Le tecniche molecolari nella diagnosi delle STD

Dott. Stefano Grandesso
U.O.C. Laboratorio Analisi SSD Microbiologia
Ospedale dell’Angelo – Mestre
Azienda ULSS 12 Veneziana
IST curabili:
448 milioni di nuovi casi all’anno
(O.M.S. - 2005)

<table>
<thead>
<tr>
<th>Infezioni</th>
<th>Casi/anno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonas</td>
<td>248 milioni</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>102 milioni</td>
</tr>
<tr>
<td>Gonococco</td>
<td>87 milioni</td>
</tr>
<tr>
<td>Sifilide</td>
<td>10 milioni</td>
</tr>
<tr>
<td>HSV</td>
<td>26 milioni</td>
</tr>
</tbody>
</table>
### Chlamydia and Gonorrhea

**Age- and sex-specific rates:**

**United States, 2008**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Rate (per 100,000 population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-14</td>
<td>2800</td>
</tr>
<tr>
<td>15-19</td>
<td>1056.1</td>
</tr>
<tr>
<td>20-24</td>
<td>78.3</td>
</tr>
<tr>
<td>25-29</td>
<td>277.1</td>
</tr>
<tr>
<td>30-34</td>
<td>78.3</td>
</tr>
<tr>
<td>35-39</td>
<td>34.4</td>
</tr>
<tr>
<td>40-44</td>
<td>10.4</td>
</tr>
<tr>
<td>45-54</td>
<td>2.7</td>
</tr>
<tr>
<td>55-64</td>
<td>211.7</td>
</tr>
<tr>
<td>65+</td>
<td>103.3</td>
</tr>
<tr>
<td>Total</td>
<td>278.3</td>
</tr>
</tbody>
</table>

**Source:** CDC/NCHSTP 2008 STD Surveillance Report
Trend delle infezioni da Neisseria gonorrhoeae e Chlamydia trachomatis

STI sentinel surveillance network 2000-2004

_Eurosurveillance 2006_
# Evidence of the Impact of Selected Infectious Agents on Reproductive Sequelae

<table>
<thead>
<tr>
<th>Health Effects</th>
<th>Chlam</th>
<th>GC</th>
<th>GBS</th>
<th>HIV</th>
<th>Syphilis</th>
<th>HSV</th>
<th>BV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous abortion</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Birth defects</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Fetal infection</td>
<td>—</td>
<td>—</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑↑↑</td>
<td>↑↑↑</td>
<td>—</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>↑↑</td>
<td>↑↑</td>
<td>—</td>
<td>—</td>
<td>↑↑↑↑</td>
<td>↑↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Growth restriction</td>
<td>—</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>↑↑↑↑</td>
<td>↑↑</td>
<td>—</td>
</tr>
<tr>
<td>Perinatal mortality</td>
<td>—</td>
<td>—</td>
<td>↑↑↑</td>
<td>↑</td>
<td>↑↑↑↑</td>
<td>↑↑</td>
<td>—</td>
</tr>
</tbody>
</table>

Baecher-Lind, 2009
Evidence of the Impact of Selected Infectious Agents on Reproductive Sequelae

<table>
<thead>
<tr>
<th>Health Effects</th>
<th>Chlam</th>
<th>GC</th>
<th>GBS</th>
<th>HIV</th>
<th>Syphilis</th>
<th>HSV</th>
<th>BV</th>
</tr>
</thead>
</table>

Intrapartum Infection: ↑↑↑ ↑↑↑ ↑↑↑ ↑↑↑ ↑↑↑ ↑

Baecher-Lind, 2009
L'infezione colpisce prevalentemente le giovani donne di età inferiore ai 25 anni ed i sintomi sono generalmente lievi, aspecifici o addirittura inesistenti: il

- nelle donne
  70-80%

- nell'uomo
  30-50%

delle infezioni decorre in modo asintomatico, comportando, sovente, una sottostima della diffusione dell'infezione e quindi una maggiore facilità della trasmissione.

Van Bergen 2006; Manavi 2006
Chlamydia trachomatis e Neisseria gonorrhoeae

PID

Infertilità tubarica

GEU

Algie pelviche croniche

Sindrome di Fitz Hugh Curtis

De Barberyrac, 2007
Honey, 2002
Kobayashi, 2006
Chlamydia t. e Neisseria g. – Nell’uomo

C. t. è responsabile di circa il 50% delle uretriti non specifiche (non gonococciche e post gonococciche).

L’uretrite acuta, simile a quella gonococcica, è rara.

**Sintomi**

- Disuria ± ++
- Bruciore minzionale + +++
- Dolore uretrale ± ++
- Secrezioni + chiare e vischiose +++ purulente
- Eritema del meato urinario ± ++

**Foto**

I. Dal Conte, M. Cusini

**Un uomo con un’uretrite asintomatica (30-50% dei casi) costituisce un importante reservoir di infezione!**

Florian, 2006
Linfogranuloma venereo

Malattia sistemica causata dai serovars L₁, L₂ e L₃ di C.t., non comune nelle nazioni industrializzate ma largamente diffuso in Asia, Africa e Asia. Clinicamente il quadro è quello di una linfadenopatia acuta a volte preceduta da una lesione primaria (ulcera erpetiforme, papula, pustola) o di una proctite ulcerativa con secrezioni rettali purulente.

Artrite reattiva

Una rara complicanza di un’infezione non trattata è rappresentata dalla sindrome di Reiter, un’artrite reattiva che associa la triade uretrite (a volte cervicite nelle donne), congiuntivite e lesioni mucocutanee non dolorose. Più frequente nell’uomo (rapporto 5:1 negli uomini rispetto alle donne). Ha una distribuzione mondiale, con un picco tra i 20 e i 40 anni e si manifesta più frequentemente in pazienti con predisposizione genetica: il gene HLA-B27 è presente nel 70-80% dei pazienti di razza bianca con tale sindrome.
Chlamydia trachomatis - Tecniche diagnostiche

- isolamento del microrganismo in colture cellulari
- metodi in immunofluorescenza
- tecniche immunoenzimatiche
- tecniche di biologia molecolare
- sierodiagnosi
• Necessità di diagnosticare le infezioni asintomatiche soprattutto nelle popolazioni più giovani

• Il ricorso ad una visita specialistica e l’esecuzione di prelievi “invasivi” sono poco accettati
Diagnosi

Necessità di tecniche diagnostiche

- Non invasive
- Ad elevata sensibilità e specificità
- Di facile esecuzione
- A costi contenuti
**Strategie diagnostiche nell’infezione da Chlamydia trachomatis**

*(Da Hamdad e coll. 2004, mod.)*

<table>
<thead>
<tr>
<th>Infezioni del basso apparato genitale</th>
<th>Screening</th>
<th>Infezioni dell’apparato genitale alto</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocervice, uretra</td>
<td>Endocervice</td>
<td>1° getto urine</td>
</tr>
<tr>
<td>Coltura cellulare</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>IFD</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>EIA</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Ibridazion</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>NAATs</strong></td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

+++: molto sensibile, ++: sensibile, +: media sensibilità, +/-: poco sensibile, -: non indicato
From the NIH: Proceedings of a Workshop on the Importance of Self-Obtained Vaginal Specimens for Detection of Sexually Transmitted Infections

Original Studies

Evaluation of Self-Collected Vaginal Swab, First Void Urine, and Endocervical Swab Specimens for the Detection of Chlamydia Trachomatis and Neisseria Gonorrhoeae in Adolescent Females

Junyong Fang, MD, PhD, Constance Husman, CRNP, MSH, Lalitha DeSilva, MD, MPH, Ruzhang Chang, MS, and Ligia Peralta, MD
Department of Pediatrics, University of Maryland School of Medicine, Baltimore, Maryland, USA
Results
Chlamydia prevalence in the sampled population was 11.1%. Sensitivities of vaginal, urine, and cervical AC2 were 97.2%, 91.7%, and 91.7%, respectively. The sensitivity of the DNA probe was derived from the literature and estimated at 68.8%. The self-obtained vaginal AC2 strategy was the least expensive and the most cost-effective, preventing 17 more cases of pelvic inflammatory disease than the next least expensive strategy.

Conclusions
Use of a vaginal swab to detect Chlamydia in this STD clinic population was cost-saving and cost-effective.
In conclusion, vaginal sampling for detecting *C. trachomatis* infection in women is a reliable and convenient method. The present study showed **self-collected vaginal sampling to be as sensitive a method as clinician-collected endocervical sampling** and combined FCU/vaginal sampling. The combination of vaginal specimens and FCU did not add any further advantage.
ABBANDONARE I TEST CON SCARSE PERFORMANCE

In generale la coltura, i test EIA e i test in IF non rilevano dal 20 al 30% delle infezioni e sono scoraggiati dalla recenti LG (SIGN 2009)

- Test EIA sensibilità 85% (PERDONO TROPPPI POSITIVI !!!) e specificità 97% (DANNO TROPPPI FALSI POSITIVI !!!)
- L’isolamento colturale è tecnicamente complesso, costoso, con una specificità circa 100% e sensibilità relativamente bassa (60-90%): forse a torto viene ancora considerato il gold standard nelle problematiche medico-legali (abusi sessuali).
Il loro limitato utilizzo dovuto al costo elevato, alla complessità ed ai problemi di contaminazione è stato risolto con la standardizzazione e l’introduzione in commercio di test dal costo contenuto, notevolmente semplificati e automatizzati.
NAAT e CHLAMYDIA

L’introduzione dei test di amplificazione degli acidi nucleici (NAATs = PCR, SDA, Ibridazione) per lo screening di C. trachomatis ha migliorato notevolmente l’identificazione delle infezioni asintomatiche, che sono frequenti (70-80%).

I test NAATs (PCR sensibilità almeno 98%, specificità circa 100%) offrono pertanto sostanziali benefici e sono raccomandati da recenti LG (SIGN 2009)
Pooling di urine 1° getto o di tamponi cervico-vaginali/uretrali (da 4 a 10 campioni)

- Riduzione costi dal 50 al 63 % per pool di 4-5 e del 30-50% per pool di 8-10
- Riduzione tempo tecnici dal 50 al 60%
- Sensibilità 96-99%

Currie M.J. : J Clin Microbiol 2004
Shipitsyna E.: Acta Derm Venereol. 2007
Tecniche ad elevata sensibilità e specificità

<table>
<thead>
<tr>
<th>Method</th>
<th>Turn-around time</th>
<th>Advantages</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture</td>
<td>72 h</td>
<td>Specificity, strain</td>
<td>Sensitivity 80–85%</td>
</tr>
<tr>
<td>Antigen detection</td>
<td>45 min</td>
<td>Simple, unit test</td>
<td>Sensitivity 75–80%</td>
</tr>
<tr>
<td>DFA</td>
<td></td>
<td></td>
<td>Subjective reading</td>
</tr>
<tr>
<td>EIA</td>
<td>4 h</td>
<td>Automation</td>
<td>Sensitivity 75–80%</td>
</tr>
<tr>
<td>Point of care</td>
<td>30 min</td>
<td>Low cost, unit test</td>
<td>Low specificity (confirmatory test)</td>
</tr>
<tr>
<td>Molecular methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA probing</td>
<td>2 h</td>
<td>Easy to perform</td>
<td>Sensitivity 75–80%</td>
</tr>
<tr>
<td>Hybrid capture</td>
<td>4 h</td>
<td>Sensitivity 95%</td>
<td>Only for cervical specimens (FDA)</td>
</tr>
<tr>
<td>NAAT (real-time PCR, SDA, TMA, NASBA)</td>
<td>2–4 h</td>
<td>Specificity 99%</td>
<td>Contamination, costly processing of specimen</td>
</tr>
</tbody>
</table>

Le tecniche di amplificazione degli acidi nucleici rappresentano il "GOLD STANDARD" per la diagnosi delle infezioni da Chlamydia trachomatis

L’uso di metodi non amplificati, soprattutto nello screening di popolazioni a medio-basso rischio o asintomatiche, si traduce in una diminuzione della prevalenza dell’infezione (fino al 30–40%)

Bébéar: Clin Microb Infect 2009
Chlamydia trachomatis: diagnosi microbiologica

Acta Derm Venereol 2007; 87: 140–143

INVESTIGATIVE REPORT

Pooling Samples: the Key to Sensitive, Specific and Cost-effective Genetic Diagnosis of Chlamydia trachomatis in Low-Resource Countries

Elena SHIPITSYNA1, Kira SHALEPO1, Alevtina SAVICHEVA1,2, Magnus UNEMO2,3 and Marius DOMEIKA2

