of a first passed urine specimen and *Neisseria gonorrhoeae* was not cultured from a urethral swab. Seven of 54 men had a history of urethritis in the previous three months, suggesting that they were suffering from chronic or recurrent urethritis.

Before storage at −20°C, the urine specimens were tested for *Chlamydia trachomatis* by standard laboratory methods; positive results with IDEIA, an amplified enzyme immunoassay (Novo Nordisk Diagnostics, Cambridge, UK), were confirmed using direct fluorescent antibody (MicroTrak; Syva, Palo Alto, California, USA).

The research ethics committee of the United Bristol Healthcare NHS Trust granted permission to test urine specimens from men with urethritis for *M genitalium*.

Urine specimens were brought to room temperature and mixed thoroughly. One millilitre of urine was centrifuged at 15 000 g for five minutes. The pellet was washed twice in phosphate buffered saline, resuspended in 20 μl 10% Chelex 100® resin (BioRad Laboratories, Hemel Hempstead, Hertfordshire, UK) in deionised water and held at 100°C for 10 minutes. The slurry was pulse centrifuged to pellet the resin, and 5 μl of the supernatant used in the PCR and 100°C for 5 minutes. The slurry was pulse centrifuged to pellet the resin, the 5 μl of the supernatant used in the PCR and 100°C for 5 minutes. The slurry was pulse centrifuged to pellet the resin, the 5 μl of the supernatant used in the PCR.

Both the 16S rRNA gene PCR and an adhesin gene based PCR were performed on each specimen. The adhesin gene based assay was performed using reagent concentrations and cycling times optimised for assay components and cyclers used locally (those used for the 16S PCR). The concentration of MgCl₂ was 4.5 mM (as published) and the concentration of each primer was reduced from 200 nM to 20 nM. The annealing temperature for the primers was 60°C and 40 cycles of PCR were performed (as recommended by JS Jensen, personal communication, 1996). Primer sequences were as follows:

- **MgPa1:** 5′-AGT TGA TGA AAC CTT AAC CCC TTG G-3′.
- **MgPa3:** 5′-CCG TTG AGG GGT TTT CCA TTT TTG C-3′.

The modified hot start was not used. Sensitivity of the PCR was confirmed to be comparable to that obtained in the author’s laboratory by use of positive control DNA kindly donated by JS Jensen (Statens Seruminstitut, Copenhagen, Denmark).

Specimens positive by either or both the adhesin gene and 16S based tests were retested by a heminested adhesin gene PCR. Again, the assay was adapted to local practice: primer concentrations were reduced from 1 μg/reaction to 20 nM and dNTP concentrations from 200 μM to 20 μM in a reaction volume of 50 μl. Thirty five cycles were performed at each stage of the PCR, with hold times as in the 16S PCR. Primer sequences were as follows:

- **MgP1:** 5′-GGT TAA CTT ACC TAG TGG GGA GTT CACATA-3′.
- **Mg2:** 5′-CGT CTT GAG CTT GGA CAT CA-3′.
**Table 2** Comparison of *Mycoplasma genitalium* detection in men with urethritis by two different PCR assays

<table>
<thead>
<tr>
<th></th>
<th>16S based PCR</th>
<th>MgPa based PCR (non-nested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>55</td>
</tr>
</tbody>
</table>

**RESULTS**

**Assay validation**

DNA preparations from all nine strains of *M. genitalium* and none of the other organisms were positive by PCR, producing the expected 341 bp product. Preparations from all other organisms tested gave no observable PCR products.

The nucleic acid obtained from *M. genitalium* strain TW48-5G was estimated by the GeneQuant instrument as 66% pure. The A260/A280 ratio was 1.190, and the concentration of DNA was calculated as 72 µg/ml. Each genome copy is 580 kbp or 0.476 fg. Using these figures the sensitivity of the PCR is 24 fg *M genitalium* DNA, equivalent to about 50 genome copies. This was similar to the sensitivity of 7 fg achieved using both the comparator and confirmatory adhesin gene based tests when challenged with a dilution series of the same DNA extract.

**Usefulness of the test with clinical specimens**

Tables 2 and 3 show the results of these investigations. Nine of 54 specimens (16.7%) gave a positive result with the 16S rDNA based PCR for *M. genitalium* when products were examined by gel electrophoresis. Eight of these were also positive by the MgPa gene PCR method of Jensen et al., and all were confirmed using the MgPa gene heminested PCR. Eight men were positive for *C. trachomatis*. Two of the *M. genitalium* positive men were also infected with *C. trachomatis*. One patient had Gram negative diplococci (presumptively *N. gonorrhoeae*) on a urethral smear but was not positive for *M. genitalium* or *C. trachomatis*.

**DISCUSSION**

The *M. genitalium* 16S based PCR was tested against a range of target and non-target microorganisms. A product of the correct size was obtained from all *M. genitalium* isolates tested and from no other mollicute. The modified hot start procedure was necessary to prevent amplification of DNA from a bacillus sp isolate. No amplification was seen with the other organisms tested, either with or without the hot start.

“This assay is useful as a confirmatory assay or as an alternative to MgPa based assays, and may be less susceptible to intraspecies genetic polymorphism”

**Table 3** *Mycoplasma genitalium* and *Chlamydia trachomatis* detection in men with urethritis

<table>
<thead>
<tr>
<th></th>
<th><em>M. genitalium</em> (16S based PCR)</th>
<th><em>C. trachomatis</em> (EIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td><em>C. trachomatis</em> (EIA)</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

EIA, enzyme immunoassay; PCR, polymerase chain reaction.

