Le tecniche molecolari nella diagnosi delle STD

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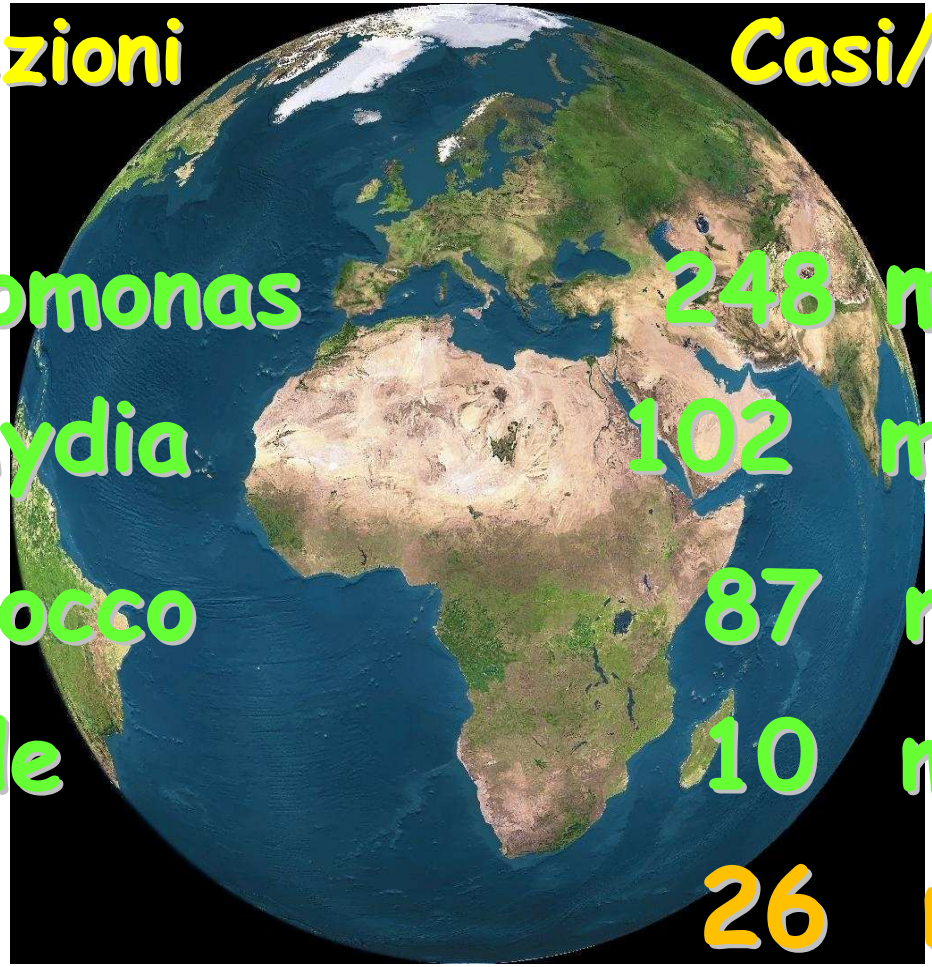
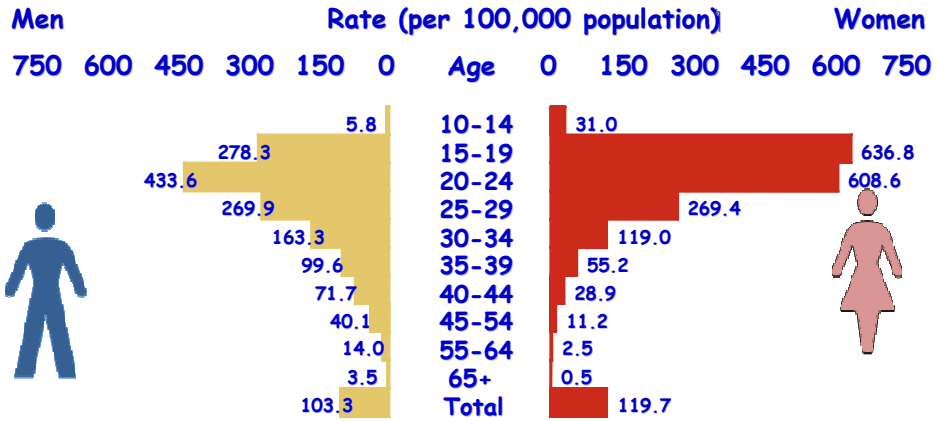
IST curabili:
448 milioni di nuovi casi all'anno
(O.M.S. - 2005)

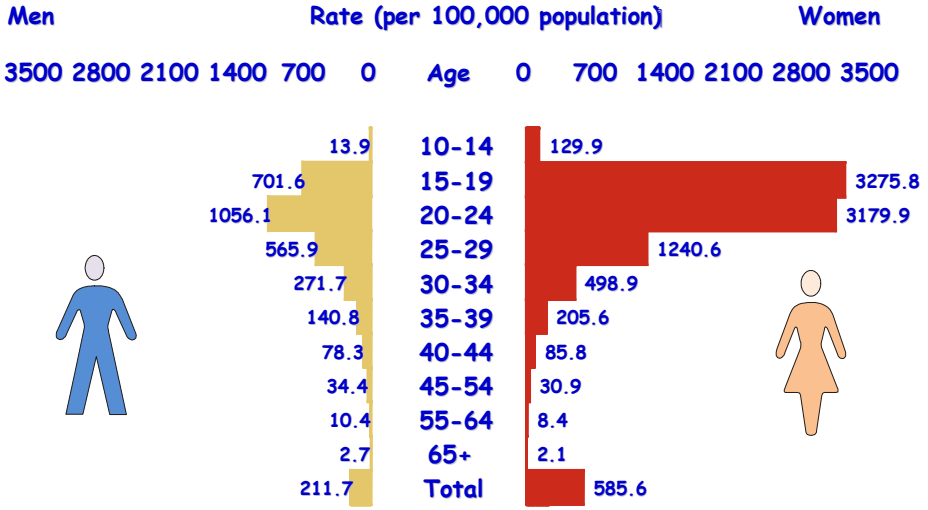


Infezioni	Casi/anno
Trichomonas	248 milioni
Chlamydia	102 milioni
Gonococco	87 milioni
Sifilide	10 milioni
HSV	26 milioni

Chlamydia and Gonorrhea Age- and sex-specific rates: United States, 2008



Gonorrea

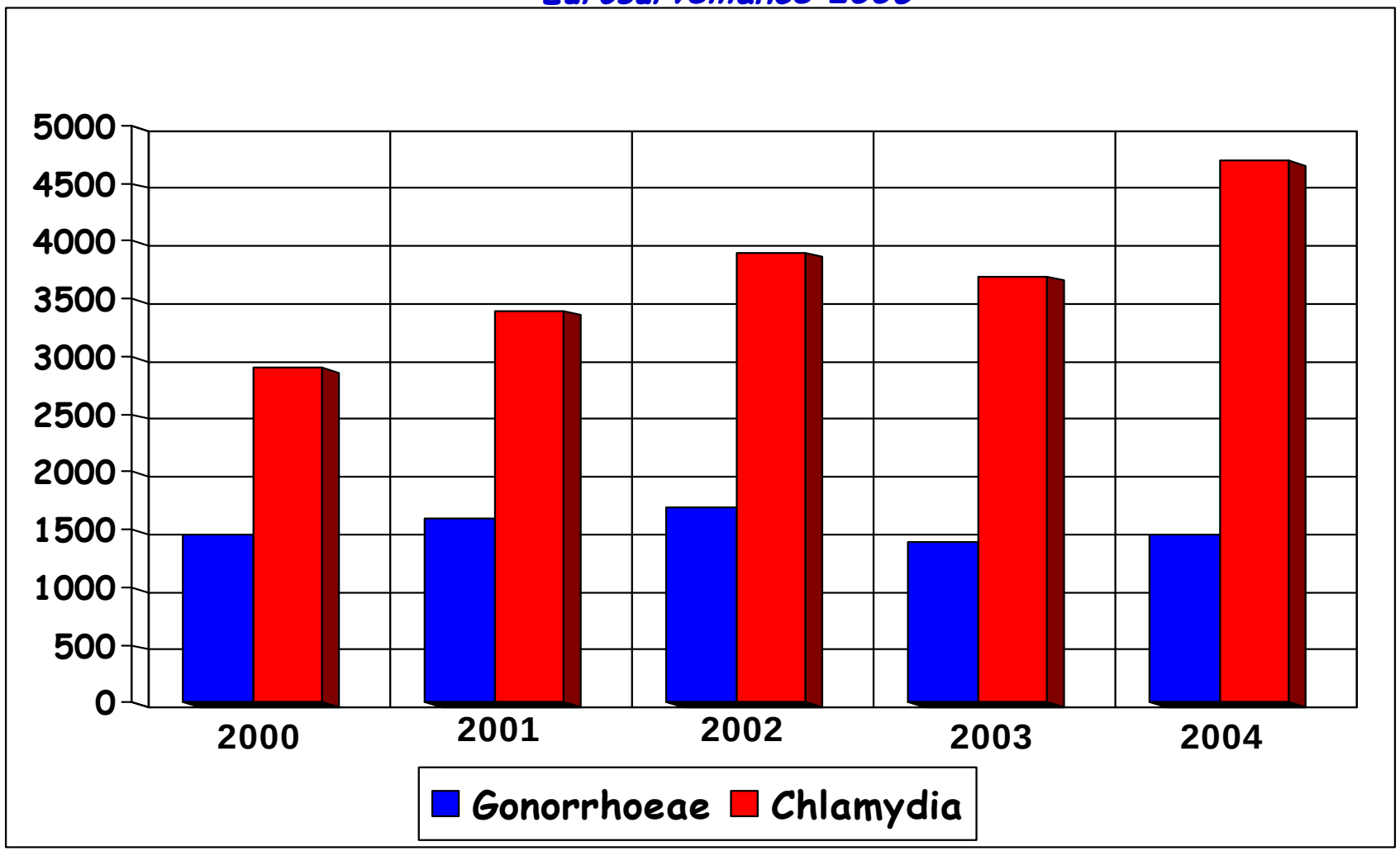


Chlamydia

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

Health Effects	Chlam	GC	GBS	HIV	Syphilis	HSV	BV
Spontaneous abortion	–	–	–	–	–	↑	–
Birth defects	–	–	–	–	↑↑↑	↑↑↑	–
Fetal infection	–	–	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑
Preterm delivery	↑↑	↑↑	–	–	↑↑↑	↑↑↑	↑↑
Growth restriction	–	↑	–	–	↑↑↑	↑↑↑	
Perinatal mortality	–	–	↑↑↑	↑	↑↑↑	↑↑	–

Baecher-Lind, 2009

Evidence of the Impact of Selected Infectious Agents on Reproductive Sequelae

Health Effects	Chlam	GC	GBS	HIV	Syphilis	HSV	BV
Intrapartum infection	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑	↑↑↑	–