1Laboratory of Microbiology, DO Ott Research Institute of Obstetrics and Gynecology RAMS, St Petersburg, Russia. 2Department of Medical Sciences, Uppsala University, Uppsala, and 3Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Pooling of Clinical Specimens Prior to Testing for Chlamydia trachomatis by PCR Is Accurate and Cost Saving

Marian J. Currie,1* Michelle McNiven,2 Tracey Yee,2 Ursula Schiemer,2 and Francis J. Bowden1

ACT Pathology, The Canberra Hospital, and Academic Unit of Internal Medicine, Australian National University, Canberra, Australia
**Chlamydia trachomatis: PCR real time**

Recentemente introdotte, le tecniche di PCR real time permettono di quantificare il prodotto dell’amplificazione: l’aumento di un segnale di emissione fluorescente è direttamente proporzionale alla quantità di amplificato prodotto

- presentano sensibilità, specificità e riproducibilità più elevate rispetto alla PCR tradizionale
- non richiedono manipolazioni post-amplificazione con conseguente diminuzione dei tempi e dei rischi di contaminazione ambientale
- rappresentano uno strumento prezioso per migliorare la diagnostica e fornire al clinico risultati attendibili e in tempi utili
- costituiscono un valido contributo al monitoraggio della terapia antibiotica.
Comparison of the Abbott RealTime CT New Formulation Assay with Two Other Commercial Assays for Detection of Wild-Type and New Variant Strains of *Chlamydia trachomatis*

Jens Kjølseth Møller, Lisbeth Nørøm Pedersen, and Kenneth Persson

Department of Clinical Microbiology, Aarhus University Hospital, Skejby, Denmark, and Department of Clinical Microbiology, Malmö University Hospital, Malmö, Sweden

Received 25 July 2009/Returned for modification 8 September 2009/Accepted 29 November 2009

In an analytical-method comparison study of clinical samples, the Abbott RealTime CT new formulation assay (m2000 real-time PCR), consisting of a duplex PCR targeting different parts of the cryptic plasmid in *Chlamydia trachomatis*, was compared both with version 2 of the Roche Cobas TaqMan CT assay, comprising a duplex PCR for a target in the cryptic plasmid and the *omp1* gene, and with the Gen-Probe Aptima Combo 2 assay (AC2) targeting the *C. trachomatis* 23S rRNA molecule. First-catch urine samples from Sweden were tested in Malmö, Sweden, for *C. trachomatis* with the m2000 real-time PCR assay and with an in-house PCR for the new variant *C. trachomatis* strain with a deletion in the cryptic plasmid. Aliquots of the urine samples were sent to Aarhus, Denmark, where they were further examined with the TaqMan CT and AC2 assays. A positive prevalence of 9.4% (148/1,632 urine samples examined) was detected according to the combined reference standard. The sensitivities and specificities of the three assays were as follows: for the Abbott m2000 assay, 95.3% (141/148) and 99.9% (1,483/1,485), respectively; for the Roche TaqMan assay, 82.4% (122/148) and 100.0% (1,485/1,485); and for the Gen-Probe AC2 assay, 99.3% (147/148) and 99.9% (1,484/1,485). The plasmid mutant strain was detected in 24% (36/148) of the *C. trachomatis*-positive samples. There is a difference in sensitivity between the new formulations of the Abbott and the Roche assays, but both assays detected the wild-type and new variant *C. trachomatis* strains equally well.
Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR Chlamydia trachomatis kit for detection of C. trachomatis

Poonam Sachdeva, Achchhe Lal Patel, Divya Sachdev, Mashook Ali, Aruna Mittal and Daman Saluja

1Dr B R Ambedkar Center for Biomedical Research, University of Delhi, Delhi, India
2Institute of Pathology (ICMR), Safdarjung Hospital Campus, New Delhi, India

To improve the control of Chlamydia trachomatis infection in India, a rapid, specific and cost-effective method is much needed. We developed an in-house PCR assay by targeting a unique genomic sequence encoding a protein from the C. trachomatis phospholipase D endonuclease superfamily that produces an amplified fragment of 388 bp. The specificity of the primers was confirmed using genomic DNA from other sexually transmitted disease-causing and related microorganisms and from humans. The assay was highly sensitive and could detect as low as 10 fg C. trachomatis DNA. Clinical evaluation of the in-house-developed PCR was carried out using 450 endocervical specimens that were divided in two groups. In group I (n=274), in-house PCR was evaluated against the direct fluorescence assay. The resolved sensitivity of the in-house PCR method was 97.22% compared with 88% for the direct fluorescent antibody assay. In group II (n=176), the in-house PCR was compared with the commercial Roche AMPLICOR MWP CT detection kit. The resolved sensitivity of the in-house PCR assay reported here was 93.1% and the specificity was 97.40%, making it a cost-effective alternative for routine diagnosis of genital infection by C. trachomatis. The method should facilitate early detection leading to better prevention and treatment of genital infection in India.
**Conclusioni 1**

- La diagnosi precoce delle infezioni da *Chlamydia trachomatis* è un approccio indispensabile per prevenire la diffusione dell'infezione e le sue complicanze.

- L'età inferiore ai 25 anni e il sesso femminile rappresentano i maggiori fattori di rischio. Si stima che oltre il 70% delle infezioni siano diagnosticate in donne d'età compresa tra i 15 e i 24 anni, mentre negli uomini la maggiore prevalenza è segnalata tra i 20 ed i 29 anni (CDC).

- Sono auspicabili programmi di screening che, utilizzando come criterio di selezione la giovane età in combinazione o meno con altri fattori di rischio potrebbero diagnosticare la maggior parte delle infezioni.
Negli ultimi anni l’introduzione di tecniche diagnostiche basate sull’amplificazione degli acidi nucleici (NAATs) ha permesso di utilizzare, per la diagnosi delle infezioni da *Chlamydia trachomatis*, campioni alternativi, rispetto al tampone endocervicale o uretrale, come le urine o i tamponi vaginali e vulvari.

L’elevata sensibilità (>90%) e specificità (>98%) rendono i NAATs i migliori test per la diagnosi delle infezioni da *Chlamydia trachomatis*.

La dimostrazione di anticorpi specifici anti-*Chlamydia trachomatis* a titolo elevato si è dimostrata correlata all’infertilità da danno tubarico.

La presenza di Ab antiHSP-60 può essere un efficiente marcatore diagnostico di infezione cronica.
Neisseria gonorrhoeae

- Decremento delle infezioni fino agli anni 1995-96
- Aumento delle infezioni dal 1997
- Ridotta sensibilità ai chinoloni (ceppi importati dell’Asia)

Berglund - 2001
Diagnosi microbiologica: Neisseria gonorrhoeae

La scelta dei campioni e il metodo di raccolta dipendono dalle metodiche utilizzate dal laboratorio, dal quadro clinico, dall’età, dal sesso e dall’orientamento sessuale del paziente. Tutti i siti in cui sono presenti dei sintomi (secrezioni e/o dolore) dovrebbero essere indagati.

La diagnosi di infezione gonococcica è basata su:

- esame microscopico previa colorazione (Gram, Giemsa, Blu di metilene),
- esame colturale,
- test rapidi per la ricerca di antigeni,
- NAATs .

Domeika: Euro Surveill. 2007
CDC MMWR Recomm Rep. 2006
Bignell: Sex Transm Infect. 2006
Ng: Can J Infect Med Microbiol 2005
Diagnosi microbiologica: Neisseria gonorrhoeae

**Esame microscopico**
Molto utile nelle secrezioni uretrali. Il vetrino, che deve essere allestito al momento del prelievo e non dal tampone inviato al Laboratorio, viene esaminato, dopo colorazione, al microscopio per evidenziare la presenza dei tipici diplococchi Gram negativi prevalentemente all’interno dei PMN.

**Esame colturale**
Rappresenta, ancor oggi, il “gold standard” per la diagnosi di infezione gonococcica ed è anche il solo metodo che consenta di testare la sensibilità agli antibiotici.

**Tecniche di amplificazione degli acidi nucleici (NAATs)**
La ricerca può essere eseguita, oltre che dal tampone uretrale o cervico-vaginale, anche dal primo getto di urina, da tamponi orofaringei, rettali e congiuntivali o da altri campioni biologici.
Diagnosi microbiologica: Neisseria gonorrhoeae

**Diagnosi molecolare**

La sensibilità resta molto elevata (>90%), ma la specificità non lo è altrettanto.

Rischio di falsi positivi soprattutto per i prelievi ano-rettali e faringei.

Possibili reazioni crociate con altre Neisserie non patogene.

*Wiley: Sex Health. 2008*
*Tapsall: Future Microbiol. 2006*
*CDC MMWR Recomm Rep. 2006*
NAAts: *Neisseria gonorrhoeae* - Limiti

Si possono avere falsi risultati positivi, dovuti a cross-reazioni con Neisserie commensali, come ad esempio quelle presenti nella cavità orale (elevata omologia di sequenze con *N. meningitidis*), in quanto le sequenze target utilizzate dalle NAATs possono essere comuni a Neisserie non patogene.

La falsa positività può essere dovuta a sequenze target, non presenti in alcuni sottotipi di *N. gonorrhoeae* e presenti invece in ceppi di Neisserie commensali.

Fredlung H: APMIS, 2004
**Diagnosi molecolare**

In popolazioni con bassa prevalenza delle infezioni gonococciche, il PPV potrebbe essere <80% per cui è raccomandata la conferma colturale.

Questi test non sono validati per i prelievi ano-rettali o faringei: l’esame colturale rappresenta ancora il gold standard per la diagnosi in siti extra-genitali.

In realtà, nella pratica clinica, non dovrebbero essere utilizzati come unico saggio diagnostico di routine, ma dovrebbero sempre essere confermati dall’esame colturale.

Non consentono di sorvegliare la resistenza agli antibiotici.

European Guideline (IUST/WHO) on the Diagnosis and Treatment of Gonorrhoea in Adult. 2009
CDC MMWR Recomm Rep. 2006
Nucleic Acid Amplification Tests for Diagnosis of Neisseria gonorrhoeae Oropharyngeal Infections

Laura H. Bachmann,1,2,5* Robert E. Johnson,4 Hong Cheng,1 Lauri E. Markowitz,4 John R. Papp,4 and Edward W. Hook III1,2,3

University of Alabama at Birmingham School of Medicine, Department of Medicine, Division of Infectious Diseases, Birmingham, Alabama; University of Alabama at Birmingham School of Public Health, Birmingham, Alabama; Jefferson County Department of Health, Birmingham, Alabama; Centers for Disease Control and Prevention, Atlanta, Georgia; and Birmingham Veterans Administration Medical Center, Birmingham, Alabama

Received 14 August 2008/Returned for modification 10 November 2008/ Accepted 22 January 2009

The optimal methods for the diagnosis of pharyngeal Neisseria gonorrhoeae infection are uncertain. The objective of this study was to define the performance of culture and nucleic acid amplification tests (NAATs) for the diagnosis of pharyngeal N. gonorrhoeae. In this cross-sectional study, males and females >15 years old who acknowledged performing fellatio or cunnilingus (in the previous 2 months) were recruited from three clinics (two human immunodeficiency virus clinics and one sexually transmitted diseases clinic) located in Birmingham, AL. The test performance of culture for N. gonorrhoeae, the Gen-Probe Aptima Combo 2 transcription-mediated amplification assay (TMA), the BD ProbeTec ET amplified DNA strand displacement assay (SDA), and the Roche Cobas Amplicor PCR was defined by using a rotating “gold standard” of any positive results by two or three of the three tests that excluded the test being evaluated. A total of 961 evaluable test sets were collected. On the basis of a rotating gold standard of positive results by two of three comparator tests, the sensitivity and specificity were as follows: culture for N. gonorrhoeae, 59.8% and 99.4%, respectively; PCR, 80.3% and 73.0%, respectively; TMA, 83.6% and 98.6%, respectively; and SDA, 92.2% and 96.3%, respectively. On the basis of a rotating gold standard of positive results by three of three comparator tests, the sensitivity and specificity were as follows: culture for N. gonorrhoeae, 62.1% and 99.8%, respectively; PCR, 81.3% and 71.3%, respectively; TMA, 100% and 96.2%, respectively; and SDA, 97.1% and 94.2%, respectively. In conclusion, currently available NAATs are more sensitive than culture for the detection of pharyngeal gonorrhea in at-risk patients. PCR is substantially less specific than culture, TMA, or SDA and should not be used for the detection of pharyngeal gonorrhea.
Comparison of COBAS AMPLICOR *Neisseria gonorrhoeae* PCR,
Including Confirmation with *N. gonorrhoeae*-Specific 16S
rRNA PCR, with Traditional Culture

Dirk S. Luijt,\(^1\) Petra A. J. Bos,\(^1\) Anton A. van Zwet,\(^1\) Pieter C. van Voorst Vader,\(^2\)
and Jurjen Schirm\(^1\)*

*Regional Public Health Laboratory\(^1\)* and *Department of Dermatology, University Hospital Groningen,\(^2\)*
*Groningen, The Netherlands*

Received 28 May 2004/Returned for modification 23 August 2004/Accepted 22 October 2004

A total of 3,023 clinical specimens were tested for *Neisseria gonorrhoeae* by using COBAS AMPLICOR (CA) PCR and confirmation of positives by *N. gonorrhoeae*-specific 16S rRNA PCR. The sensitivity of CA plus 16S rRNA PCR was 98.8%, compared to 68.2% for culture. Confirmation of CA positives increased the positive predictive value from 54.8 to 96.6%.
Cross reazioni con N. cinerea, N. flava, Lactobacillus spp.