**Take home messages**

- Mg3: 5’-GTA ATT AGT TAC TCA GTA GA-3’.
- The assay retained a very low minimum level of detection.

In the study of urine specimens, 16S rDNA based PCR performed slightly better than did the non-nested adhesin gene based PCR. This could result from heterogeneity in the adhesin gene, although no such heterogeneity has been reported in this region. The proportion of men identified as *M. genitalium* positive is low compared with that found in many of the previous PCR based studies of comparable subjects; the range for men with urethritis being 10–50%. The specimens had been stored at −20°C, and both the storage conditions and the freeze thaw cycle may have caused degradation of *M genitalium* DNA. This is unlikely to have affected the comparative sensitivities of the assays and the prevalence is compatible with a later, unpublished, survey of unfrozen samples from men in the Bristol area. The low prevalence may be accounted for by the small size of the study, but is also compatible with variations of *M genitalium* prevalence in men with NGU between populations or over time caused by geographical variation, ethnic composition, local sexual practices, the prevalence of virulent strains, or other, unknown, factors. Such factors may also account for the low prevalence of *C. trachomatis* compared with other studies of comparable subjects in Bristol. It is possible that this is because we used IDEIA, which has a reported sensitivity of 80% in a similar population. However, this loss of sensitivity is unlikely to account for the large discrepancy between the observed and expected *C. trachomatis* prevalence in the group studied. It has been proposed that the prevalence of chlamydial infection in men may be lower in those without symptoms or signs”; we did not collect this information and it is possible that the inclusion of men with NGU but no signs or symptoms is the cause of the unexpectedly low rates of both *M. genitalium* and *C. trachomatis*.

In conclusion, on a small sample of patients, the novel PCR assay appears to be at least as sensitive as a previously published non-nested assay, detects DNA from a range of *M. genitalium* isolates, and shows no crossreactivity with the common microorganisms tested. It is useful as a confirmatory assay or as an alternative to MgPa based assays, and may be less susceptible to intraspecies genetic polymorphism.

**ACKNOWLEDGEMENTS**

This study was funded by a Public Health Laboratory Service Research and Development grant. Dr V Battu helped with clinical information concerning men attending the Milne Centre. *Mycoplasma genitalium* G37 broth culture was kindly donated by Dr M Sills of Norwich Public Health Laboratory. *Mycoplasma genitalium* TW10, R32, UTMB, and TW48 were kindly donated as lyophilised cultures by Dr J Tully, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. *Mycoplasma genitalium* G37, M-2341, M-2321, M-2288, and M-2300 were kindly donated as lyophilised cultures by Dr WJ Tully, National Institutes of Health, Bethesda, Maryland, USA. *Mycoplasma genitalium* G37, M-2341, M-2321, M-2288, and M-2300 were kindly donated as lyophilised cultures by Dr WJ Tully, National Institutes of Health, Bethesda, Maryland, USA.

**Authors’ affiliations**

K Eastick, J P Leeming, M R Miller, Public Health Laboratory, Level 8, Bristol Royal Infirmary, Maudlin Street, Bristol BS2 8HW, UK
E O Cuil, Public Health Laboratory, Myrtle Road, Kingsdown, Bristol BS2 8EL, UK
P J Horner, The Milne Centre for Sexual Health, Bristol Royal Infirmary, Maudlin Street, Bristol BS2 8HW, UK
REFERENCES


New JCP online submission and review system

We are pleased to inform authors and reviewers of the new online submission and review system at JCP. Developed by HighWire Press (CA, USA), Bench Press is a fully integrated electronic system that utilises the web to allow rapid and efficient submission of manuscripts. It also allows the peer review process to be conducted entirely online. We are one of the first journals in the BMJ Special Journals group to go online in this way. The aim, apart from saving trees, is to speed up the often frustratingly slow process (for both authors and editors) from submission to publication. Many reviewers might appreciate this too.

Authors may submit their manuscript in any standard word processing software. Acceptable standard graphic formats include: jpeg, tiff, gif, and eps. The text and graphic files are automatically converted to PDF for ease of distribution and reviewing purposes. Authors are asked to approve their submission before it formally enters the reviewing process. On approval by the authors, the submission is passed to the editor and/or reviewers via the web. All transactions are secure.

To access the system click on “SUBMIT YOUR MANUSCRIPT HERE” on the JCP homepage: HYPERLINK http://www.jcp.bmjpath.com, or you can access Bench Press directly at HYPERLINK http://submit.jcp.bmjpath.com.

We are very excited with this new development and would encourage authors and reviewers to use the online system whenever possible. As editors, we will use it all the time, the up side being lack of need to travel to the editorial office to deal with papers, the down side having no more excuses to postpone decisions on papers because we are “at a meeting”!

The system is very easy to use and should be a big improvement on the current peer review process. Full instructions can be found on Bench Press http://submit.jcp.bmjpath.com and JCP online at http://www.jcp.bmjpath.com. Please contact Natalie Davies, Project Manager, HYPERLINK mailto:nlindell@bmjgroup.com for any further information.

Reference

www.molpath.com