Baecher-Lind, 2009

Chlamydia trachomatis

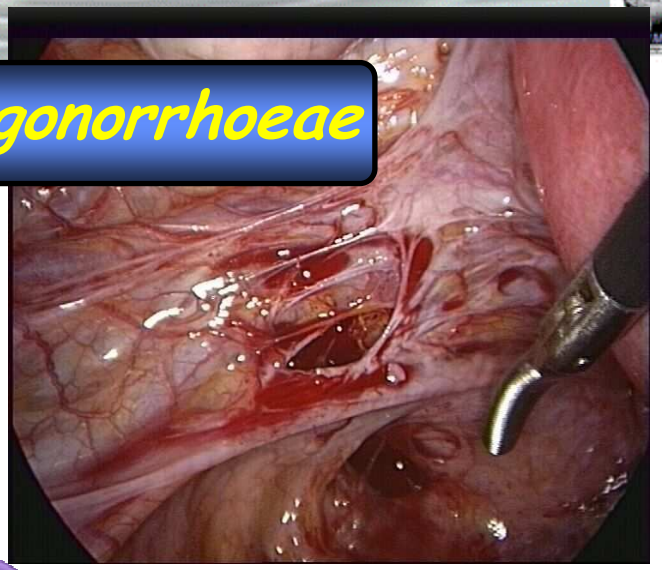
L'infezione colpisce prevalentemente le giovani donne di età inferiore ai 25 anni ed i sintomi sono generalmente lievi, aspecifici o addirittura inesistenti: il

- 70-80% nella donna
- 30-50% nell'uomo

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

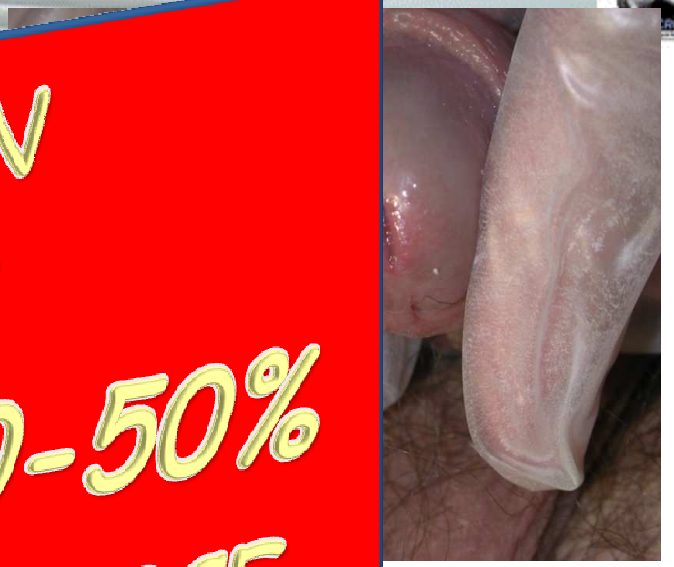
Chlamydia t. e Neisseria g. – Nell'uomo

C.t. è
non
gon

Si
Di
Br
mi
Do
ur
Se
Er
me
ur

UN UOMO CON UN'URETRITE ASINTOMATICA (30-50% DEI CASI) COSTITUISCE UN'IMPORTANTE

RESERVOIR DI INFEZIONE!

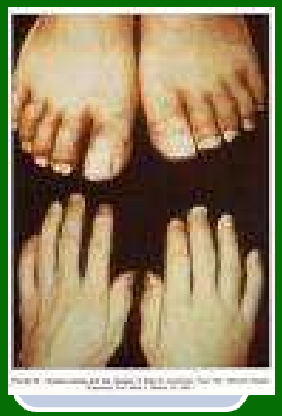


Florian, 2006

Chlamydia trachomatis e Neisseria gonorrhoeae – In entrambi i sessi

Linfogranuloma venereo

Malattia sistemica causata dai serovars L_1, L_2 e L_3 di C.t., non comune nelle nazioni industrializzate ma largamente diffuso in Asia, Africa e Asia. Clinicamente il quadro è quello di una linfoadenopatia acuta a volte preceduta da una lesione primaria (ulcera erpetiforme, papula, pustola) o di una proctite ulcerativa con secrezioni rettali purulente.

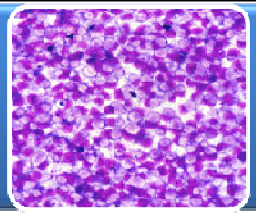


Artrite reattiva

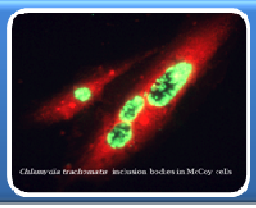
Una rara complicanza di un'infezione non trattata è rappresentata dalla sindrome di Reiter, un'artrite reattiva che associa la triade uretrite (a volte cervicite nelle donne), congiuntivite e lesioni mucocutanee non dolorose. Più frequente nell'uomo (rapporto 5:1 negli uomini rispetto alle donne). Ha 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.

Ozgul, 2006

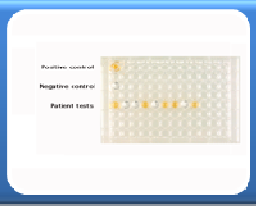
Chlamydia trachomatis - Tecniche diagnostiche



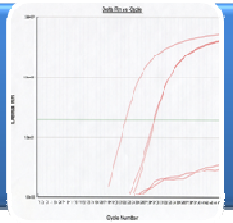
isolamento del microrganismo in colture cellulari



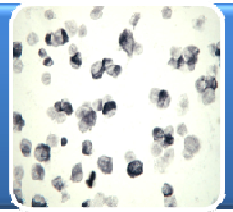
metodi in immunofluorescenza



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

} BIOLOGIA MOLECOLARE

Strategie diagnostiche nell'infezione da Chlamydia trachomatis

(Da Hamdad e coll. 2004, mod.)

	Infezioni del basso apparato genitale	Screening			Infezioni dell'apparato genitale alto	
	Endocervice, uretra	Endocervice	1° getto urine	Vagina, vulva	Endocervice	Biopsia tubarica
Coltura cellulare	+++	+	-	-	+/-	-
IFD	+++	+	-	-	-	-
EIA	++	+	-	-	-	-
Ibridazion	++	+	-	-	-	-
NAATs	+++	+++	+++	+++	+++	+++

+++: molto sensibile, ++: sensibile, +:media sensibilità, +/-:poco sensibile, -:non indicato

Sexually Transmitted Diseases, January 2008, Vol. 35, No. 1, p.8–19
DOI: 10.1097/OLQ.0b013e318156d98d
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From the NIH: Proceedings of a Workshop on the Importance of Self-Obtained Vaginal Specimens for Detection of Sexually Transmitted Infections

MARCIA M. HOBBS, PHD,* BARBARA VAN DER POL, MPH, PHD,† PATRICIA TOTTEN, PHD,‡
CHARLOTTE A. GAYDOS, DRPH,§ ANNA WALD, MD, MPH,‡ TERRI WARREN, RN, ANP,‖ RACHEL L. WINER, PHD,‡
ROBERT L. COOK, MD, MPH,¶ CAROLYN D. DEAL, PHD,# M. ELIZABETH ROGERS, BS,#
JULIUS SCHACHTER, PHD,** KING K. HOLMES, MD, PHD,‡ AND DAVID H. MARTIN, MD††

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

Sexually Transmitted Diseases, July 2008, Vol. 35, No. 7, p.649–655
DOI: 10.1097/OLQ.0b013e31816ddb9a
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Cost-Effectiveness of Screening Strategies for Chlamydia trachomatis *Using Cervical Swabs, Urine, and Self-Obtained Vaginal Swabs in a Sexually Transmitted Disease Clinic Setting*

DIANE R. BLAKE, MD,* NANCY MALDEIS, MPH,† MATHILDA R. BARNES, BS,† ANDREW HARDICK, BS,†
THOMAS C. QUINN, MD, ScD,†‡ AND CHARLOTTE A. GAYDOS, MS, MPH, DrPH†

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.

Sampling for *Chlamydia trachomatis* infection – a comparison of vaginal, first-catch urine, combined vaginal and first-catch urine and endocervical sampling

L Falk MD PhD*†, **B-I Coble** MD PhD†, **P-A Mjörnberg** MD‡ **and H Fredlund** MD PhD§**

*Research & Development, Department of Local Health Care, County of Östergötland, Linköping University, Linköping; †Department of Dermatology and Venereology, Linköping University Hospital, Linköping; ‡Department of Dermatology and Venereology, Ryhov County Hospital, Jönköping; §Department of Laboratory Medicine, Clinical Microbiology Section, Örebro University Hospital, Örebro; **Örebro University, Örebro, Sweden

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.

International Journal of STD & AIDS Volume 21 April 2010

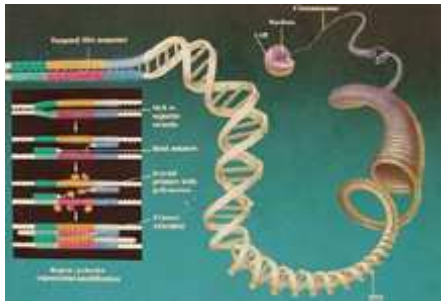
ABBANDONARE I TEST CON SCARSE PERFORMANCE

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 TROPPI POSITIVI !!!) e specificità 97% (DANNO TROPPI 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).

A costi contenuti e di facile esecuzione

NAAts



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

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

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%)

Hadgu: Eur J Clin Microbiol Infect Dis. 2008
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 SHIPITSYNA[1], Kira SHALEPO[1], Alevtina SAVICHEVA[1,2], Magnus UNEMO[2,3] and Marius DOMEIKA[2]
[1]Laboratory of Microbiology, DO Ott Research Institute of Obstetrics and Gynecology RAMS, St Petersburg, Russia, [2]Department of Medical Sciences, Uppsala University, Uppsala, and [3]Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2004, p. 4866–4867 Vol. 42, No. 10
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.10.4866–4867.2004
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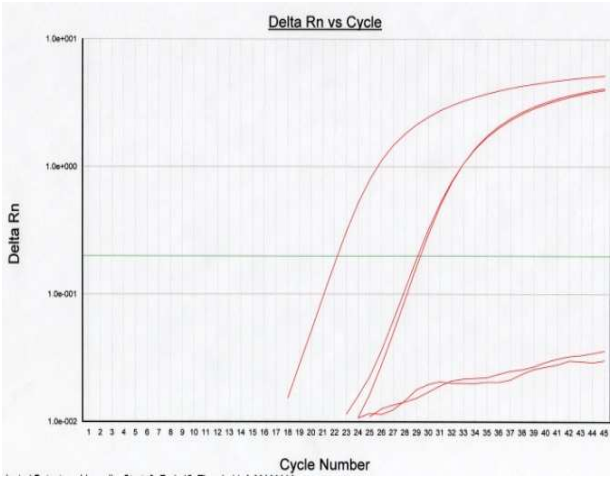
Pooling of Clinical Specimens Prior to Testing for *Chlamydia trachomatis* by PCR Is Accurate and Cost Saving

Marian J. Currie,[1]* Michelle McNiven,[2] Tracey Yee,[2] Ursula Schiemer,[2] and Francis J. Bowden[1]
ACT Pathology, The Canberra Hospital,[2] and Academic Unit of Internal Medicine, Australian National University,[1] 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.*



JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2010, p. 440–443 Vol. 48, No. 2
0095-1137/10/$12.00 doi:10.1128/JCM.01446-09
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Comparison of the Abbott RealTi*m*e CT New Formulation Assay with Two Other Commercial Assays for Detection of Wild-Type and New Variant Strains of *Chlamydia trachomatis*▽

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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 RealTi*m*e CT new formulation assay (*m*2000 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 *ompl* 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 *m*2000 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 9.1% (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 *m*2000 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.