### TABLE 1. Test characteristics

<table>
<thead>
<tr>
<th>Specimen type and/or criterion</th>
<th>Value for test method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N. gonorrhoeae culture</td>
</tr>
<tr>
<td>True positive</td>
<td></td>
</tr>
<tr>
<td>No. of positive results</td>
<td>58</td>
</tr>
<tr>
<td>No. of negative results</td>
<td>27</td>
</tr>
<tr>
<td>True negative</td>
<td></td>
</tr>
<tr>
<td>No. of positive results</td>
<td>0</td>
</tr>
<tr>
<td>No. of negative results</td>
<td>2,938</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>68.2</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>100</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>99.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only specimens which were repeatedly reactive in the CA N. gonorrhoeae PCR test were considered to be CA N. gonorrhoeae PCR positive.

<sup>b</sup> Specimens which were repeatedly positive by CA N. gonorrhoeae PCR and positive by 16SrRNA N. gonorrhoeae PCR were considered to be N. gonorrhoeae PCR positive. All other specimens were N. gonorrhoeae PCR negative.

<sup>c</sup> Specimens were considered to be true N. gonorrhoeae positive or true N. gonorrhoeae negative as described in the text.
Test di sensibilità agli antibiotici

I test di sensibilità agli antibiotici rivestono una particolare importanza sia per la scelta della terapia sia per la sorveglianza ed il monitoraggio delle resistenze.

Si dovrebbero sempre saggiare i seguenti gruppi:

- penicillina (benzilpenicillina, ampicillina)
- cefalosporine (ceftriaxone, cefotaxime, cefixime)
- tetracicline (tetraciclini, doxiciclina)
- macrolidi (eritromicina, azitromicina)
- fluorochinoloni (ciprofloxacina, ofloxacina)
Il virione:
- Sprovvisto di envelope
- Ha un capsido icosaedrico costituito da L1 e L2
- Genoma: dsDNA, circolare di 7900 bp

Le caratteristiche più importanti di questi virus sono:
- Specie-specificità (virus ampiamente diffusi in natura, infettano l’uomo e diverse specie di animali)
- Tropismo tissutale: cellule epiteliali (epiteli pavimentosi pluristratificati: cute, epitelio ano-genitale, epitelio prime vie aeree e prime vie digestive)
- Potere oncogeno
Classificazione

12 Generi: identificati con la lettera dell’alfabeto greco. Omologia di sequenza di L1 > 60%

Specie: 60-70%
Tipo: 71-89%
Subtipo: 90-98%
Variante: > 98%
HPV: una ricerca in accelerazione

1907
Eziopatogenesi virale delle verruche cutanee

1930
Prime identificazioni di PV animali (Shope)

1949
Virioni di HPV in verruche cutanee (ME)

1962-67
Ciclo biologico di HPV

1965
Caratterizzazione strutturale e molecolare di HPV

1965
Ipotesi sul ruolo eziopatogenetico di HPV su ca cervicali

1975
Ipotesi sul ruolo eziopatogenetico di HPV su ca cervicali

1976-77
HPV 16 nei ca.cervicali

1983
HPV 18 nei ca.cervicali

1984
HPV 18 nei ca.cervicali

1984
HPV 16 nei ca.cervicali

1999
HPV in 99.7% dei ca.cervicali

2004
Famiglia Papillomaviridae

2006
Disponibilità vaccino

2004
Produzione VLPs (L1+L2)
Storia naturale dell’infezione

~ entro 1 anno  ~ 1-5 anni  Fino a decadi

Infezione persistente  CIN 2, 3  Ca. cervicale

CIN I

Clearance dell’infezione (80%)
**Tecniche di ibridazione**

**DIRETTA**

**Southern Blot**

Impiego limitato, costoso, laborioso, necessaria una notevole quantità di campione

**Ibridazione in situ (ISH)**

Rileva DNA e/o RNA in preparati citologici o istologici preservando la morfologia cellulare e tissutale

**CON AMPLIFICAZIONE DEL SEGNALE**

**Hybrid Capture 2 (HC 2)**

Ibridazione in fase liquida tra HPV-DNA e 2 cocktail di sonde a RNA (alto e basso rischio)

L’ibrido viene catturato da Ab adesi in una fase solida e rilevato con reazione chemiluminescente utilizzando Ab marcati

Automatizzabile ma non consente la genotipizzazione specifica né consente di identificare le infezioni miste
HPV e rischio oncogeno

- HPV alto rischio: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68...

- HPV basso rischio: 6, 11, 26, 42, 43, 44, 53, 54, 55, 62, 66...
Il carcinoma della cervice è la seconda causa di morte per tumore tra le donne (dopo il carcinoma della mammella)

500.000 nuovi casi di carcinoma della cervice uterina ogni anno nel mondo

Nel 99.7% dei casi di carcinoma della cervice si trova DNA di HPV

Il 53% dei carcinomi della cervice correlati ad HPV sono associati al 16, il 15% al 18, il 9% al 45, il 6% al 31 e il 3% al 33 (fonte IARC)

HPV è causa necessaria del cancro cervicale invasivo

Walboomers, J.Pathol 189:12-19, 1999

Roden & Wu, Nature Reviews Cancer 6, 753–763 (Oct 2006)
Presenza di HPV-DNA in Carcinomi

Diagnosi di infezione

- **Gli HPV non possono essere coltivati in vitro**
  - Il loro ciclo replicativo è strettamente dipendente dai processi maturativi dei cheratinociti di cui non è possibile promuovere la differenziazione in vitro.

- **La diagnosi sierologica è poco affidabile**
  - proteine capsidiche antigenicamente simili nei diversi genotipi di HPV (scarsa specificità)
  - proteine capsidiche non sempre espresse in tutte le lesioni (solo in infezioni produttive) (scarsa sensibilità)
Metodi Indiretti

Evidenziano lesioni clinicamente apparenti [infezioni cliniche] o modificazioni cellulari e di tessuto indotte dal virus [infezioni subcliniche]. Non evidenziano le infezioni latenti (non produttive).

- Ispezione clinica e colposcopica
- Indagine microscopica di strisci cellulari (Pap-test)
- Indagine microscopica di preparati istologici

Non sono in grado di evidenziare la presenza virale prima che questa abbia indotto alterazioni della morfologia cellulare
Diagnosi citologica
Metodi diretti

Tecniche di biologia molecolare per la:

✓ rilevazione del genoma di HPV in campioni biologici diversi (tamponi genitali cervicali, vaginali, vulvari, anali, biopsie)

✓ identificazione del genotipo virale

Le tecniche molecolari oggi maggiormente impiegate per la ricerca e la tipizzazione dei diversi genotipi di HPV si dividono in:

- Tecniche di ibridazione
- Tecniche di amplificazione
Il ruolo delle infezioni multiple di HPV

25% delle infezioni, più frequenti negli immunocompromessi

La contemporanea presenza e attiva replicazione di più ceppi potenzialmente oncogeni nel medesimo micro-ambiente potrebbe agire in modo sinergico al processo patogenetico

Diversi studi hanno mostrato la presenza di più tipi virali in carcinomi cervicali

Le infezioni miste possono complicare l’uso delle procedure di tipizzazione mediante sequenziamento
SCREENING PRIMARIO

Sostituzione o integrazione del Pap-Test nello screening primario con genotipizzazione HPV

Pro

- Test molecolari più sensibili del Pap-Test per le CIN di alto grado
- VPN del test HPV-DNA prossimo al 100%
- Allungamento dell’intervallo di screening in caso di negatività del Pap-Test e di HPV

Contro

- Aumento del numero dei casi HPV positivi e citologia/colposcopia negativi
- Aumento del numero dei pazienti da seguire con follow-up
5.3.2 Signal amplification

The most widely-used signal amplification method for HPV is the Digene® Hybrid Capture® 2 (HC2) HPV Test (Qiagen). The assay uses a simple alkaline lysis to release sample DNA for hybridization to the probe cocktail of RNA probes complementary to 13 HR HPV types [16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68]. Ribonucleic acid (RNA)-DNA hybrids indicate the presence of HPV DNA, as these are never found naturally. A monoclonal antibody specific for RNA-DNA hybrids bound to microtitre plates is used to capture these hybrids. After washing to remove non-specifically bound material, the DNA-RNA hybrids are detected with the same monoclonal antibody conjugated to alkaline phosphatase. Localized enzyme is detected and quantitated with the use of a chemiluminescence detection system. This semi-quantitative assay is designed for clinical use as an indicator of the risk of cervical neoplasia. It is very reproducible with a very good inter-laboratory comparison and very good negative predictive values for high grade lesions. A low-cost version has been designed to be performed in low-resource settings requiring minimal equipment and training (1). The assay does not distinguish specific types, and, for this reason, is not envisaged to play a role in epidemiological studies required for HPV type-specific vaccine assessment, and so will not be discussed further in this manual.
5.3.3 Target amplification

Target amplification systems most commonly use a PCR. While type-specific PCR assays have been developed to detect, and in some cases to quantitate, the presence of a single HPV type, epidemiologic studies generally need to address the presence of many of the most important types found in the genital tract. This is achieved by amplifying a portion of the HPV genome that is relatively conserved. When the L1 region is targeted (Figure 5.1B), the assays are referred to as L1 consensus PCR. The first consensus PCR assay was described by Manos and colleagues (2). This assay used a set of degenerate primers, MY09/MY11, and resulted in a 450 bp amplicon amenable to typing determination by subsequent molecular techniques like restriction fragment length polymorphism, DNA sequencing and reverse blotting hybridization (RBH) with type-specific oligonucleotide probes. The degenerate MY09/MY11 primers were subsequently replaced by a set of 18 defined primers, PGM09/11, to improve specificity and sensitivity (3). Additional primer sets targeting the same region of L1 are widely used. These include GP5+/6+ producing an amplicon of ~160 bp (4), and SPF10, producing an amplicon of 65 bp (5). The PGM09/11 system is commercially available as the Research Use Only (RUO) Linear Array HPV Genotyping Test (Roche), the SPF system is commercially available as INNO-LiPA HPV Genotyping v2 (Innogenetics), and GP5+/6+ systems are commercially available as RUO HPV Genotyping LQ Test (Qiagen) and Multiplex HPV Genotyping Kit (Multimetrix, Heidelberg). The GP5+/6+ and SPF primers may be preferred for paraffin-embedded material to improve sensitivity of the assay with degraded nucleic acids, owing to the small size of the amplicons.
Detection of the amplicon can be done by gel electrophoresis and ethidium bromide or GelRed staining under ultraviolet (UV) light for the PGMY and GP5+/6+ reactions. (Gel detection of PGMY products is illustrated later in the chapter as part of the protocol, see Figure 5.4). DNA hybridization in microplate enzyme-linked immunosorbent assay (ELISA)-type format is preferred for the SPF system owing to the very small size of the amplicon. Detection of the amplicon confirms the presence of HPV, with type-specific hybridization required to determine its type(s). Typing of the consensus PCR products is most commonly accomplished using reverse blotting hybridization (RBH), in which amplicon with affinity label is hybridized to an array of unlabelled type-specific probes, and detected with colorimetric or chemiluminescent methods.

The number and variety of HPV detection and typing assays, using target amplification, being developed and introduced are increasing. Laboratories must validate the sensitivity and specificity of the assay they use, whether they rely on in-house or on commercial assays, to ensure that the assay as performed in their hands allows the laboratory to be proficient in HPV testing. The approach to assay validation is covered in chapter 6. Participation in HPV proficiency testing allows laboratories to verify that they have successfully implemented HPV detection and typing assays.
5.4 HPV detection with PGMY09/11 PCR

5.4.1 Principle of assay

The PGMY primer PCR system amplifies mucosal HPV types, producing a 450 bp amplicon. Including primers for a cellular target, histocompatibility leukocyte antigen (HLA), producing an amplicon of 230 bp, confirms that DNA has been extracted in sufficient amounts and that inhibitors are not preventing amplification. Amplicons are detected after agarose gel electrophoresis and GelRed staining under UV transillumination, and recorded with a camera. Samples with an HPV band are considered adequate and should be typed by RBH following protocol in section 5.5. Samples without HPV and HLA bands cannot be interpreted and are considered inadequate.