Journal of Medical Microbiology (2009), 58, 867–873 DOI 10.1099/jmm.0.008698-0

Comparison of an in-house PCR assay, direct fluorescence assay and the Roche AMPLICOR *Chlamydia trachomatis* kit for detection of *C. trachomatis*

Poonam Sachdeva,[1] Achchhe Lal Patel,[1] Divya Sachdev,[1] Mashook Ali,[1] Aruna Mittal[2] and Daman Saluja[1]

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

Correspondence
Daman Saluja
dsalujach@yahoo.com

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 368 bp. The specificity of the primers was confirmed using genomic DNA from other sexually transmitted disease-causing and related micro-organisms 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 97.46%, 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.

Received 11 December 2008
Accepted 9 March 2009

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.

Conclusioni 2

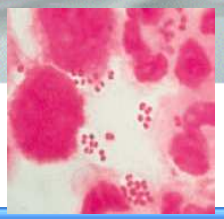
- Negli ultimi anni l'introduzione di tecniche diagnostiche basate sull'amplificazione degli acidi nucleici (NAATs) ha permesso di utilizzare, per la diagnosi delle infezioni da *Chlamydia trachomatis*, campioni alternativi, rispetto al tampone endocervicale o uretrale, come le urine o i tamponi vaginali 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



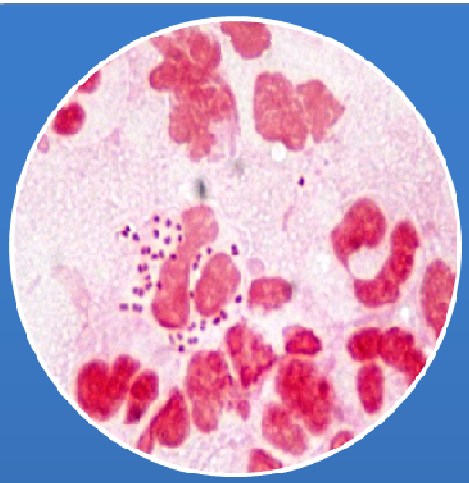
La scelta dei campioni e il metodo di raccolta dipendono dalle metodiche utilizzate dal laboratorio, dal quadro clinico, dall'età, dal sesso e dall'orientamento sessuale del paziente.
Tutti i siti in cui sono presenti dei sintomi (secrezioni e/o dolore) dovrebbero essere indagati.

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 .

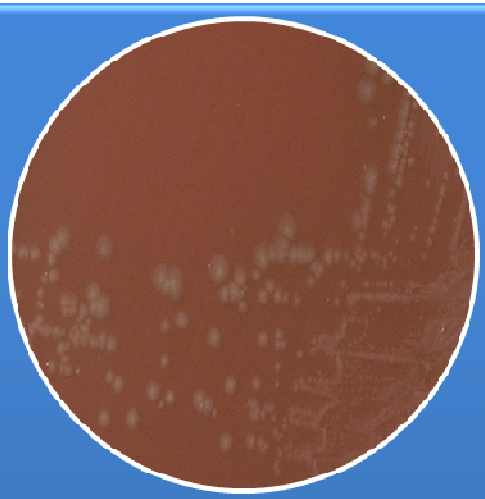
Domeika: Euro Surveill. 2007
CDC MMWR Recomm Rep. 2006
Bignell: Sex Transm Infect. 2006
Ng: Can J Infect Med Microbiol 2005

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 cha 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.

Whiley: 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 ol Gonorrhoea in Adult. 2009
CDC MMWR Recomm Rep. 2006

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Nucleic Acid Amplification Tests for Diagnosis of *Neisseria gonorrhoeae* Oropharyngeal Infections▽

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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 the specificity were as follows: culture for *N. gonorrhoeae*, 50.0% and 99.4%, respectively; PCR, 80.3% and 73.0%, respectively; TMA, 83.6% and 98.6%, respectively; and SDA, 93.2% 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*, 65.4% and 99.8%, respectively; PCR, 91.9% and 71.8%, 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.

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2005, p. 1445–1447 Vol. 43, No. 3
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.3.1445–1447.2005
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Comparison of COBAS AMPLICOR *Neisseria gonorrhoeae* PCR, Including Confirmation with *N. gonorrhoeae*-Specific 16S rRNA PCR, with Traditional Culture

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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%.

TABLE 1. Test characteristics

Specimen type[c] and/or criterion	Value for test method		
	N. gonorrhoeae culture	CA *N. gonorrhoeae* PCR[a]	Final *N. gonorrhoeae* PCR result[b]
True positive			
No. of positive results	58	85	84
No. of negative results	27	0	1
True negative			
No. of positive results	0	70	3
No. of negative results	2,938	2,868	2,935
Sensitivity (%)	68.2	100	98.8
Specificity (%)	100	97.6	99.9
Positive predictive value (%)	100	54.8	96.6
Negative predictive value (%)	99.1	100	100

[a] Only specimens which were repeatedly reactive in the CA *N. gonorrhoeae* PCR test were considered to be CA *N. gonorrhoeae* PCR positive.
[b] 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.
[c] Specimens were considered to be true *N. gonorrhoeae* positive or true *N. gonorrhoeae* negative as described in the text.

Cross reazioni con N. cinerea, N. flava, Lactobacillus spp.

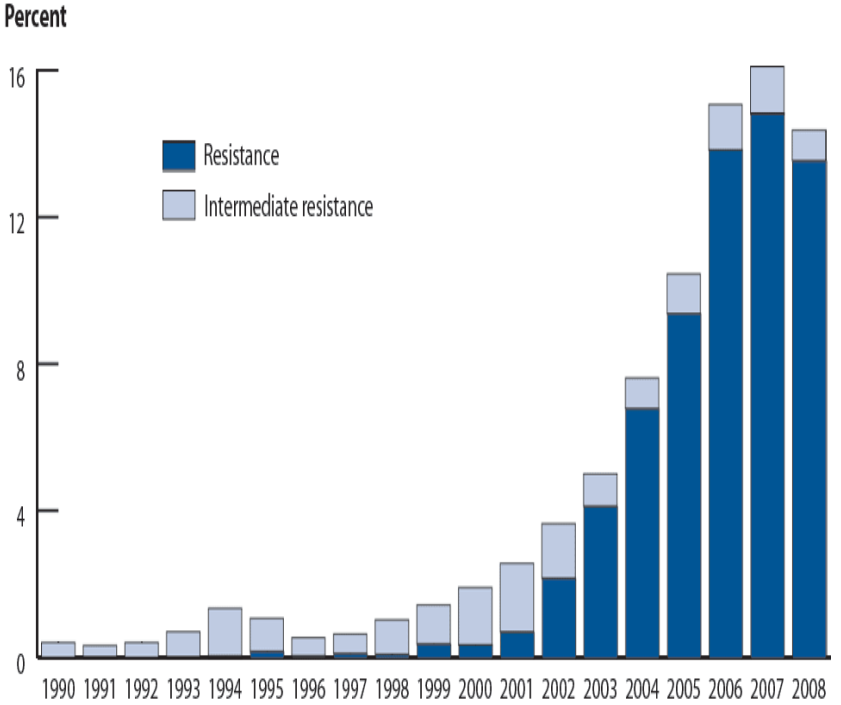
Test di sensibilità agli antibiotici

Vengono sempre più frequentemente descritte resistenze acquisite sia di tipo cromosomico sia di tipo plasmidico.

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 (tetraciclina, doxiciclina)*
- *macrolidi (eritromicina, azitromicina)*
- *fluorochinoloni (ciprofloxacina, ofloxacina)*

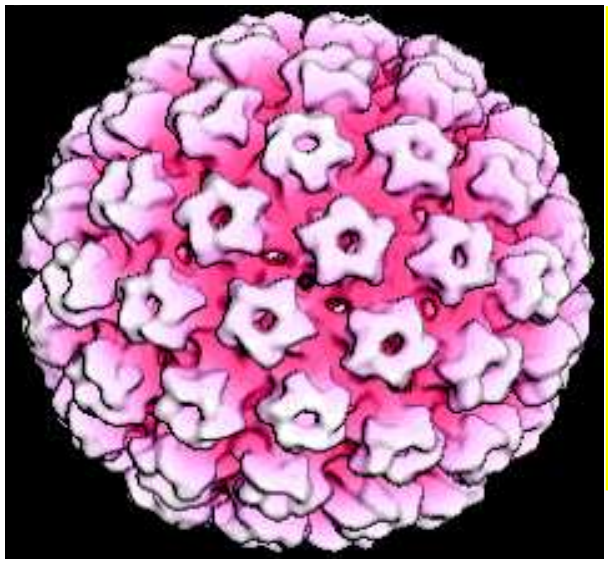


Percentuale di ceppi resistenti o sensibilità intermedia alla ciprofloxacina 1990–2008
Gonococcal Isolate Surveillance Project (GISP)

I Papillomavirus

55 nm

Famiglia *Papillomaviridae (2004)*



Il virione:

- **Sprovvisto di envelope**
- **Ha un capside 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)

2) Tropismo tissutale: cellule epiteliali (epiteli pavimentosi pluristratificati: cute, epitelio ano-genitale, epitelio prime vie aeree e prime vie digestive)

3) Potere oncogeno

Classificazione

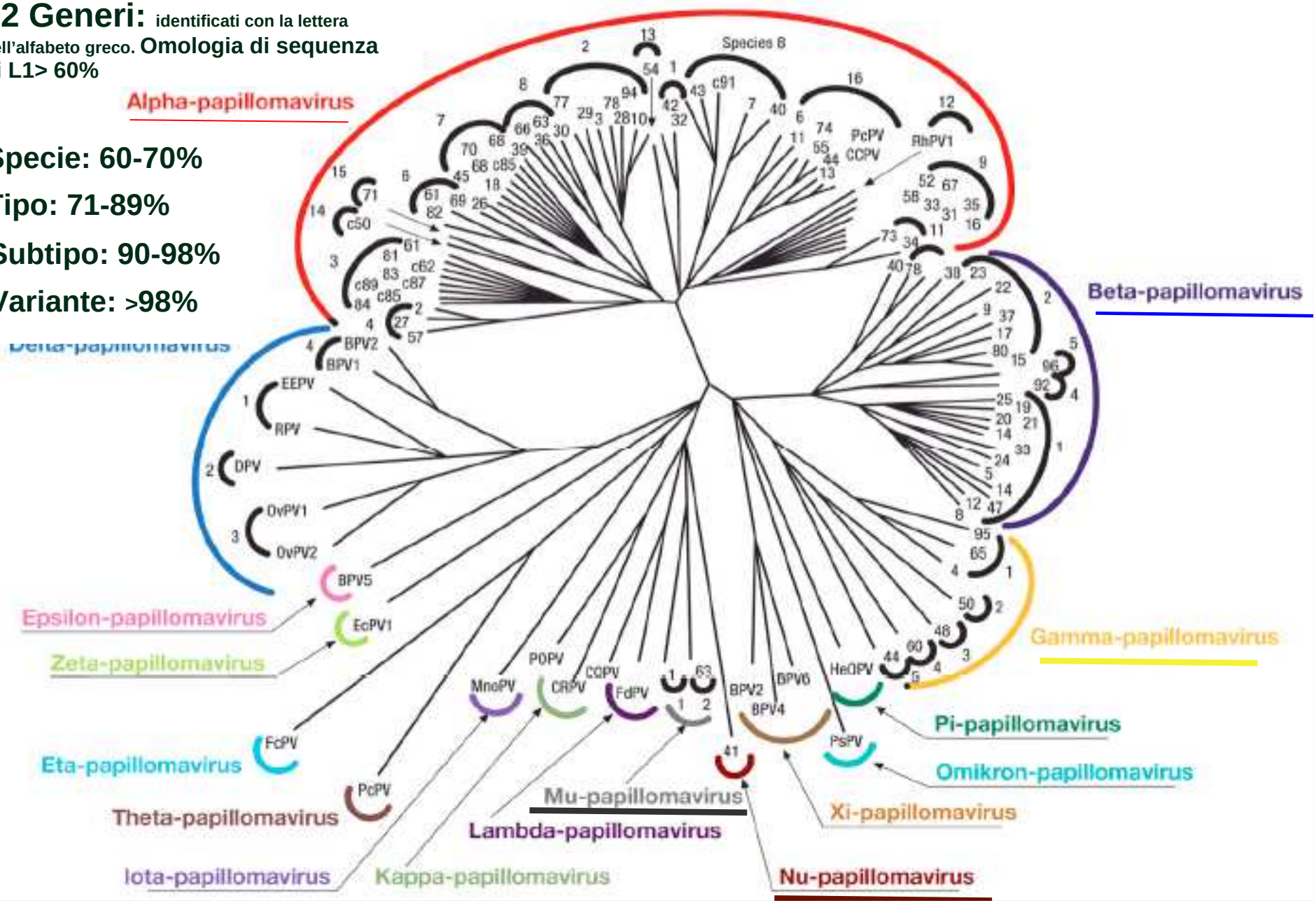
12 Generi: identificati con la lettera dell'alfabeto greco. **Omologia di sequenza di L1> 60%**

Specie: 60-70%

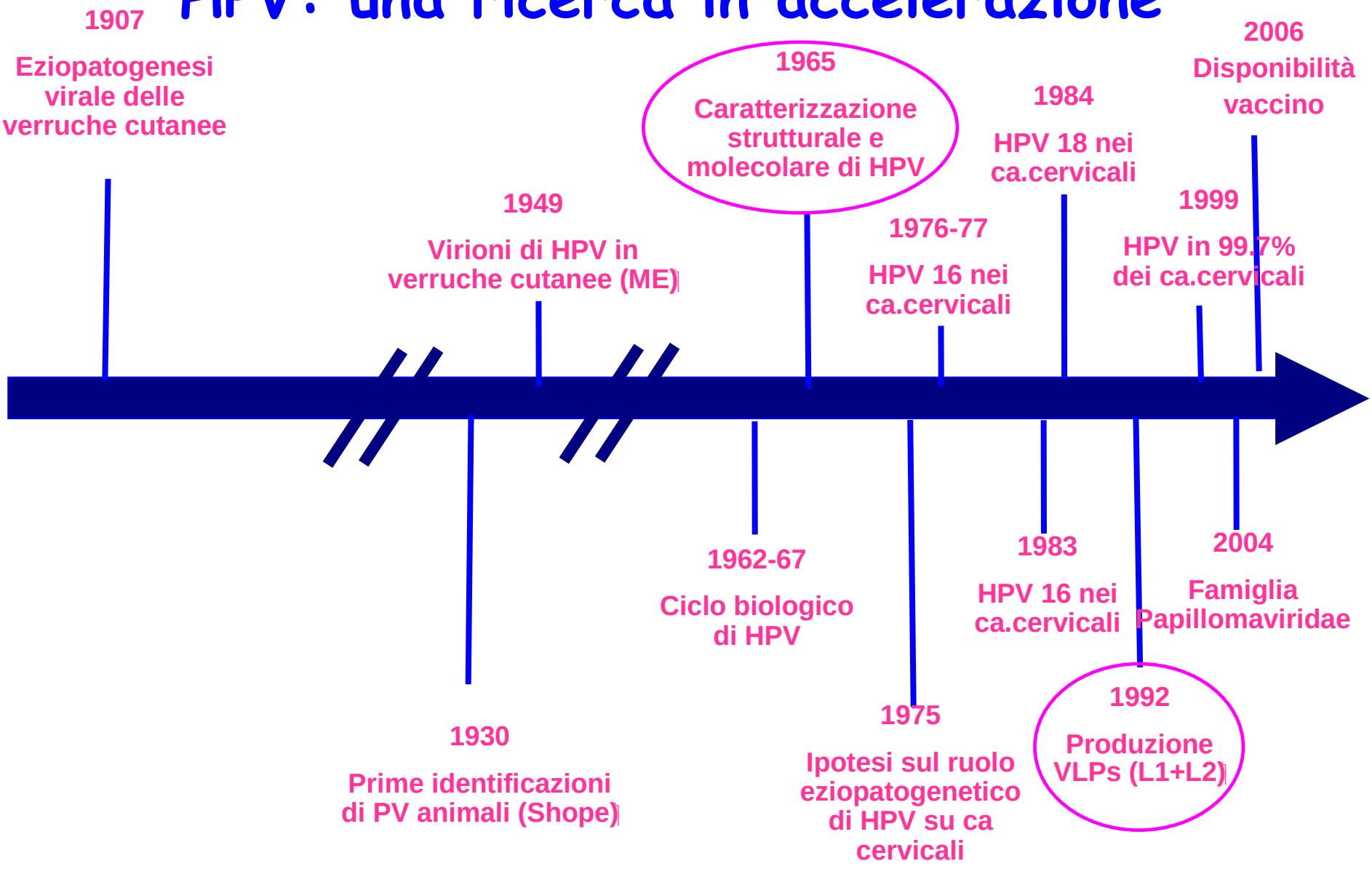
Tipo: 71-89%

Subtipo: 90-98%

Variante: >98%

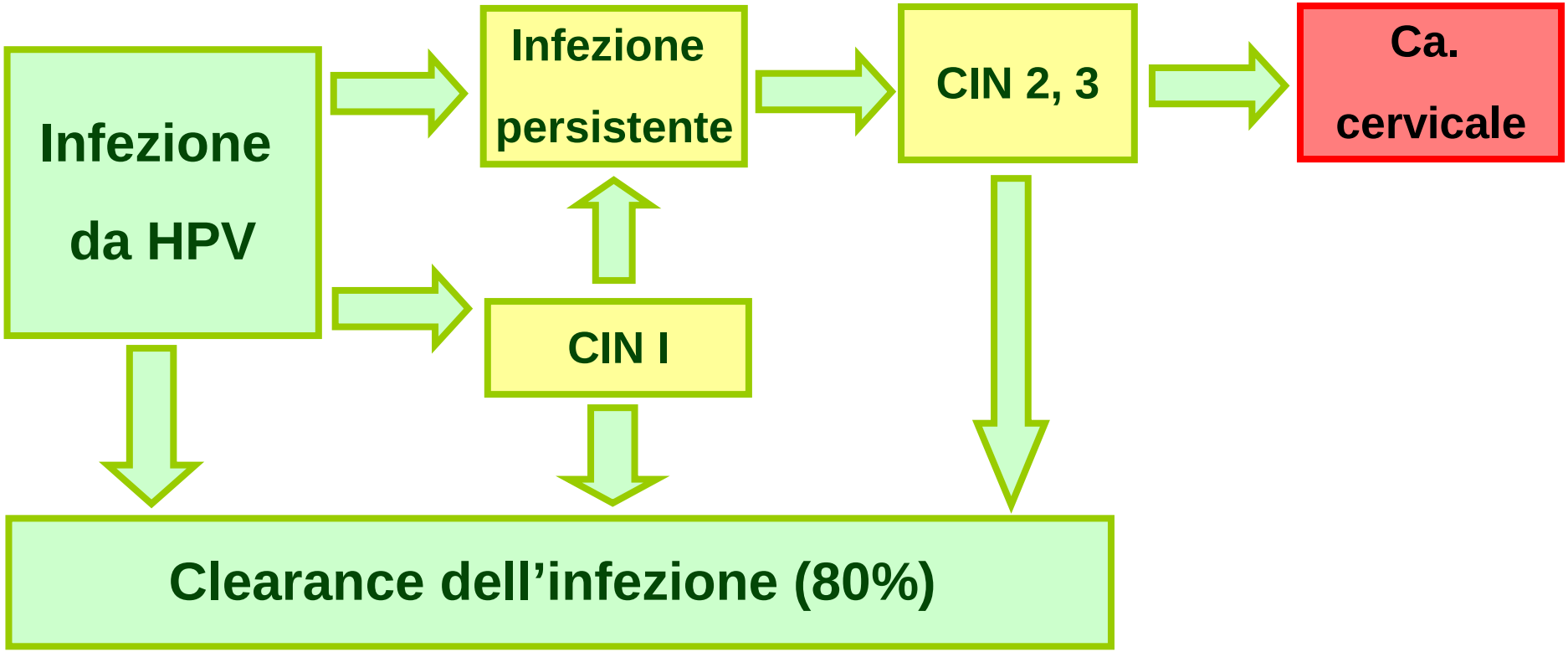


HPV: una ricerca in accelerazione



Storia naturale dell'infezione

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



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) →

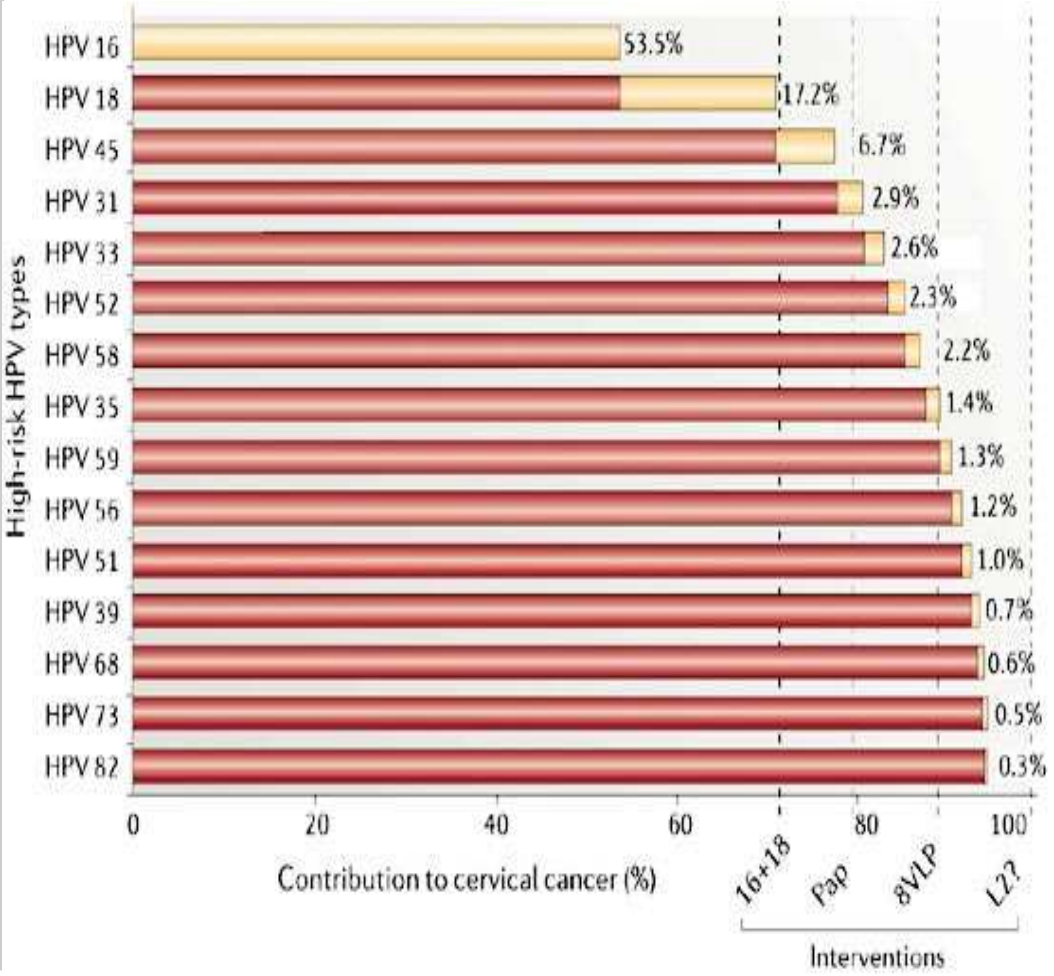
- Ibridazione in fase liquida tra HPV-DNA e 2 cocktail di sonde a RNA (alto e basso rischio)
- L'ibrido viene catturato da Ab adesi in una fase solida e rilevato con reazione chemiluminescente utilizzando Ab marcati
- Automatizzabile ma non consente la genotipizzazione specifica né consente di identificare 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…**