Gel electrophoretic analysis is used to limit the number of samples subjected to RBH to further diminish the cost of typing. If the prevalence of infection is high, then RBH can be used directly after the PCR. In this case, RBH negatives should be verified by gel electrophoresis. A sample with an HPV band that is RBH negative indicates that a type not included in RBH may be present.
Famiglia Herpesviridae

IL VIRIONE
• Diametro 200 nm
• Provvisto di envelope
• Capside icosaedrico
• Genoma: una molecola lineare di DNA a doppia elica

CARATTERISTICHE COMUNI:
- Diffusione geografica
- Strutturali e morfologiche
- Storia naturale dell’infezione (infezione primaria, latenza, riattivazione)

Sieroprevalenza Herpes Simplex 2
Prevalenza dell’infezione

Popolazione USA di 15 e 49 anni

1% Infezione clinica
4% Infezione subclinica (colposcopia)
10% Infezione subclinica (PCR)
60% Infezione pregressa (Ab)
25% Nessuna infezione attuale o pregressa

Mod. da Koutsky L, Am J Med 1997
Eliminazione virale asintomatica

- Avviene nella maggior parte degli individui infetti (>90%), meno comune nell’infezione da HSV1
- Più frequente durante il primo anno dall’acquisizione dell’infezione
- L’assenza di sintomi favorisce il contagio a partner sieronegativi
Il 90% degli individui infetti non sanno di esserlo

- Molte infezioni sono lievi e misconosciute
- I pz attribuiscono i sintomi ad altro


- Follicollite
- Zipper burn
- Emorroidi
- Pantaloni stretti
- Allergia condoms
- Infezione fungina
- Vaginite
- Infezione urinaria
- Sintomi mestruali
- Emorroidi
- Irritazione da depilazione
- allergia

Utilità clinica della corretta diagnosi

- Il rischio di trasmissione si riduce se si sa di essere infettanti
- Il pz può astenersi dai rapporti sessuali durante la fase attiva dell’infezione
- Il pz può ricevere la terapia soppressiva
- Le coppie possono essere istruite sull’uso del preservativo
- Una corretta diagnosi evita l’errata gestione clinica dei segni/sintomi
METODI DIAGNOSTICI DISPONIBILI

 DIAGNOSI DIRETTA
- ISOLAMENTO VIRALE
- RICERCA DIRETTA ANTIGENI VIRALI
- BIOLOGIA MOLECOLARE

 DIAGNOSI INDIRETTA
- SIEROLOGIA
DIAGNOSI VIROLOGICA DIRETTA

Il campione biologico migliore

Tampone su lesione

Scraping

Il prelievo deve essere eseguito prima di iniziare il trattamento farmacologico e possibilmente quando le lesioni sono ancora in fase florida

Il prelievo di sangue è utile solo in casi selezionati
RICERCA DEL GENOMA VIRALE - PCR-

- **Presenta una elevata sensibilità e rapidità (tempi di risposta in 6-8 ore)**

- **Non è necessario l’invio immediato al Laboratorio, il campione può essere conservato a +4°C anche per alcuni giorni**

- **Non sono consigliate particolari precauzioni di trasporto**
Trichomonas vaginalis infection:
the most prevalent nonviral sexually transmitted infection receives the least public health attention

Barbara Van Der Pol, CID 2007, 44:23-25.
• *Trichomonas vaginalis* non è fisiologicamente presente a livello vaginale

• si trasmette per via sessuale come *Chlamydia trachomatis* e *Neisseria gonorrhoeae*

• epidemiologia: 170 - 190 milioni casi/anno (stima WHO), maggiore incidenza in popolazioni a basso standard

• Sintomatologia: forme asintomatiche (30%) → vaginiti, cerviciti, uretriti: leucorrea, irritazione vulvare, disuria, dispareunia, perdite maleodoranti (se associato a vaginosi batterica)

*Barbara Van Der Pol, CID, 2007, 44: 25-25*
• Corso naturale dell’infezione: guarigione spontanea, ma in assenza di terapia l’infezione può persistere per oltre 3 mesi

• Complicanze: PID, parto pretermine, basso peso alla nascita


Barbara Van Der Pol, CID, 2007, 44: 25-25
DIAGNOSI

Clinica
“Falsi negativi”: casi asintomatici

“Falsi positivi”: vaginiti da altre cause o vaginosi batterica

Microbiologica
• Esame microscopico a fresco
• Isolamento colturale
• PCR
• Test rapidi
Diagnosi microbiologica

Esame microscopico a fresco
400x: numerosi leucociti e Trichomonas mobili flagellati

Sensibilità: 60-70% in pazienti sintomatiche e con personale esperto

Specificità: 100%

Esame microscopico dopo colorazione May Grünwald-Giemsa
Esame colturale in terreno di Diamond’s (CDC): gold standard
Anaerobiosi: non è essenziale (è sufficiente chiudere bene la provetta)
Esaminare dopo 3-5 giorni
Sensibilità >>> esame microscopico.
Fattore limitante: inoculo (falsi negativi in caso di bassa carica)
Importante soprattutto in pazienti asintomatiche

Specificità: 100%
Growth of *Trichomonas vaginalis* in Commercial Culture Media

SHELDON M. GELBART, JESSICA L. THOMASON,* PETER J. OSYPOWSKI, ARLENE V. KELLETT, JANINE A. JAMES, AND FREDRIK F. BROEKHUIZEN

*Department of Obstetrics and Gynecology, University of Wisconsin Medical School, Milwaukee, Wisconsin 53201-0342*

---

**Diamond's:**

Recupero: 6 ceppi/6

Motilità: lieve perdita, evidente dopo 72h

Fase esponenziale: <96 h

7 gg: perdita completa della motilità
1086 campioni biologici testati

Positivi: 14.5% con il test molecolare
7.0 % dell’esame microscopico a fresco
Comparison between the Gen-Probe Transcription-Mediated Amplification
Trichomonas vaginalis Research Assay and Real-Time PCR for
Trichomonas vaginalis Detection Using a Roche LightCycler
Instrument with Female Self-Obtained Vaginal Swab
Samples and Male Urine Samples

Andrew Hardick, Justin Hardick, Billie Jo Wood, and Charlotte Gaydos

Transcription-mediated amplification (Gene-Probe, Inc)
sensibilità = 96.7%
specificità = 97.5%
Two Unusual Occurrences of Trichomoniasis: Rapid Species Identification by PCR

A. P. Bellanger, O. Cabaret, J. M. Costa, F. Foulet, S. Bretagne, and F. Botterel

Laboratoire de Parasitologie-Mycologie, Hôpital Henri Mondor (AP-HP), Créteil, France

Received 15 February 2008/Returned for modification 3 June 2008/Accepted 9 July 2008

PCR analysis in two unusual occurrences of trichomoniasis, trichomonal empyema due to *Trichomonas tenax* and *Trichomonas vaginalis* in an infant urine sample, allowed us to obtain rapid and accurate trichomonad species identification. The weak sensitivity of wet preparations and the low viability of the flagellates can be remedied by the PCR method.
TRICHOMONAS

Comparison of a TaqMan-based real-time polymerase chain reaction with conventional tests for the detection of Trichomonas vaginalis

A Pillay, F Radebe, G Fehler, Y Htun, R C Ballard

Objective: To compare a TaqMan-based real-time polymerase chain reaction (PCR) with conventional PCR, culture, and wet-mount microscopy for the diagnosis of trichomoniasis in women.

Methods: Vaginal swabs from 119 women were tested for Trichomonas vaginalis by wet mount and culture. Paired vaginal lavage and urine specimens were tested by conventional and real-time PCR.

Results: Using an expanded “gold standard”, defined as a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers, the overall prevalence of T vaginalis in the study population was 65.5% (78/119). The detection rate of T vaginalis was 65.5% (78/119) and 36.9% (44/119) by conventional PCR using vaginal washings and urine specimens, respectively; 68.9% (82/119) by real-time PCR using vaginal washings and 61.3% (73/119) by real-time PCR using urine specimens. The sensitivities of conventional PCR using vaginal washings and urine and real-time PCR using vaginal washings and urine, compared with the gold standard were 100%, 56.4%, 100% and 76.7%, and the specificities of these tests were 100%, 97.6%, 82.9% and 97%, respectively.

Conclusions: The real-time PCR test proved to be significantly more sensitive than culture and wet-mount microscopy, although its specificity was slightly lower than these tests. In addition, it was more sensitive, rapid and less time consuming than conventional PCR for the detection of T vaginalis.
### Table 2  Performance of diagnostic tests for *T vaginalis* (n = 119)*

<table>
<thead>
<tr>
<th>Assay or test</th>
<th>True positive</th>
<th>False positive</th>
<th>False negative</th>
<th>True negative</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture, vaginal swab</td>
<td>34</td>
<td>0</td>
<td>44</td>
<td>41</td>
<td>43.6 (32.4 to 55.3)</td>
<td>100 (91.4 to 100)</td>
</tr>
<tr>
<td>Wet preparation, vaginal swab</td>
<td>34</td>
<td>1</td>
<td>44</td>
<td>40</td>
<td>43.6 (32.4 to 55.3)</td>
<td>97.6 (87.1 to 99.9)</td>
</tr>
<tr>
<td>Conventional PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal washing</td>
<td>78†</td>
<td>0</td>
<td>0</td>
<td>41</td>
<td>100 (95.4 to 100)</td>
<td>100 (91.4 to 100)</td>
</tr>
<tr>
<td>Urine</td>
<td>44‡</td>
<td>1</td>
<td>34</td>
<td>40</td>
<td>56.4 (44.7 to 67.6)</td>
<td>97.6 (87.1 to 99.9)</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal washing</td>
<td>78†</td>
<td>7</td>
<td>0</td>
<td>34</td>
<td>100 (95.4 to 100)</td>
<td>82.9 (67.9 to 92.8)</td>
</tr>
<tr>
<td>Urine</td>
<td>66§</td>
<td>1</td>
<td>20</td>
<td>32</td>
<td>76.7 (66.4 to 85.2)</td>
<td>97.0 (84.2 to 99.9)</td>
</tr>
</tbody>
</table>

*PCR: polymerase chain reaction.

*The gold standard was a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers (n = 78).

†Includes all 34 culture-positive specimens.

‡Six culture-positive specimens were negative by this assay.

§One culture-positive specimen was negative by this assay.
Detection of Trichomonosis in Vaginal and Urine Specimens from Women by Culture and PCR

LISA F. LAWING,¹ SPENCER R. HEDGES,² AND JANE R. SCHWEBKE¹,³*

Departments of Medicine/Infectious Diseases¹ and Microbiology,² University of Alabama at Birmingham, Birmingham, Alabama 35294, and Jefferson County Department of Health, Birmingham, Alabama 35202³

Received 17 May 2000/Returned for modification 4 July 2000/Accepted 8 August 2000

Vaginal trichomonosis is a highly prevalent infection which has been associated with human immunodeficiency virus acquisition and preterm birth. Culture is the current “gold standard” for diagnosis. As urine-based testing using DNA amplification techniques becomes more widely used for other sexually transmitted diseases (STDs) such as gonorrhea and chlamydia, a similar technique for trichomoniasis would be highly desirable. Women attending an STD clinic for a new complaint were screened for Trichomonas vaginalis by wet-preparation (wet-prep) microscopy and culture and for the presence of T. vaginalis DNA by specific PCR of vaginal and urine specimens. The presence of trichomoniasis was defined as the detection of T. vaginalis by direct microscopy and/or culture from either vaginal samples or urine. The overall prevalence of trichomoniasis in the population was 28% (53 of 190). The sensitivity and specificity of PCR using vaginal samples were 89 and 97%, respectively. Seventy-four percent (38 of 51) of women who had a vaginal wet prep or vaginal culture positive for trichomonads had microscopic and/or culture evidence of the organisms in the urine. Two women were positive for trichomonads by wet prep or culture only in the urine. The sensitivity and specificity of PCR using urine specimens were 64 and 100%, respectively. These results indicate that the exclusive use of urine-based detection of T. vaginalis is not appropriate in women. PCR-based detection of T. vaginalis using vaginal specimens may provide an alternative to culture.
**TABLE 1. Comparison of diagnostic tests for T. vaginalis in females**