HPV e carcinoma della cervice

- **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*)**



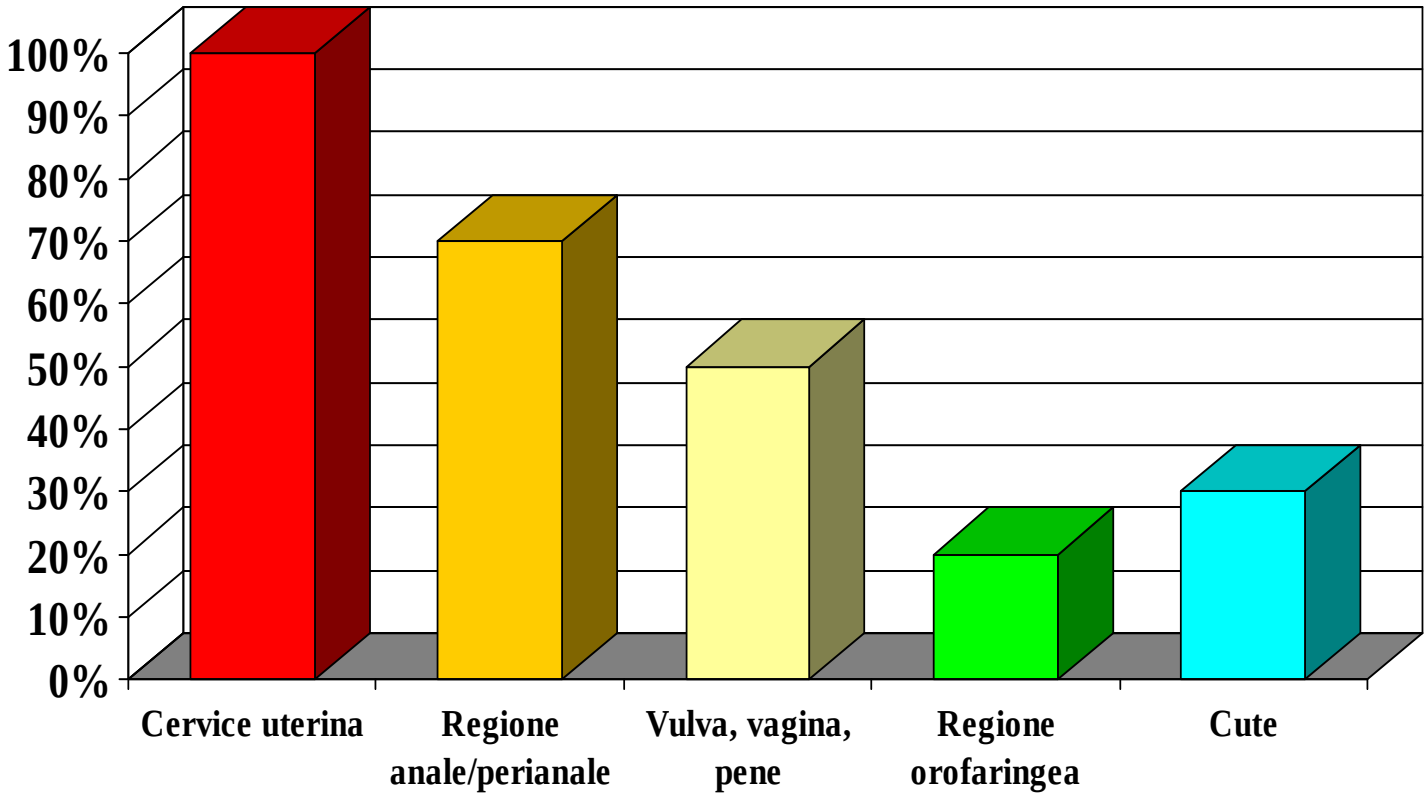
Cumulative %
53.5
70.7
77.4
80.3
82.9
85.2
87.4
88.8
90.2
91.4
92.4
93.1
93.7
94.2
94.5

- **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



Walboomers et al., J.Pathol., 1999; zur Hausen, Proc.Assoc.Am.Physicians, 1999; Picconi et al., J.Med.Virol., 2000.

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)**

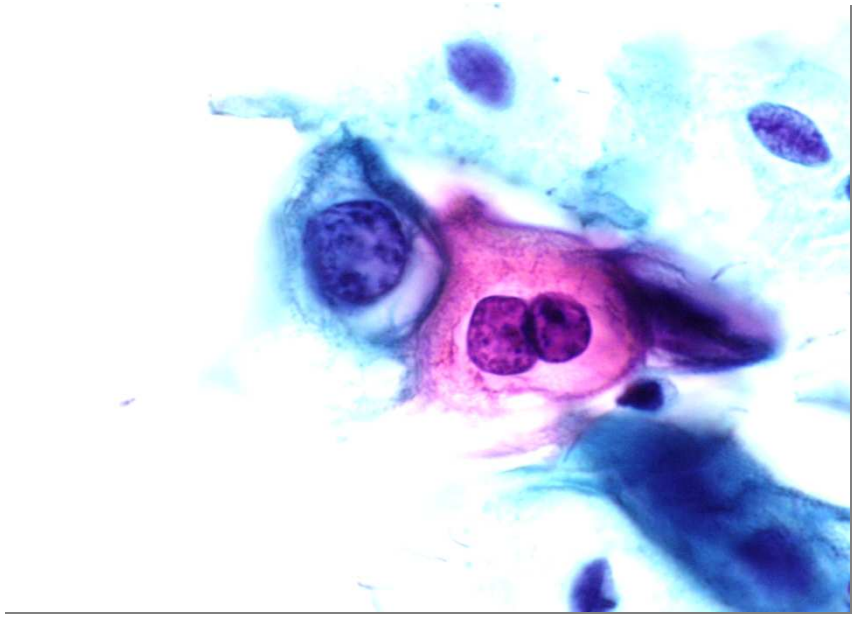
Metodi Indiretti

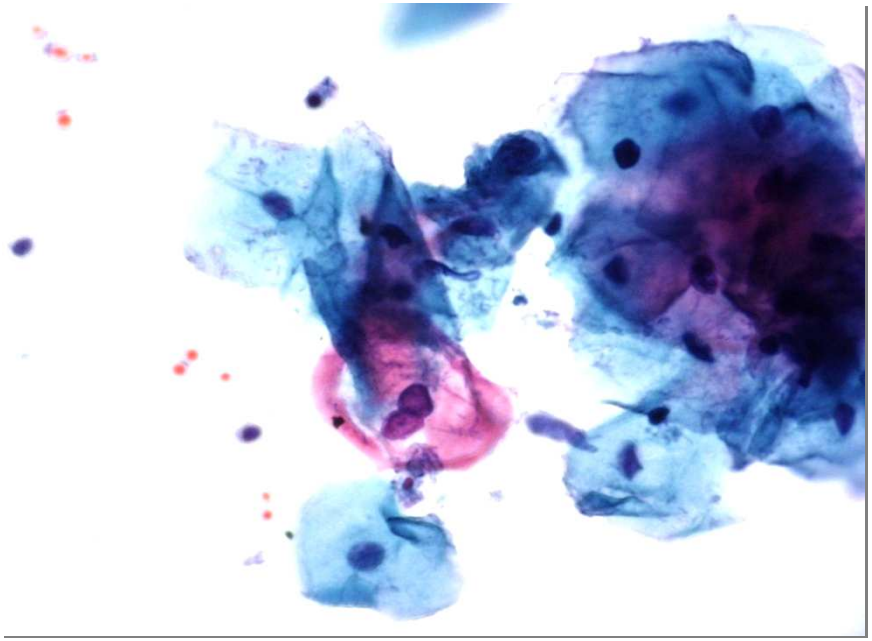
Evidenziano lesioni clinicamente apparenti [infezioni cliniche] o modificazioni cellulari e di tessuto indotte dal virus [infezioni subcliniche]. Non evidenziano le infezioni latenti (non produttive).

- **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

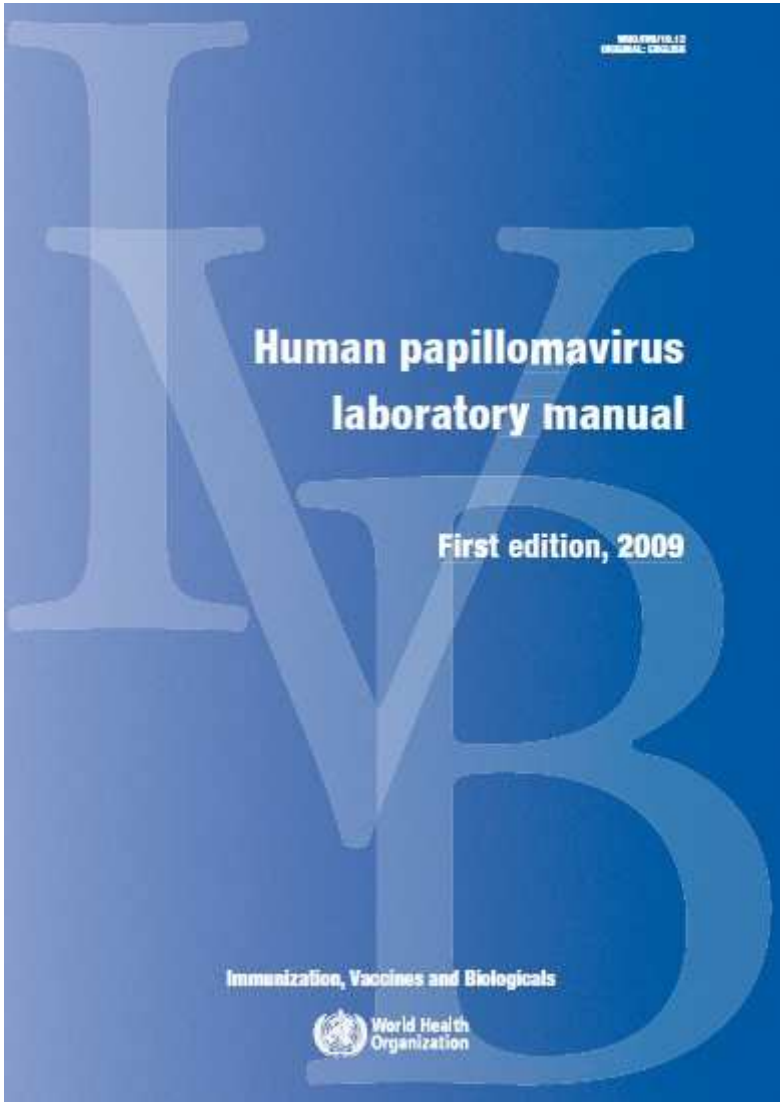
Sostituzione o integrazione del Pap-Test nello screening primario con genotipizzazione HPV

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


Human papillomavirus
laboratory manual
First edition, 2009
Immunization, Vaccines and Biologicals
World Health Organization

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, PGMY09/11, 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 PGMY09/11 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.

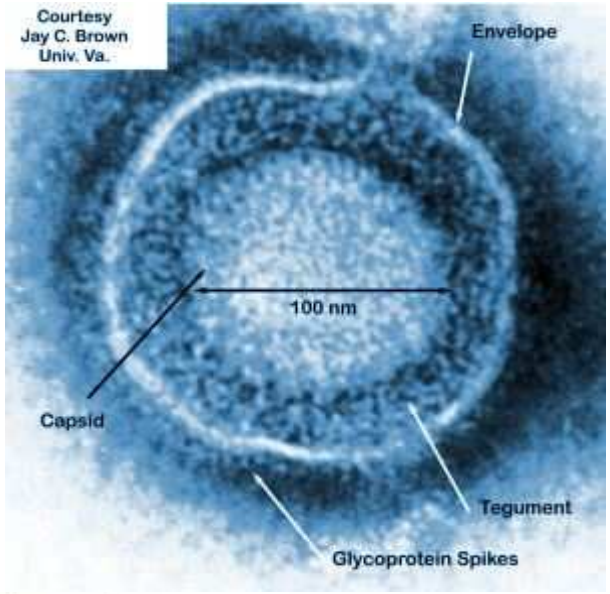
5.4 HPV detection with PGMY09/11 PCR

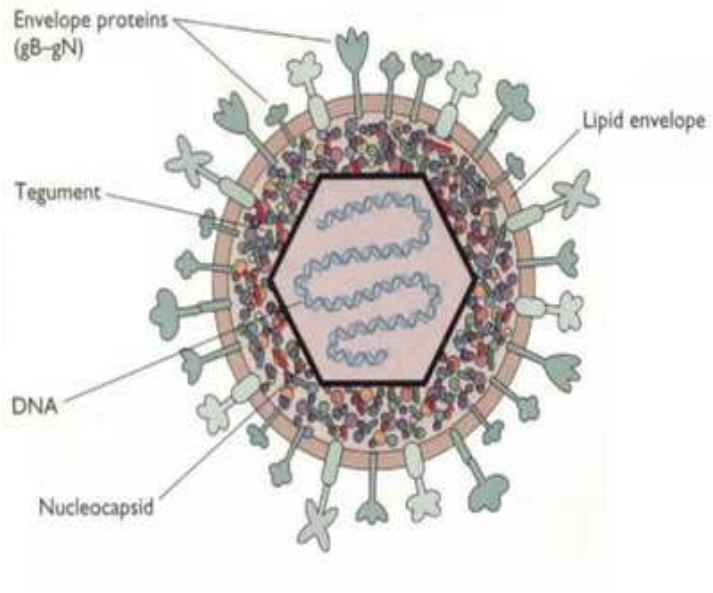
5.4.1 Principle of assay

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





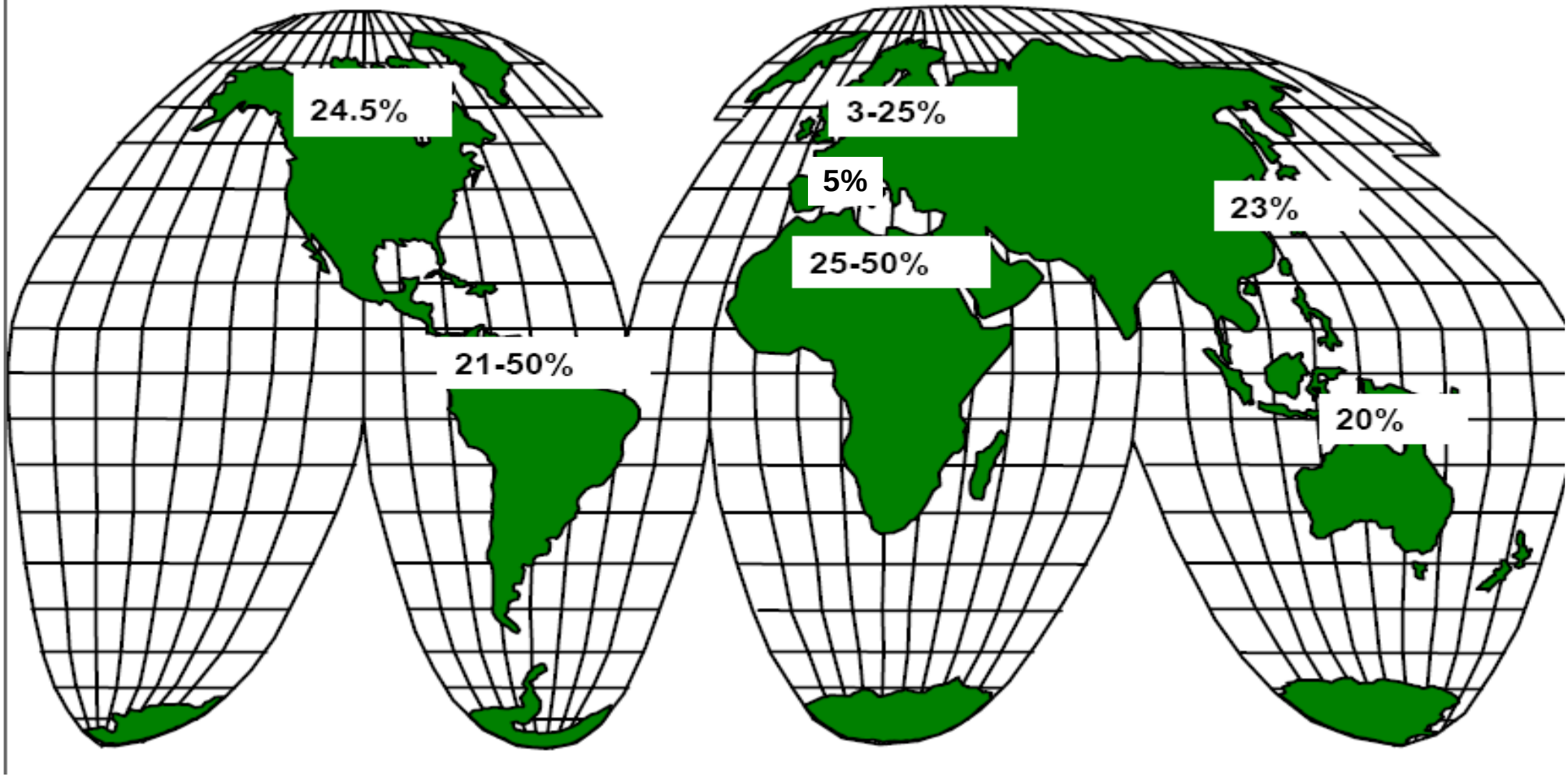
IL VIRIONE

- 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

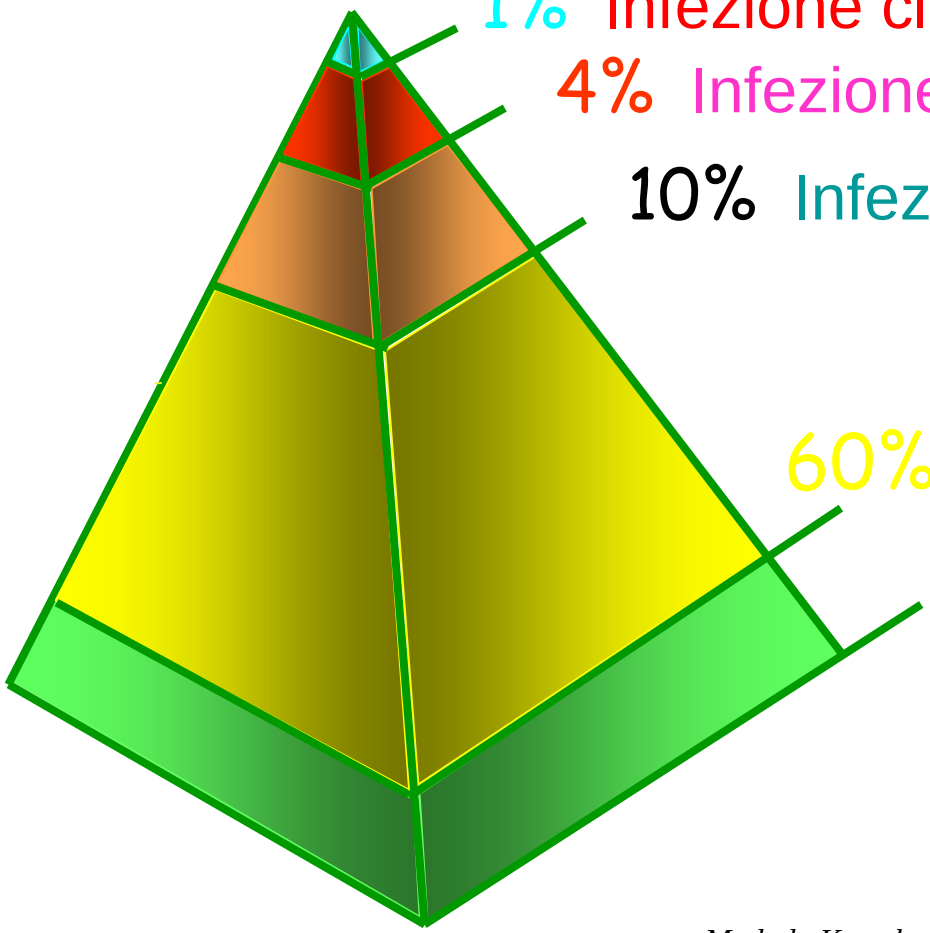


In Italia la prevalenza dell'infezione da HSV2 è molto più alta nei soggetti assistiti nelle cliniche per malattie sessualmente trasmesse: 44%

(Suligoi B. et al. J Med Virol. 2002 Jul;67(3):345-8;)

Prevalenza dell'infezione

Popolazione USA di ♂ e ♀ di età compresa tra 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

Fleming DT et al. *N Engl J Med.* 1997;337:1105-1111

- Follicollite
- Zipper burn
- Emorroidi
- Pantaloni stretti
- Allergia condoms

- Infezione fungina
- Vaginite
- Infezione urinaria
- Sintomi mestruali
- Emorroidi
- Irritazione da depilazione
- allergia