<table>
<thead>
<tr>
<th>Diagnostic method</th>
<th>No. true positive</th>
<th>No. false positive</th>
<th>No. false negative</th>
<th>No. true negative</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>Positive</td>
</tr>
<tr>
<td>Vaginal swab culture</td>
<td>50</td>
<td>0</td>
<td>3</td>
<td>137</td>
<td>94.3</td>
<td>100</td>
<td>96.6-100</td>
</tr>
<tr>
<td>Vaginal PCR</td>
<td>47</td>
<td>4</td>
<td>6</td>
<td>133</td>
<td>88.7</td>
<td>97.1</td>
<td>92.2-99.1</td>
</tr>
<tr>
<td>Vaginal wet prep</td>
<td>31</td>
<td>0</td>
<td>22</td>
<td>137</td>
<td>58.5</td>
<td>100</td>
<td>96.6-100</td>
</tr>
<tr>
<td>Urine culture</td>
<td>32</td>
<td>0</td>
<td>21</td>
<td>137</td>
<td>60.4</td>
<td>100</td>
<td>96.6-100</td>
</tr>
<tr>
<td>Urine PCR</td>
<td>34</td>
<td>0</td>
<td>19</td>
<td>137</td>
<td>64.2</td>
<td>100</td>
<td>96.6-100</td>
</tr>
<tr>
<td>Urine wet prep</td>
<td>31</td>
<td>0</td>
<td>22</td>
<td>137</td>
<td>58.5</td>
<td>100</td>
<td>96.6-100</td>
</tr>
</tbody>
</table>

\( n = 190 \). The gold standard is trichomonads visualized from any wet prep or culture \( n = 53 \). CI, confidence interval.
**Micooplasmì**

**Famiglia:** *Mycoplasmataceae*

**Classe:** Mollicutes

**Caratteristiche:**
- i più piccoli batteri capaci di vita autonoma
- assenza di parete cellulare
- pleiomorfi
- particolari esigenze nutrizionali (steroli)

**Origine:**
forse evoluzione degenerativa da batteri Gram+ appartenenti al gruppo dei Lattobacilli
## Micoplasmi genitali

<table>
<thead>
<tr>
<th>Micoplasma</th>
<th>Frequenza</th>
<th>Metabolismo</th>
<th>Atmosfera</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. urealyticum</em></td>
<td>Comune</td>
<td>Urea</td>
<td>Anaerobia</td>
</tr>
<tr>
<td><em>M. hominis</em></td>
<td>Comune</td>
<td>Arginina</td>
<td>Aerobica</td>
</tr>
<tr>
<td><em>M. fermentans</em></td>
<td>Occasionale</td>
<td>Glucosio+arginina</td>
<td>Anaerobica</td>
</tr>
<tr>
<td><em>M. genitalium</em></td>
<td>Comune</td>
<td>Glucosio</td>
<td>Anaerobica</td>
</tr>
<tr>
<td><em>M. penetrans</em></td>
<td>Occasionale</td>
<td>Glucosio+arginina</td>
<td>Anaerobica</td>
</tr>
</tbody>
</table>
**U. urealyticum**

14 serovar, divisi in 2 biovar per le caratteristiche genotipiche:

Biovar 1: serovar (parvo) 1,3,6,14
Biovar 2: serovar (T960) 2,4,5,7,8,9,10,11,12,13

Attualmente diviso in 2 specie:

*U. parvum* (ex- *U. urealyticum* biovar 1), la maggioranza degli isolati

*U. urealyticum* (ex- *U. urealyticum* biovar 2)
Detection of *Ureaplasma* biovars and polymerase chain reaction-based subtyping of *Ureaplasma parvum* in women with or without symptoms of genital infections

M. A. De Francesco · R. Negrini · G. Pinsi · L. Peroni · N. Manca
Fig. 1 *Ureaplasma parvum* serovars and T960 biovar distribution by age. The numbers of genital *Ureaplasma* isolates were 24, 32, 39, 34 and 29 for each year age group, respectively.
### Table 2  Relationship between vaginal flora changes and *Ureaplasma* biovars and serovars

<table>
<thead>
<tr>
<th>Vaginal flora</th>
<th>Parvo biovar Serovar 1</th>
<th>Parvo biovar Serovar 3/14</th>
<th>Parvo biovar Serovar 6</th>
<th>T960 biovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>31/94 (33%)</td>
<td>26/94 (28%)*</td>
<td>28/94 (30%)**</td>
<td>9/94 (9%)*</td>
</tr>
<tr>
<td>Absence of lactobacilli</td>
<td>13/45 (29%)</td>
<td>21/45 (47%)</td>
<td>3/45 (2%)</td>
<td>10/45 (22%)</td>
</tr>
</tbody>
</table>

*P*<0.05; **P*<0.01

### Table 3  Relationship between clinical symptomatology and *Ureaplasma* biovars and serovars

<table>
<thead>
<tr>
<th></th>
<th>Parvo biovar Serovar 1</th>
<th>Parvo biovar Serovar 3/14</th>
<th>Parvo biovar Serovar 6</th>
<th>T960 biovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic subjects</td>
<td>26/80 (32%)</td>
<td>35/80 (44%)*</td>
<td>3/80 (4%)**</td>
<td>16/80 (20%)*</td>
</tr>
<tr>
<td>Asymptomatic subjects</td>
<td>18/59 (31%)</td>
<td>12/59 (20%)</td>
<td>26/59 (44%)</td>
<td>3/59 (5%)</td>
</tr>
</tbody>
</table>

*P*<0.05; **P*<0.01
Patologie associate

<table>
<thead>
<tr>
<th>Patologia</th>
<th><em>U. urealyticum</em></th>
<th><em>M. hominis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>UNG</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Prostatite</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Epididimite</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Cervicite</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>MIP</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Vaginosi batterica</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Parto pretermine</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Aborto spontaneo</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Febbre post-partum</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Infertilità</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Artrite reattiva</td>
<td>?</td>
<td>-</td>
</tr>
</tbody>
</table>
Campioni biologici: tampone vaginale e cervicale

Esame colturale:
• inoculazione in brodo urea-arginina
• semina in piastra di terreno agarizzato A7
• incubazione del brodo a 37°C per 24 h
• incubazione delle piastre a 37°C per 48 h in anaerobiosi o microaerofilia
• esame del viraggio di colore del brodo
• osservazione microscopica delle colonie cresciute su terreno solido
Coltura in brodo e isolamento su terreno solido selettivo

Inseminare il brodo urea-arginina immediatamente dopo il prelievo

Prelevare con una pipetta Pasteur una aliquota di brodo

Incubare il brodo 24 ore a 37°C con il flacone tappato

Incubare l’agar per 24-48 ore a 37°C in anaerobiosi o in microaerofilìa

Seminare l’agar con 3 gocce non confluenti, senza spanderle
Osservazione del viraggio dell’indicatore presente nel brodo

Il colore del brodo

- Rosso lampone
- Rosso arancio
- Giallo limone
- Giallo arancione
Semina in microgallerie contenenti substrati e antibiotici in forma liofila e incubate a 37°C per 48h. Identificazione in base alle reazioni biochimiche e alla sensibilità agli antibiotici.

Affidabile????
Mycoplasma genitalium

- Identificato per la prima volta negli anni ’80
- Estremamente difficile la coltura e l’isolamento
- Diagnosi solo mediante amplificazione genica
- Ad oggi dimostrata l’associazione con:
  - uretrite non gonococcica
  - cervicite
  - MIP
  - endometrite
  - infertilità da fattore tubarico
Etiologies of Nongonococcal Urethritis: Bacteria, Viruses, and the Association with Orogenital Exposure

Catriona S. Bradshaw,1,2 Sepehr N. Tabrizi,3,4 Timothy R. H. Read,1 Suzanne M. Garland,3,4 Carol A. Hopkins,1 Lorna M. Moss,1 and Christopher K. Fairley1,2
1Melbourne Sexual Health Centre, The Alfred Hospital, 2School of Population Health and 3Department of Obstetrics and Gynaecology, University of Melbourne, and 4Department of Microbiology and Infectious Diseases, The Royal Women’s Hospital, Victoria, Australia

336 • JID 2006:193 (1 February) • Bradshaw et al.

Results. C. trachomatis (20%), M. genitalium (9%), adenoviruses (4%), and HSV-1 (2%) were more common in cases with NGU (n = 329) after age and sexual risk were adjusted for (P ≤ .01); U. urealyticum, U. parvum, and G. vaginalis were not. Infection with adenoviruses or HSV-1 was associated with distinct clinical features, oral sex, and male partners, whereas infection with M. genitalium or C. trachomatis was associated with unprotected vaginal sex. Oral sex was associated with NGU in which no pathogen was detected (P ≤ .001). Fewer than 5 polymorphonuclear leukocytes (PMNLs) per high-power field (HPF) on urethral smear were present in 32%, 37%, 38%, and 44% of cases with C. trachomatis, M. genitalium, adenoviruses, and HSV, respectively.
Management of Women with Cervicitis

Jeanne M. Marrazzo¹ and David H. Martin²

¹Department of Medicine, University of Washington, Seattle; and ²Louisiana State University Health Sciences Center, New Orleans

In the past several years, the collective understanding of cervicitis has extended beyond the recognition of Chlamydia trachomatis and Neisseria gonorrhoeae as the prime etiologic suspects. Trichomonas vaginalis and herpes simplex virus cause cervicitis, and both Mycoplasma genitalium and bacterial vaginosis have emerged as new candidate etiologic agents or conditions. However, major gaps in our knowledge of this common condition remain. Putative etiologic agents have not been identified in many women with cervicitis. Moreover, cervicitis occurs in a relatively small proportion of women with chlamydia or gonorrhea. Finally, scant research has addressed the clinical response of nonchlamydial and nongonococcal cervicitis to antibiotic therapy, and there are no data on the benefit of sex partner treatment for such women. New research into the etiology, immunology, and natural history of this common condition is needed, especially in view of the well-established links between cervicitis and an increased risk of upper genital tract infection and human immunodeficiency virus type 1 acquisition.
**Results:** Overall prevalence of infection with *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium* was found to be 11.1%, 4.6%, 15.3%, and 19.2%, respectively. Prevalence in women with cervicitis was 15.8%, 6%, 18.9%, and 28.6% for *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, and *M. genitalium*, respectively. Percentages of coinfections were high. *C. trachomatis* and *M. genitalium* were significantly associated with cervicitis in univariate analysis, but only *M. genitalium* was significantly associated with cervicitis (AOR: 2.5) in multiple logistic regression models.
Mycoplasma genitalium Compared to Chlamydia, Gonorrhea and Trichomonas as an Etiologic Agent of Urethritis in Men Attending STD Clinics

Charlotte Gaydos¹,* , Nancy E. Maldeis², Andrew Hardick¹, Justin Hardick¹, and Thomas C. Quinn¹,³


Results—The overall prevalences of infection with C. trachomatis, N. gonorrhoeae, T. vaginalis, and M. genitalium, were 20.3%, 12.8%, 3.4%, and 15.2% respectively. Prevalences in men with urethritis were 32.7%, 24.2%, 5.2%, and 22.2% for C. trachomatis, N. gonorrhoeae, T. vaginalis, and M. genitalium, respectively. Percentages of coinfections were high. All men with N. gonorrhoeae had urethritis. C. trachomatis and M. genitalium were found to be significantly associated with urethritis in univariate analysis and in multiple logistic regression analysis.

Conclusion—The association of M. genitalium with urethritis in this study provides confirmation of the importance of screening men for M. genitalium as a cause of non-gonococcal urethritis and supports treatment considerations for urethritis for agents other than gonococci and chlamydia.
Certezze:
causa di uretrite maschile e di PID e infertilità femminile

Incertezze:
causa di cervicite (prevalenza troppo alta, imprecisa definizione di cervicite, diverse popolazioni esaminate)

Auspicio:
disponibilità di test commerciali per saggire in modo sistematico tutte le donne sintomatiche
Clinical Presentation of *Mycoplasma genitalium* Infection versus *Neisseria gonorrhoeae* Infection among Women with Pelvic Inflammatory Disease

Vanessa L. Short¹, Patricia A. Totten², Roberta B. Ness¹, Sabina G. Astete², Sheryl F. Kelsey¹, and Catherine L. Haggerty¹

¹Department of Epidemiology, University of Pittsburgh, Pittsburgh, Pennsylvania

²Department of Medicine, Division of Infectious Diseases, University of Washington, Seattle

Presentazione clinica più simile alla MIP da *C. trachomatis*
Mycoplasma genitalium is associated with symptomatic and asymptomatic non-gonococcal urethritis in men

H Moi,¹ N Reinton,² A Moghaddam²  


Key messages

► Mycoplasma genitalium is associated with non-gonococcal urethritis (NGU) in men with and without clinical symptoms  
► M genitalium is associated with the symptoms of NGU.  
► M genitalium is associated with the severity of NGU.