Ashley RL, Wald A. *Clin Microbiol Rev* 1999;12:1-8

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

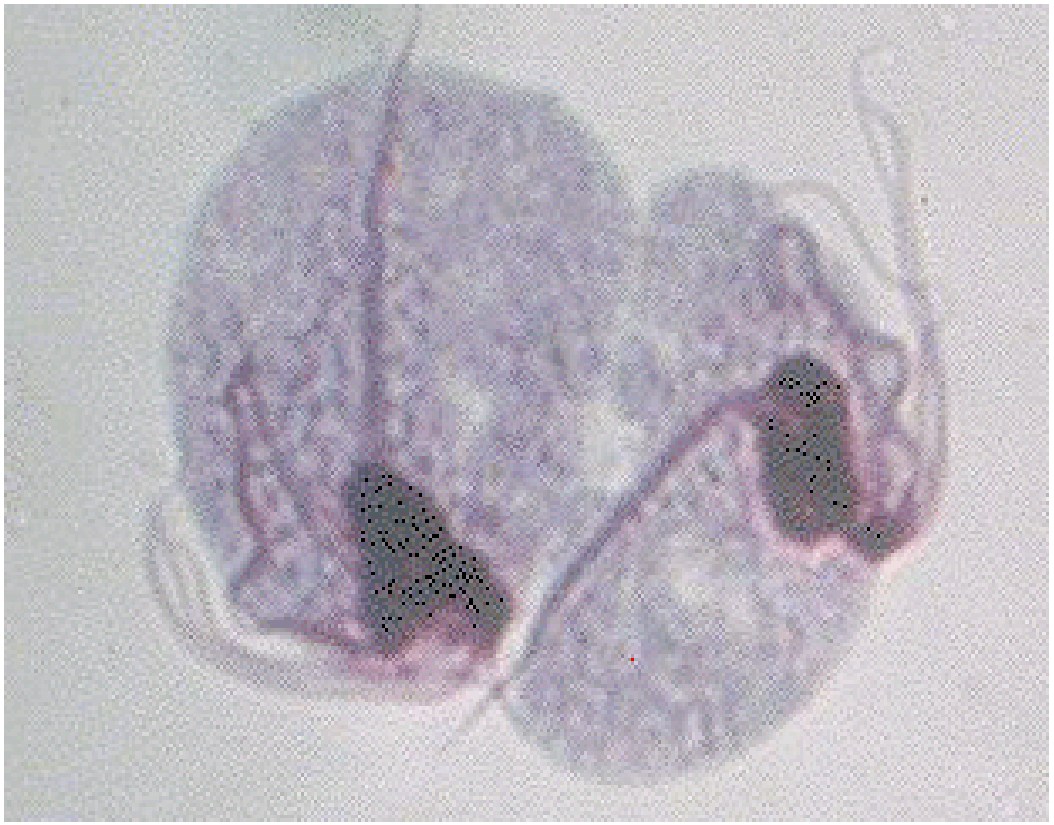
RICERCA DEL GENOMA VIRALE - PCR-

- 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**

- ***T. vaginalis* aumenta il rischio di trasmissione sessuale di HIV, aumentando il carico virale a livello cervico-vaginale e del liquido seminale** *(Sharif et al, Clin Microbiol Rev, 2009, 22:37-45)*

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

DIAGNOSI

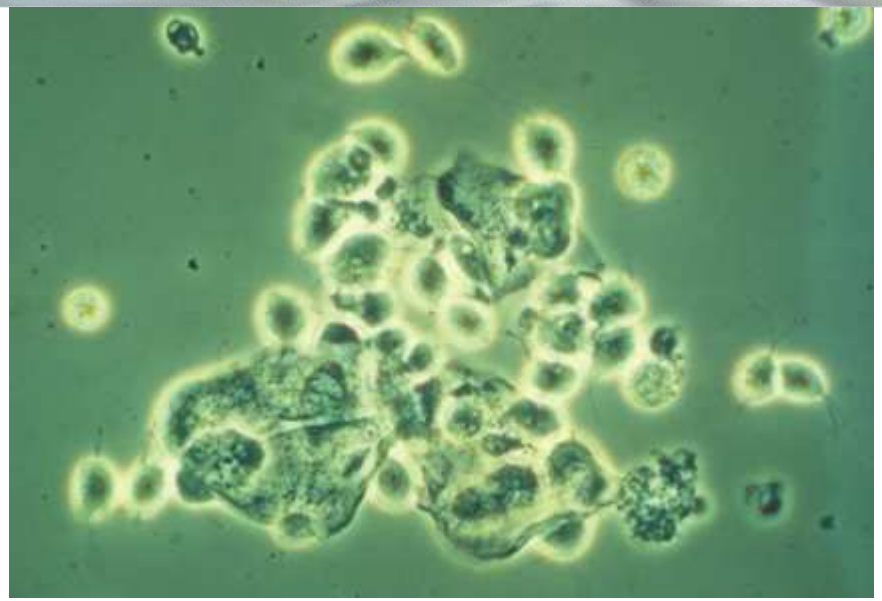
Clinica

"Falsi negativi": casi asintomatici

"Falsi positivi": vaginiti da altre cause o vaginosi batterica

Microbiologica

- Esame microscopico a fresco
- Isolamento colturale
- PCR
- Test rapidi



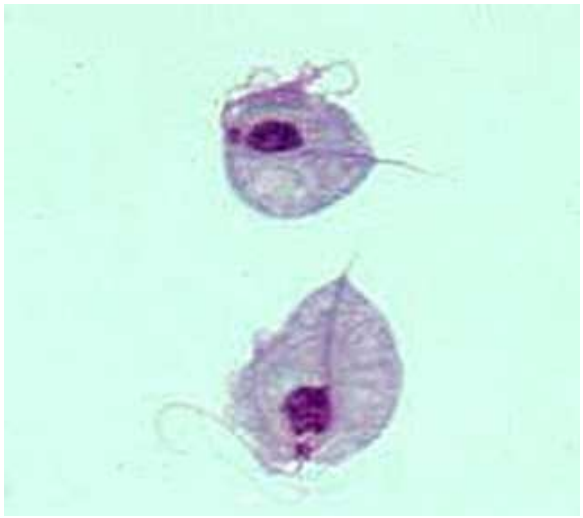
Diagnosi microbiologica

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

JOURNAL OF CLINICAL MICROBIOLOGY, June 1989, p. 1230–1233 Vol. 27, No. 6
0095-1137/89/061230-04$02.00/0
Copyright © 1989, American Society for Microbiology

Evaluation of Six Media for the Growth of *Trichomonas vaginalis* from Vaginal Secretions

GEORGE P. SCHMID,[1]* LINDA C. MATHENY,[2] AKBAR A. ZAIDI,[1] AND STEPHEN J. KRAUS[1]

Division of Sexually Transmitted Diseases, Center for Prevention Services, Centers for Disease Control, Atlanta, Georgia 30333,[1] and DeKalb County Board of Health, Decatur, Georgia 30030[2]

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%

JOURNAL OF CLINICAL MICROBIOLOGY, May 1990, p. 962–964
0095-1137/90/050962-03$02.00/0
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Vol. 28, No. 5

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

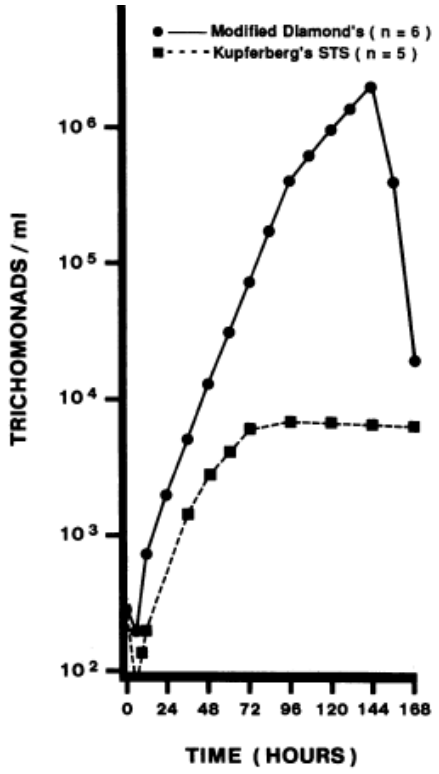

FIG. 1. Growth of *T. vaginalis* in modified Diamond's and Kupferberg's media. Each curve shows the average of the composite counts of the six isolates inoculated into the two media. One strain of *T. vaginalis* could not be recovered from Kupferberg's medium after initial inoculation.

Diamond's:

Recupero: 6 ceppi/6

Motilità: lieve perdita, evidente dopo 72h

Fase esponenziale: <96 h

7 gg: perdita completa della motilità

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2008, p. 3368–3374 Vol. 46, No. 10
0095-1137/08/$08.00+0 doi:10.1128/JCM.00564-08
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Impact of *Trichomonas vaginalis* Transcription-Mediated Amplification-Based Analyte-Specific-Reagent Testing in a Metropolitan Setting of High Sexually Transmitted Disease Prevalence▽

Erik Munson,[1,2]* Maureen Napierala,[1] Robin Olson,[1] Tina Endes,[1]† Timothy Block,[1] Jeanne E. Hryciuk,[1] and Ronald F. Schell[3,4,5]

1086 campioni biologici testati

Positivi: 14.5% con il test molecolare
7.0 % dell'esame microscopico a fresco

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2006, p. 4197–4199 Vol. 44, No. 11
0095-1137/06/$08.00+0 doi:10.1128/JCM.01447-06
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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%

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2008, p. 3159–3161 Vol. 46, No. 9
0095-1137/08/$08.00+0 doi:10.1128/JCM.00322-08
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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

Sex Transm Infect 2007;83:126–129. doi: 10.1136/sti.2006.022376

See end of article for authors' affiliations

Correspondence to: Dr A Pillay, Centers for Disease Control and Prevention, Division of STD Prevention, Laboratory Reference and Research Branch, 1600 Clifton Road, MS-G39, Atlanta, GA 30333, USA; apillay@cdc.gov

Accepted 20 October 2006
Published Online First 7 November 2006

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)*

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

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.

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2000, p. 3585–3588 Vol. 38, No. 10
0095-1137/00/$04.00+0
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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. SCHWEBKE[1,3]*

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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 trichomonosis 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 trichomonosis was defined as the detection of *T. vaginalis* by direct microscopy and/or culture from either vaginal samples or urine. The overall prevalence of trichomonosis 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.

1° Congresso NEWMICRO
Biotecnologie e diagnostica molecolare nella diagnosi delle malattie infettive

TABLE 1. Comparison of diagnostic tests for *T. vaginalis* in females[a]

Diagnostic method	No. true positive	No. false positive	No. false negative	No. true negative	Sensitivity		Specificity		Predictive value (%)	
					%	95% CI	%	95% CI	Positive	Negative
Vaginal swab culture	50	0	3	137	94.3	83.4–98.5	100	96.6–100	100	97.9
Vaginal PCR	47	4	6	133	88.7	76.3–95.3	97.1	92.2–99.1	92.2	95.7
Vaginal wet prep	31	0	22	137	58.5	44.2–71.6	100	96.6–100	100	86.2
Urine culture	32	0	21	137	60.4	46.0–73.2	100	96.6–100	100	86.7
Urine PCR	34	0	19	137	64.2	49.7–76.5	100	96.6–100	100	87.8
Urine wet prep	31	0	22	137	58.5	44.2–71.6	100	96.6–100	100	86.2

[a] $n = 190$. The gold standard is trichomonads visualized from any wet prep or culture ($n = 53$). CI, confidence interval.