Mycoplasma genitalium in women with lower genital tract inflammation

H Moi,¹ N Reinton,² A Moghaddam²  


Key messages

► Mycoplasma genitalium is associated with lower genital tract inflammation in women.  
► Cervical swabs have higher sensitivity than urine for detecting M genitalium by PCR.  
► Urine is more specific than cervical swabs in the analysis of the association of M genitalium infection with lower genital tract inflammation.
2009 European Guideline on the Management of Male Non-gonococcal Urethritis

M Shahmanesh MD FRCP*, H Moi MD PhD†, F Lassau MD‡ and M Janier MD PhD‡

*Department of Genitourinary Medicine, Whittall Street Clinic, Birmingham, UK; †Olafstikliniken Oslo, Oslo, Norway; ‡STD Clinic, Hôpital Saint-Louis AP-HP, Paris, France

Keywords: urethritis (male), Chlamydia trachomatis, Mycoplasma genitalium, NGU (non-gonococcal urethritis), doxycycline, azithromycin


Table 1 Prevalence of the most common pathogens detected from patients with NGU

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. trachomatis</td>
<td>11–43%</td>
<td>7,8,11,12,16,19,23–25,27,82</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>9–25%</td>
<td>5–7,12,13,16,22,23,25,27,38,83,84</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>2–4%</td>
<td>27,32</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td>1–20%</td>
<td>28,82,85–87</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>2–3%</td>
<td>27,33</td>
</tr>
</tbody>
</table>

Tetracyclines and azithromycin are generally effective against C. trachomatis though sporadic reports of treatment failure have been reported with tetracyclines. While in general treatments that are effective against C. trachomatis appear to be also effective in NGU, tetracyclines and azithromycin in the doses used do not consistently eradicate M. genitalium (IIa, B).
STD4D ACE Detection

Simultaneous identification from a single sample:

✓ Mycoplasma hominis
✓ Mycoplasma genitalium
✓ Ureaplasma urealyticum
✓ Ureaplasma parvum

Diagnosi di infezione da *M. genitalium*
Caratteristiche del sistema

- dotato di controllo interno (controllo di PCR)
- validato per l’uso nei seguenti campioni:
  urina, tamponi del tratto vaginale (uretrali, vaginali, endocervicali) e campioni per citologia in fase liquida
- Geni target:

  Mycoplasma hominis  \[\rightarrow\] gap (gliceraldeide-3-fosfato deidrogenasi)
  Mycoplasma genitalium  \[\rightarrow\] gyrA (subunità A della DNA girasi)
  Ureaplasma urealyticum  \[\rightarrow\] ureasi
  Ureaplasma parvum  \[\rightarrow\] ureasi
### Automatic target identification

#### RESULTATI

<table>
<thead>
<tr>
<th>Lane</th>
<th>L2</th>
<th>L3</th>
<th>L4</th>
<th>L5</th>
<th>L6</th>
<th>L7</th>
<th>L8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal control</td>
<td>+</td>
<td>101</td>
<td>+</td>
<td>105</td>
<td>+</td>
<td>105</td>
<td>+</td>
</tr>
<tr>
<td>M. hominis</td>
<td>+</td>
<td>105</td>
<td>+</td>
<td>110</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>U. parvum</td>
<td>+</td>
<td>32</td>
<td>-</td>
<td>+</td>
<td>79</td>
<td>+</td>
<td>73</td>
</tr>
<tr>
<td>Unidentified</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Legend:**
- 

1~6: Clinical samples, 7: Negative control
6 different STD pathogen from a single sample:

- *Trichomonas vaginalis*
- *Mycoplasma genitalium*
- *Mycoplasma hominis*
- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Ureaplasma urealyticum*
Confronto fra metodo Seegene e metodo DID su 171 campioni

<table>
<thead>
<tr>
<th>Microrganismo</th>
<th>M. hominis</th>
<th>U. urealyticum</th>
<th>U. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STD4D</td>
<td>DID</td>
<td>STD4D</td>
</tr>
<tr>
<td>POSITIVI</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>TOTALE</td>
<td>STD4D vs DID 6-2</td>
<td>STD4D vs DID 13-11</td>
<td>STD4D</td>
</tr>
</tbody>
</table>
Clinical Study

Mycoplasma Genitalium Among Women With Nongonococcal, Nonchlamydial Pelvic Inflammatory Disease

Catherine L. Haggerty,1,2 Patricia A. Totten,3 Sabina G. Astete,3 and Roberta B. Ness1

1 University of Pittsburgh, Pittsburgh, PA 15260, USA
2 Department of Epidemiology, University of Pittsburgh, 130 DeSoto Street, 516B Parran Hall Pittsburgh, PA 15261, USA
3 Department of Medicine. Division of Infectious Diseases, University of Washington, Seattle, WA, USA

Received 2 February 2006; Accepted 20 February 2006

Pelvic Inflammatory disease (PID) is a frequent condition of young women, often resulting in reproductive morbidity. Although Neisseria gonorrhoeae and/or Chlamydia trachomatis are/is recovered from approximately a third to a half of women with PID, the etiologic agent is often unidentified. We need PCR to test for M. genitalium among a pilot sample of 50 women with nongonococcal, nonchlamydial endometritis enrolled in the PID evaluation and clinical health (PEACH) study. All participants had pelvic pain, pelvic organ tenderness, and leukorrhea, mucopurulent cervicitis, or untreated cervicitis. Endometritis was defined as ≥5 surface epithelium neutrophils per ×400 field absent of menstrual endometrium and/or ≥2 stromal plasma cells per ×120 field. We detected M. genitalium in 7 (14%) of the women tested: 6 (12%) in cervical specimens and 4 (8%) in endometrial specimens. We conclude that M. genitalium is prevalent in the endometrium of women with nongonococcal, nonchlamydial PID.

Copyright © 2006 Catherine L. Haggerty et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Real-time PCR detection of the mg219 gene of unknown function of *Mycoplasma genitalium* in men with and without non-gonococcal urethritis and their female partners in England

Victoria J. Chalker,¹ Karen Jordan,²† Tahir Ali¹ and Cathy Ison¹

¹Sexually Transmitted Bacteria Reference Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK
²Microbiology Laboratory, Northampton General Hospital Trust, Billing Road, Northampton NN1 5BD, UK

Real-time PCR was employed to detect a region of the *Mycoplasma genitalium* mg219 gene, a gene of unknown function, in clinical samples. Amplification of DNA and signal production from 15 other species of human mycoplasmas and 14 other bacteria and viruses did not occur. Using a panel of 208 genital and rectal samples, the sensitivity when compared to the modified *mmpA* gene (encoding the major surface protein MgpA) real-time PCR assay was found to be 100% and the specificity of the assay 99.5% with a positive predictive value of 80% and a negative predictive value of 100%. The mg219 gene was found to be in all strains of *M. genitalium* and was highly conserved. *M. genitalium* was detected in 3.9% (11/280, 95% CI 2.1–6.9) of all male specimens, in 7.7% (10/130, 95% CI 4.1–13.7) of patients with non-gonococcal urethritis (NGU) and in 0.7% (1/150, 95% CI <0.01–4.1) of patients without urethritis. The presence of *M. genitalium* was significantly associated with NGU (P < 0.01; 95% CI 0.88–0.98) and non-chlamydial non-gonococcal urethritis (P = 0.0005; 95% CI 0.84–0.97).
Mycoplasma genitalium Detected by Transcription-Mediated Amplification Is Associated With Chlamydia trachomatis in Adolescent Women

JILL S. HUPPERT, MD, MPH,* JOEL E. MORTENSEN, PhD,† JENNIFER L. REED, MD,‡ JESSICA A. KAHN, MD, MPH,* KIMBERLY D. RICH, MPH,§ AND MARCIA M. HOBBS, PhD$*

Objectives: The clinical significance of Mycoplasma genitalium (MG) infection in adolescent women is poorly understood. We compared the prevalence of MG with that of other sexually transmitted organisms such as Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and Trichomonas vaginalis (TV) and assessed the associations of MG with sexual behaviors, genitourinary symptoms, physical and laboratory findings.

Study Design: Women aged 14 to 21 years (n = 331) were recruited from an urban medical center. The subjects’ sexual behaviors, genitourinary symptoms, and physical findings were recorded. Endocervical swabs were collected for CT and NG testing and vaginal swabs for wet mount, Gram stain, TV and MG testing. MG infection was identified by nucleic acid amplification using a transcription-mediated amplification assay.

Results: MG was detected in 74 (22.4%), CT in 79 (24.4%), TV in 69 (20.2%) and NG in 35 (10.7%) subjects. MG infection was not associated with vaginal symptoms, physical evidence of cervicitis, or findings on wet mount or Gram stain. In logistic regression, variables positively associated with MG were current CT [odds ratio (OR), 2.3; 95% confidence interval (CI), 1.4–4.4] and recent sexual contact (≤7 days) (OR, 2.6; CI, 1.1–5.2). Dysuria (OR, 0.44; CI, 0.2–0.96) and use of hormonal contraception (OR, 0.25; CI, 0.1–1.0) were negatively associated with MG infection.

Conclusion: In adolescent women, MG infection was as common as chlamydial infection and trichomoniasis and more common than gonorrhea. MG was associated with CT and recent sexual contact but not with vaginal symptoms or signs of cervicitis.

From the *Division of Adolescent Medicine, †Laboratory Medicine, and ‡Emergency Medicine, Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine, Cincinnati, Ohio; Departments of §Medicine and ¶Microbiology and Immunology, University of North Carolina, Chapel Hill, North Carolina

ADOLESCENT WOMEN FREQUENTLY COMPLAIN of genitourinary symptoms and are at high risk for sexually transmitted infections (STI) with organisms such as Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), and Trichomonas vaginalis (TV). However, clinicians often cannot establish a definitive diagnosis for vaginitis or cervicitis. In addition, symptoms sometimes persist despite treatment. Infection with a sexually transmitted organism that is difficult to culture, such as Mycoplasma genitalium (MG), may explain genitourinary symptoms or poor response to treatment of other STI. Yet, little is known about the prevalence or significance of MG in adolescent women who are at high risk for STI.

MG has been identified as an etiologic agent of arthritis in men. However, in women, the results from studies that examined the associations between MG and genitourinary symptoms or signs are inconsistent. In one study using stored specimens that were...
Antimicrobial Susceptibilities of *Mycoplasma genitalium* Strains
Examined by Broth Dilution and Quantitative PCR

Ryoichi Hamasuna,¹,²* Jørgen Skov Jensen,³ and Yukio Osada²

Department of Urology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan¹; Division of Urology, Department of Surgery, Faculty of Medicine, University of Miyazaki, Kiyotake, Miyazaki, Japan²; and Mycoplasma Laboratory, Statens Serum Institut, DK-2300 Copenhagen, Denmark³

Received 28 May 2009/Returned for modification 6 July 2009/Accepted 1 September 2009

Only limited information regarding the antimicrobial susceptibilities of *Mycoplasma genitalium* is available because of difficulties in isolating *M. genitalium* strains from clinical specimens. Antimicrobial susceptibilities of 15 clinical isolates, 7 ATCC strains, and an early passage of the M30 strain were examined by the broth dilution method. Azithromycin, clarithromycin, ciprofloxacin, and moxifloxacin were the most active drugs against *M. genitalium*, and their MIC₉₀'s were 0.002, 0.008, 0.125, and 0.125 mg/liter, respectively.
<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Broth dilution</th>
<th>Quantitative TaqMan PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/liter)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=23</td>
<td>n=12</td>
</tr>
<tr>
<td></td>
<td>Range 50% 90%</td>
<td>Range 50% 90%</td>
</tr>
<tr>
<td>STX</td>
<td>0.008-0.125</td>
<td>0.016-0.25</td>
</tr>
<tr>
<td>MXF</td>
<td>0.016-0.25</td>
<td>0.031-0.5</td>
</tr>
<tr>
<td>GAT</td>
<td>0.031-0.5</td>
<td>0.063-0.5</td>
</tr>
<tr>
<td>LVX</td>
<td>0.125-2</td>
<td>0.5-8</td>
</tr>
<tr>
<td>CIP</td>
<td>0.063-8</td>
<td>1-16</td>
</tr>
<tr>
<td>NOR</td>
<td>1-64</td>
<td>4-89</td>
</tr>
<tr>
<td>MIN</td>
<td>0.031-0.25</td>
<td>NTb</td>
</tr>
<tr>
<td>DOX</td>
<td>0.063-1</td>
<td>0.125-1</td>
</tr>
<tr>
<td>TET</td>
<td>0.063-2</td>
<td>0.125-4</td>
</tr>
<tr>
<td>AZM</td>
<td>0.0002-250</td>
<td>0.002</td>
</tr>
<tr>
<td>CLR</td>
<td>0.0005-128</td>
<td>0.016</td>
</tr>
</tbody>
</table>

a n, numbers of tested strains.
b NT, not tested.
PCR Multiplex in STD
Nucleic Acid Amplification Tests for Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Rectal Infections

Laura H. Buchmann,1,2,4* Robert E. Johnson,3 Hong Cheng,2 Lauri Markowitz,3 John R. Papp,3 Frank J. Palella, Jr.,3 and Edward W. Hook III1,2

The University of Alabama at Birmingham School of Medicine, Department of Medicine, Division of Infectious Diseases, Birmingham, Alabama1; The University of Alabama at Birmingham School of Public Health, Birmingham, Alabama2; The Centers for Disease Control and Prevention, Atlanta, Georgia3; The Birmingham Veterans Administration Medical Center, Birmingham, Alabama4; and Northwestern University Feinberg School of Medicine, Division of Infectious Diseases, Chicago, Illinois5

Received 8 December 2009/Returned for modification 24 January 2010/Accepted 16 March 2010

It is uncertain which methods for the diagnosis of rectal gonococcal and chlamydial infection are optimal. This study evaluated the performance of culture and nucleic acid amplification tests (NAATs) for rectal chlamydial and gonococcal diagnosis. From July 2003 until February 2007, 441 rectal test sets were collected from individuals attending a sexually transmitted disease clinic and three HIV clinics who gave a history of anal intercourse or were women at high risk for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* infections. Rectal swab specimens were tested using culture and commercial NAATs employing transcription-mediated amplification (TMA), strand displacement amplification (SDA), and PCR amplification. Test performance was evaluated using a rotating standard by which patients were classified as infected if either two or three comparator tests were positive. Test sensitivities for the detection of *N. gonorrhoeae* ranged from 66.7% to 71.9% for culture to 100% for TMA. Specificities were 99.7% to 100% for culture and greater than 95.5% for all three NAATs. Test sensitivities for *C. trachomatis* ranged from 36.1% to 45.7% for culture and among NAATs from 91.4% to 95.8% for PCR to 100% for TMA. Specificities of the NAATs ranged from 95.6% to 98.5% (two-of-three standard) and from 88.8% to 91.8% (three-of-three standard). Over 60% and 80% of gonococcal and chlamydial infections, respectively, among men who have sex with men and over 50% of chlamydial infections in women would have been missed if the rectal site had not been tested. Currently available NAATs are more sensitive for the detection of chlamydial and gonococcal infection at the rectal site than is culture.
TABLE 2. Estimates of SDA, PCR, TMA, and culture sensitivities and specificities for detection of *N. gonorrhoeae* or *C. trachomatis* by reference standard

<table>
<thead>
<tr>
<th>Standard</th>
<th>Test</th>
<th>No. infected</th>
<th>% Sensitivity (95% CI)</th>
<th>No. uninfected</th>
<th>% Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For N. gonorrhoeae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected if any two of three comparator tests are positive; otherwise, uninfected</td>
<td>SDA</td>
<td>34</td>
<td>97.1 (84.7-99.9)</td>
<td>341</td>
<td>98.8 (97.0-99.7)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>35</td>
<td>91.4 (76.9-98.2)</td>
<td>340</td>
<td>98.5 (96.6-99.5)</td>
</tr>
<tr>
<td></td>
<td>TMA</td>
<td>33</td>
<td>100.0 (89.4-100.0)</td>
<td>342</td>
<td>98.3 (96.2-99.4)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>36</td>
<td>66.7 (49.0-81.4)</td>
<td>339</td>
<td>100.0 (98.9-100.0)</td>
</tr>
<tr>
<td>Infected if all three comparator tests are positive; otherwise, uninfected</td>
<td>SDA</td>
<td>23</td>
<td>100.0 (85.2-100.0)</td>
<td>352</td>
<td>96.0 (93.4-97.8)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>24</td>
<td>95.8 (78.9-99.9)</td>
<td>351</td>
<td>96.0 (93.4-97.8)</td>
</tr>
<tr>
<td></td>
<td>TMA</td>
<td>23</td>
<td>100.0 (85.2-100.0)</td>
<td>352</td>
<td>95.5 (92.7-97.4)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>32</td>
<td>71.9 (53.3-86.3)</td>
<td>343</td>
<td>99.7 (98.4-100.0)</td>
</tr>
<tr>
<td><strong>For C. trachomatis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected if any two of three comparator tests are positive; otherwise, uninfected</td>
<td>SDA</td>
<td>51</td>
<td>92.2 (81.1-97.8)</td>
<td>336</td>
<td>96.4 (93.8-98.1)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>57</td>
<td>80.7 (68.1-90.0)</td>
<td>330</td>
<td>98.5 (96.5-99.5)</td>
</tr>
<tr>
<td></td>
<td>TMA</td>
<td>47</td>
<td>100.0 (92.5-100.0)</td>
<td>340</td>
<td>95.6 (92.8-97.5)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>61</td>
<td>36.1 (24.2-49.4)</td>
<td>326</td>
<td>99.7 (98.3-100.0)</td>
</tr>
<tr>
<td>Infected if all three comparator tests are positive; otherwise, uninfected</td>
<td>SDA</td>
<td>21</td>
<td>100.0 (83.9-100.0)</td>
<td>266</td>
<td>89.6 (86.0-92.6)</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>22</td>
<td>95.5 (77.2-98.9)</td>
<td>365</td>
<td>91.8 (88.5-94.4)</td>
</tr>
<tr>
<td></td>
<td>TMA</td>
<td>21</td>
<td>100.0 (83.9-100)</td>
<td>366</td>
<td>88.8 (85.1-91.8)</td>
</tr>
<tr>
<td></td>
<td>Culture</td>
<td>46</td>
<td>45.7 (30.9-61.0)</td>
<td>341</td>
<td>99.4 (97.9-99.9)</td>
</tr>
</tbody>
</table>
Detection of *Chlamydia trachomatis* and *Mycoplasma hominis*, *genitalium* and *Ureaplasma urealyticum* by Polymerase Chain Reaction in patients with sterile pyuria

Fadel A. Nassar¹, Farid H Abu-Elameen², Mohammad E. Shubair³, Fadel A Sharif⁴

¹ Department of Medical Technology Islamic University of Gaza, Palestine
² Department of Medical Microbiology, Central Laboratory and Blood Bank, Al-Shifa Hospital, Palestinian Ministry of Health, Gaza, Palestine

ABSTRACT

**Purpose:** *Chlamydia trachomatis* and *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* are associated with various diseases of the urogenital tract, but they are usually not detected by routine microbiological diagnosis. To determine the occurrence of *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* in patients with sterile pyuria.

**Material/Methods:** Sterile pyuria urine samples collected during the period from February 2006 to April 2007 were tested by polymerase chain reaction (PCR) for the presence of *C. trachomatis*, *M. hominis*, *M. genitalium*, and *U. urealyticum* using specific primers for each species. A total of 206 sterile pyuria samples selected from about 2400 urine samples attending the genitourinary clinic at Al-Shifa hospital, Gaza, during the period February 2006 to April 2007 were analyzed for routine urine examination and cultured on MacConkey agar, blood agar, and subculture agar to detect the presence of bacteria and *Candida*. The 200 samples (96 male, 104 female; aged 21 years; containing more than 10 leukocytes/µL and negative for culture (showing no significant growth after 24 h) were tested by PCR for *C. trachomatis* and *M. hominis*, *M. genitalium*, and *U. urealyticum*.

**Results:** *C. trachomatis* was detected in 20 samples (10%), *U. urealyticum* in 10 samples (5%), *M. hominis* in 5 samples (3%) and *M. genitalium* in 2 samples (1%). The difference in occurrence of *C. trachomatis* was statistically insignificant between males and females (P=0.509), whereas it was significant (P=0.008) for *U. urealyticum*. *M. hominis* was detected only in samples collected from female patients. On the other hand, *M. genitalium* was detected only in males.

**Conclusion:** PCR testing of sterile pyuria showed a significant number of *C. trachomatis*, *Mycoplasma*, and *Ureaplasma* infections. Consequently, PCR is recommended for the detection of these microorganisms in the urine samples of sterile pyuria patients.
Table 2. Microorganisms identified in the 200 studied specimens.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>male</th>
<th></th>
<th>female</th>
<th></th>
<th>total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td>C. trachomatis</td>
<td>11</td>
<td>(5.5)</td>
<td>9</td>
<td>(4.5)</td>
<td>20</td>
<td>(10.0)</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td>1</td>
<td>(0.5)</td>
<td>9</td>
<td>(4.5)</td>
<td>10</td>
<td>(5.0)</td>
</tr>
<tr>
<td>M. hominis</td>
<td>0</td>
<td>(0.0)</td>
<td>6</td>
<td>(3.0)</td>
<td>6</td>
<td>(3.0)</td>
</tr>
<tr>
<td>M. genitalium</td>
<td>2</td>
<td>(1.0)</td>
<td>0</td>
<td>(0.0)</td>
<td>2</td>
<td>(1.0)</td>
</tr>
</tbody>
</table>
Multiplex PCR Testing Detection of Higher-than-Expected Rates of Cervical Mycoplasma, Ureaplasma, and Trichomonas and Viral Agent Infections in Sexually Active Australian Women

Christopher J. McIver,1,2,3 Nikolas Rismanto,1 Catherine Smith,1 Zin Wai Naing,1 Ben Rayner,1 M. Josephine Lusk,1 Pamela Konecny,2,4 Peter A. White,2 and William D. Rawlinson1,4,*

Viology Division, Microbiology Department (SEALS), Prince of Wales Hospital,1 School of Medical Sciences,2 School of Biotechnology and Biomolecular Sciences, University of New South Wales,3 and Department of Immunology and Infectious Diseases, St. George Hospital,4 Sydney, Australia

Received 28 September 2008/Returned for modification 12 November 2008/Accepted 13 February 2009

Knowing the prevalence of potential etiologic agents of nongonococcal and nontreponemal cervicitis is important for improving the efficacy of empirical treatments for this commonly encountered condition. We describe four multiplex PCRs (mPCRs), designated VDL05, VDL06, VDL07, and VDL09, which facilitate the detection of a wide range of agents either known to be or putatively associated with cervicitis, including cytomegalovirus (CMV), enterovirus (EV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), herpes simplex virus type 1 (HSV-1), and herpes simplex virus type 2 (HSV-2) (VDL05); Ureaplasma parvum, Urea

plastra urealyticum, Mycoplasma genitalium, and Mycoplasma hominis (VDL06); Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum, and group B streptococci (VDL07); and adenovirus species A to E (VDL09). The mPCRs were used to test 233 cervical swabs from 175 women attending a sexual-health clinic in Sydney, Australia, during 2006 and 2007. The agents detected alone or in combination in all cervical swabs (percentage of total swabs included CMV (6.0), EV (2.1), EBV (2.6), VZV (4.7), HSV-1 (2.6), HSV-2 (0.8), HSV-2 and VZV (0.4), U. parvum (57.0), U. urealyticum (61.0), M. genitalium (1.5), M. hominis (15.7), C. trachomatis (0.4), T. vaginalis (3.4), and group B streptococci (0.4). Adenovirus species A to E and T. pallidum were not detected. These assays are adaptable for routine diagnostic laboratories and provide an opportunity to measure the true prevalence of microorganisms potentially associated with cervicitis and other genital infections.
<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>mPCR</th>
<th>No. (%) detected</th>
<th>Total cervical swabs (n = 235)</th>
<th>Total women (n = 175)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Molllicues</strong></td>
<td>VDL06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U. parvum</td>
<td></td>
<td></td>
<td>112 (48.0)</td>
<td>93 (53.1)</td>
</tr>
<tr>
<td>U. urealyticum</td>
<td></td>
<td></td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
</tr>
<tr>
<td>M. hominis</td>
<td></td>
<td></td>
<td>15 (6.4)</td>
<td>13 (7.4)</td>
</tr>
<tr>
<td>M. genitalium</td>
<td></td>
<td></td>
<td>3 (1.3)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>U. parvum + M. hominis</td>
<td></td>
<td></td>
<td>15 (6.4)</td>
<td>13 (7.4)</td>
</tr>
<tr>
<td>U. urealyticum + M. hominis</td>
<td></td>
<td></td>
<td>2 (0.9)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>U. parvum + U. urealyticum</td>
<td></td>
<td></td>
<td>6 (2.6)</td>
<td>5 (2.9)</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>VDL05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td></td>
<td></td>
<td>14 (6.0)</td>
<td>11 (6.3)</td>
</tr>
<tr>
<td>EV</td>
<td></td>
<td></td>
<td>5 (2.1)</td>
<td>5 (2.8)</td>
</tr>
<tr>
<td>EBV</td>
<td></td>
<td></td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td></td>
<td>6 (2.6)</td>
<td>6 (3.4)</td>
</tr>
<tr>
<td>HSV-2</td>
<td></td>
<td></td>
<td>2 (0.8)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td></td>
<td>10 (4.3)</td>
<td>9 (5.1)</td>
</tr>
<tr>
<td>VZV + HSV-2</td>
<td></td>
<td></td>
<td>1 (0.4%)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td><strong>Adenovirus species</strong></td>
<td>VDL09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A, B, C, D, E</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Other agents</strong></td>
<td>VDL07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. trachomatis</td>
<td></td>
<td></td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>T. vaginalis</td>
<td></td>
<td></td>
<td>8 (3.4)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>T. pallidum</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group B streptococci</td>
<td></td>
<td></td>
<td>1 (0.4)</td>
<td>1 (0.6)</td>
</tr>
</tbody>
</table>
Table 1. CDC Recommendations for STI Screening in Pregnancy

<table>
<thead>
<tr>
<th>Condition</th>
<th>Screening recommended?</th>
<th>Preferred test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis*</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>Yes: all pregnant women</td>
<td>NAAT</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>Yes: women who are at risk† or living in a high-prevalence area</td>
<td>NAAT or culture on Thayer-Martin media</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>Yes: all pregnant women</td>
<td>HBsAg serology</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>Yes: women who are at high risk‡</td>
<td>Anti-HCV</td>
</tr>
<tr>
<td>Herpes</td>
<td>No (culture lesions if present)</td>
<td>Culture, PCR</td>
</tr>
<tr>
<td>HIV</td>
<td>Yes: all pregnant women</td>
<td>EIA, Western blot</td>
</tr>
<tr>
<td>HPV</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>Syphilis</td>
<td>Yes: all pregnant women</td>
<td>RPR or VDRL</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>No</td>
<td>—</td>
</tr>
</tbody>
</table>

*Bacterial vaginosis is not an STI, but it is more common in sexually active women

Majeroni, 2007
Prevention of Perinatal Group B Streptococcal Disease
Revised Guidelines from CDC, 2010

Continuing Education Examination available at http://www.cdc.gov/mmwr/cm/index.html

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
### TABLE 2: Performance of Nucleic Acid Amplification Tests (NAAT) compared with enriched culture for detecting group B Streptococcus (GBS)

<table>
<thead>
<tr>
<th>Test</th>
<th>Swab for NAAT and culture</th>
<th>No. positive by NAAT/No. positive by culture</th>
<th>NAAT sensitivity</th>
<th>No. negative by NAAT/No. negative by culture</th>
<th>NAAT specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Timing</td>
<td>Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAAT performed on nonenriched samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDI-Strep&lt;sup&gt;3&lt;/sup&gt;</td>
<td>IP</td>
<td>VR</td>
<td>140/149</td>
<td>94.0%</td>
<td>626/653</td>
</tr>
<tr>
<td>IDI-Strep&lt;sup&gt;4&lt;/sup&gt;</td>
<td>IP</td>
<td>V</td>
<td>35/56</td>
<td>62.5%</td>
<td>252/259</td>
</tr>
<tr>
<td>IDI-Strep&lt;sup&gt;**&lt;/sup&gt;</td>
<td>AP/IP</td>
<td>VR</td>
<td>59/68</td>
<td>86.8%</td>
<td>157/165</td>
</tr>
<tr>
<td>GeneXpert&lt;sup&gt;††&lt;/sup&gt;</td>
<td>IP</td>
<td>VR</td>
<td>23/24</td>
<td>95.8%</td>
<td>20/31</td>
</tr>
<tr>
<td>IDI-Strep&lt;sup&gt;§§&lt;/sup&gt;</td>
<td>AP/IP</td>
<td>VR</td>
<td>149/188</td>
<td>79.3%</td>
<td>575/603</td>
</tr>
<tr>
<td>GeneXpert&lt;sup&gt;§§§&lt;/sup&gt;</td>
<td>AP/IP</td>
<td>VR</td>
<td>173/190</td>
<td>91.1%</td>
<td>570/594</td>
</tr>
<tr>
<td>BD GeneOhm&lt;sup&gt;†††&lt;/sup&gt;</td>
<td>AP</td>
<td>V</td>
<td>64/83</td>
<td>77.1%</td>
<td>99/117</td>
</tr>
<tr>
<td>BD GeneOhm&lt;sup&gt;††††&lt;/sup&gt;</td>
<td>IP</td>
<td>VR</td>
<td>49/54</td>
<td>90.7%</td>
<td>121/124</td>
</tr>
<tr>
<td>GeneXpert&lt;sup&gt;†††††&lt;/sup&gt;</td>
<td>AP/IP</td>
<td>V</td>
<td>135/137</td>
<td>98.5%</td>
<td>723/726</td>
</tr>
<tr>
<td>IDI-Strep&lt;sup&gt;§§§§&lt;/sup&gt;</td>
<td>IP</td>
<td>VR</td>
<td>38/42</td>
<td>90.5%</td>
<td>148/154</td>
</tr>
</tbody>
</table>

| NAAT performed on enriched samples | | | | | | |
| BD GeneOhm<sup>††††††</sup> | AP | VR | 49/53  | 92.5% | 136/147 | 92.5% |
| BD GeneOhm<sup>†††††††</sup> | AP | VR | 55/55  | 100.0% | 55/55  | 99.3% |
| BD GeneOhm<sup>††††††††</sup> | AP | VR | 55/55  | 92.5% | 55/55  | 99.3% |
| BD GeneOhm<sup>†††††††††</sup> | AP | V/VR | 136/141 | 96.4% | 349/357 | 97.8% |

**Abbreviations:** AP = antepartum, IP = intrapartum, V = vaginal only, and VR = vaginal-rectal.

* Includes only those NAAT that are approved for use by the Food and Drug Administration.

† Compared with enriched culture of specimen collected at the same time as that used for NAAT.
Published studies on the performance of commercially available NAAT on nonenriched samples have demonstrated varying sensitivities (range: 62.5%–98.5%) and specificities (range: 64.5%–99.6%), compared with the gold standard of enrichment followed by subculture (179–188) (Table 2). Three studies have compared both intrapartum NAAT on nonenriched samples and late antepartum enriched culture results to intrapartum enriched culture (179,182,185). When comparing swabs collected at the two different time points, two of the studies found intrapartum NAAT to be slightly more sensitive (95.8% and 90.7%, respectively) than antepartum culture (83.3% and 84.3%, respectively) (182,185), although with widely overlapping confidence intervals. One study reported a statistically significant difference between the sensitivity of swabs collected intrapartum and tested with NAAT (94.0%) compared with enriched culture performed on swabs collected prenatally (54.3%) (179). The sensitivity of NAAT for GBS increases to 92.5%–100.0% with use of an enrichment step before testing the sample (177,178,188). Use of an enrichment step lengthens the time to obtain a final result; however, for antenatal testing, the accuracy of results is much more important than timeliness.

Despite the availability of NAAT for GBS, utility of such assays in the intrapartum setting remains limited. Although a highly sensitive and specific test with rapid turnaround time could be used to assess intrapartum GBS colonization and therefore obviate the need for antenatal screening, data on currently available assays do not support their use in replacement of antenatal culture or risk-based assessment of women with unknown GBS status on admission for labor. The addi-
Diagnostic Accuracy of a Rapid Real-Time Polymerase Chain Reaction Assay for Universal Intrapartum Group B Streptococcus Screening

Najoua El Helali,1 Jean-Claude Nguyen,1 Aicha Ly,1 Yves Giovangrandi2 and Ludovic Trinquart1,3

Services de Microbiologie et Gynécologie Obstétrique, Groupe Hospitalier Paris Saint-Joseph, Université Paris Descartes, Université de Recherche Clinique, Hôpital Européen Georges Pompidou, Assistance Publique Hôpitaux de Paris, and Centre d’Investigation Épidémiologique A, INSERM, Paris, France

Background. Intrapartum antibiotic prophylaxis is currently given to mothers who test positive for group B streptococcus (GBS) by antenatal culture-based screening, with a risk-based approach for cases with an unknown GBS status. A rapid real-time polymerase chain reaction (PCR) assay for the detection of GBS became available recently, making intrapartum screening possible. We aimed to assess its diagnostic accuracy and to compare it with antenatal screening.

Methods. We conducted a prospective study in a French hospital. All pregnant women giving birth at the maternity ward were considered for inclusion, except those with planned cesarean delivery, with delivery at <35 weeks gestation, and who received antibiotic therapy before admission. We performed GBS culture (the reference standard) and a molecular GBS test (Xpert GBS; Cepheid) on intrapartum specimens. Decisions about intrapartum antibiotic prophylaxis were based on the current GBS screening by culture at 35–37 weeks gestation.

Results. We prospectively enrolled 968 pregnant women from April 2007 through March 2008. The overall molecular GBS test yield was 89.2%. Among the 863 women with available results, the molecular GBS test had a sensitivity of 98.5%, specificity of 99.6%, positive predictive value of 97.8%, and negative predictive value of 99.7%. The positive predictive value of antenatal culture for identifying colonization status at delivery was low (58.9%), whereas the negative predictive value was high (99.8%).

Conclusions. This real-time PCR assay is a highly accurate test to identify intrapartum GBS carriers at point of care. This new tool could enhance the exact identification of candidates for intrapartum antibiotic prophylaxis, including women with preterm rupture of membranes or preterm labor.
### Intrapartum Screening

**GeneXpert** vs. **Culture**

<table>
<thead>
<tr>
<th>PCR Gene-Xpert</th>
<th>INTRAPARTUM</th>
<th>CULTURE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>-</strong></td>
<td>723</td>
<td>2</td>
</tr>
<tr>
<td><strong>+</strong></td>
<td>3</td>
<td>135</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>756</td>
<td>137</td>
</tr>
<tr>
<td><strong>Invalid</strong></td>
<td>41</td>
<td>1</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>53</td>
<td>5</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>825</td>
<td>143</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SEN</th>
<th>SPE</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.5</td>
<td>99.6</td>
<td>97.8</td>
<td>99.7</td>
</tr>
</tbody>
</table>

10.8% without PCR results
Prenatal Group B Streptococcus Test Using Real-time Polymerase Chain Reaction

Chi-Feng Wei, Bo-Ching She, Hung-Shuo Liang, Qing-Dong Ling, Chen-Yuan Tsai,
Chih-Wei Yen, Hsueh-Yin Wu, Ming-Song Tsai

Department of Obstetrics and Gynecology, Cathay General Hospital, St-Jhiih, Cathay Medical Research Institute, Taipei, Departments of Laboratory Medicine and Pediatrics, Cathay General Hospital, St-Jhiih, and Obstetrics and Gynecology, Cathay General Hospital, Taipei, Taiwan.

SUMMARY

Objective: The aim of this study was to evaluate the percentage of pregnant women with negative Group B Streptococcus (GBS) screening results by culture at 35 weeks' gestation, who subsequently had positive GBS test results after 39 weeks' gestation.

Materials and Methods: From 2006 to 2007, we recruited 150 pregnant women who received routine GBS culture screening at 35 weeks' gestation with negative results, and who had repeat cultures and real-time polymerase chain reaction (RT-PCR) tests for GBS after 39 weeks' gestation.

Results: Two percent of pregnant women with GBS-negative results by culture screening at 35 weeks' gestation were GBS-positive at 39 weeks' gestation.

Conclusion: It is necessary to perform a GBS test 4 weeks after an initial negative GBS culture at 35–37 weeks of gestation. RT-PCR provides a simple and rapid alternative method for detecting rectovaginal GBS colonization at the time of labor. [Taiwan J Obstet Gynecol 2009;48(2):116–119]

Key Words: group B streptococci, maternal screening, pregnant women, real-time polymerase chain reaction

Taiwan J Obstet Gynecol 2009 Jun; 48 (2): 116-119
A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization

Michael Gavino, MD; Eileen Wang, MD

Am J Obstet Gynecol 2007; 197 (4): 388 e1-4
<table>
<thead>
<tr>
<th></th>
<th>Intrapartum</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>24</td>
<td>31</td>
</tr>
</tbody>
</table>

Sensitivity was 95.8% (95% CI, 76.9-99.8); specificity was 64.5% (95% CI, 45.4-80.2); positive predictive value was 67.6%; negative predictive value was 95.2%; positive likelihood ratio was 2.7; negative likelihood ratio was 0.065.
"La qualità in medicina è difficile da definire, da misurare e da dimostrare, ma il segreto è credere che sia possibile migliorarla di giorno in giorno con l'impegno, la cultura e con interventi sul sistema"