MICOPLASMI

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

Micoplasma	Frequenza	Metabolismo	Atmosfera
U. urealyticum	Comune	Urea	Anaerobia
M. hominis	Comune	Arginina	Aerobia
M. fermentans	Occasionale	Glucosio+arginina	Anaerobia
M. genitalium	Comune	Glucosio	Anaerobia
M. penetrans	Occasionale	Glucosio+arginina	Anaerobia

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)

Eur J Clin Microbiol Infect Dis (2009) 28:641–646
DOI 10.1007/s10096-008-0687-z

ARTICLE

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

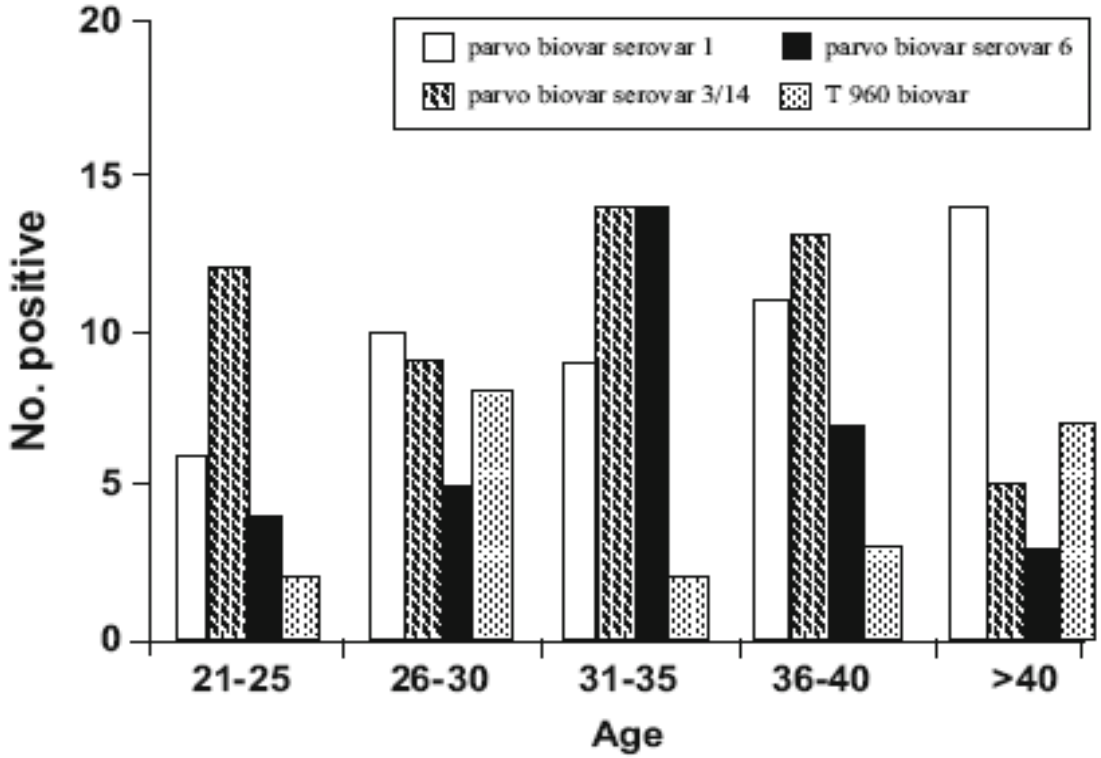


Fig. 1 *Ureaplasma parvum* serovars and T960 biovar distribution by age. The numbers of genital *Ureaplasma* isolates were 24, 32, 39, 34 and 29 for each year age group, respectively

Table 2 Relationship between vaginal flora changes and *Ureaplasma* biovars and serovars

Vaginal flora	*Ureaplasma* biovars and serovars			
	Parvo biovar Serovar 1	Parvo biovar Serovar 3/14	Parvo biovar Serovar 6	T960 biovar
Normal	31/94 (33%)	26/94 (28%)*	28/94 (30%)**	9/94 (9%)*
Absence of lactobacilli	13/45 (29%)	21/45 (47%)	3/45 (2%)	10/45 (22%)

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

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

	Ureaplasma biovars and serovars			
	Parvo biovar Serovar 1	Parvo biovar Serovar 3/14	Parvo biovar Serovar 6	T960 biovar
Symptomatic subjects	26/80 (32%)	35/80 (44%)*	3/80 (4%)**	16/80 (20%)*
Asymptomatic subjects	18/59 (31%)	12/59 (20%)	26/59 (44%)	3/59 (5%)

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

Patologie associate

Patologia	***U. urealyticum***	***M. hominis***
UNG	+++	-
Prostatite	?	?
Epididimite	?	?
Cervicite	+	+++
MIP	-	+++
Vaginosi batterica	+	+++
Parto pretermine	+	++
Aborto spontaneo	++	++
Febbre post-partum	±	+
Infertilità	+	-
Artrite reattiva	?	-

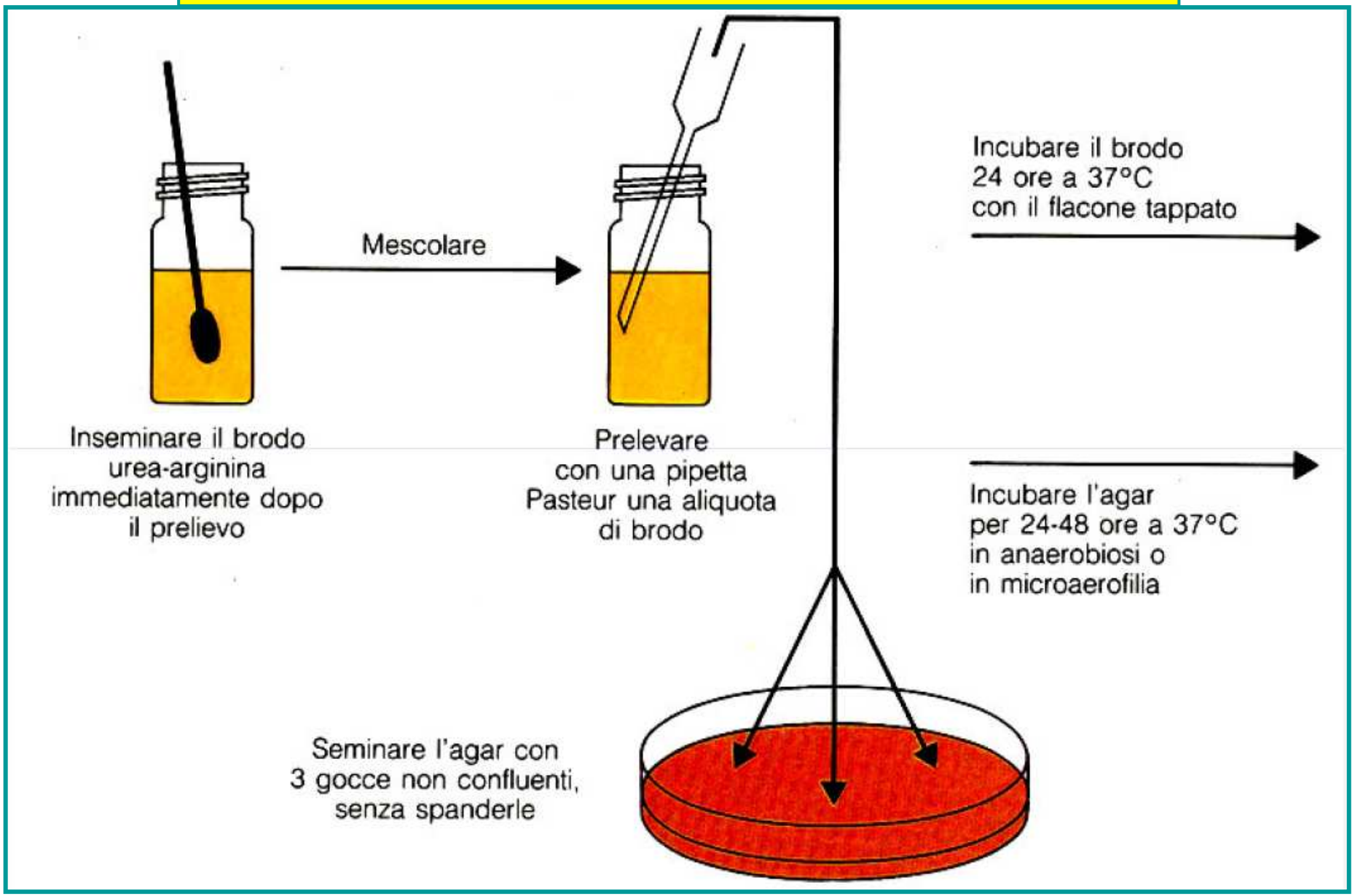
DIAGNOSI MICROBIOLOGICA

Campioni biologici: tampone vaginale e cervicale

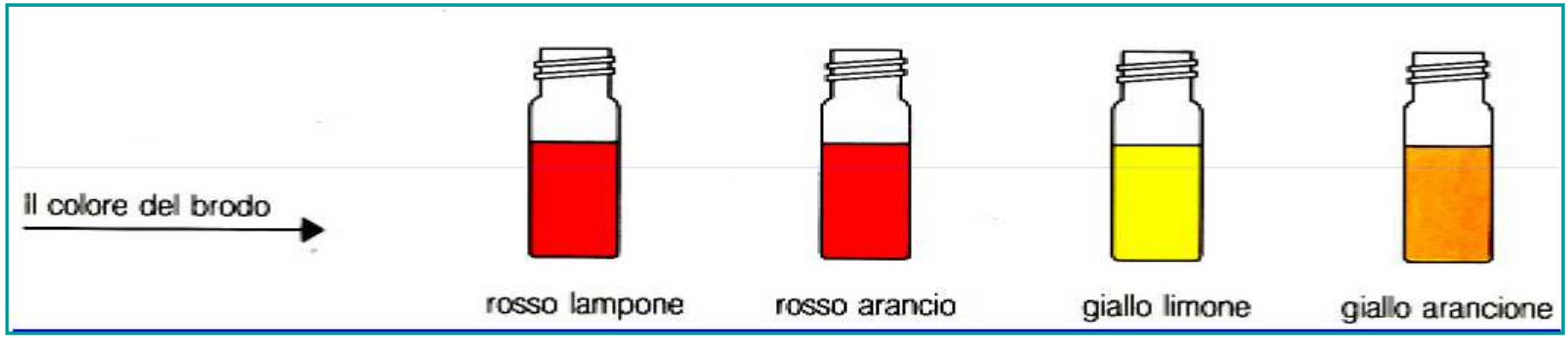
Esame colturale:

- inoculazione in brodo urea-arginina
- semina in piastra di terreno agarizzato A7
- incubazione del brodo a 37℃ per 24 h
- incubazione delle piastre a 37℃ per 48 h in anaer obiosi 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

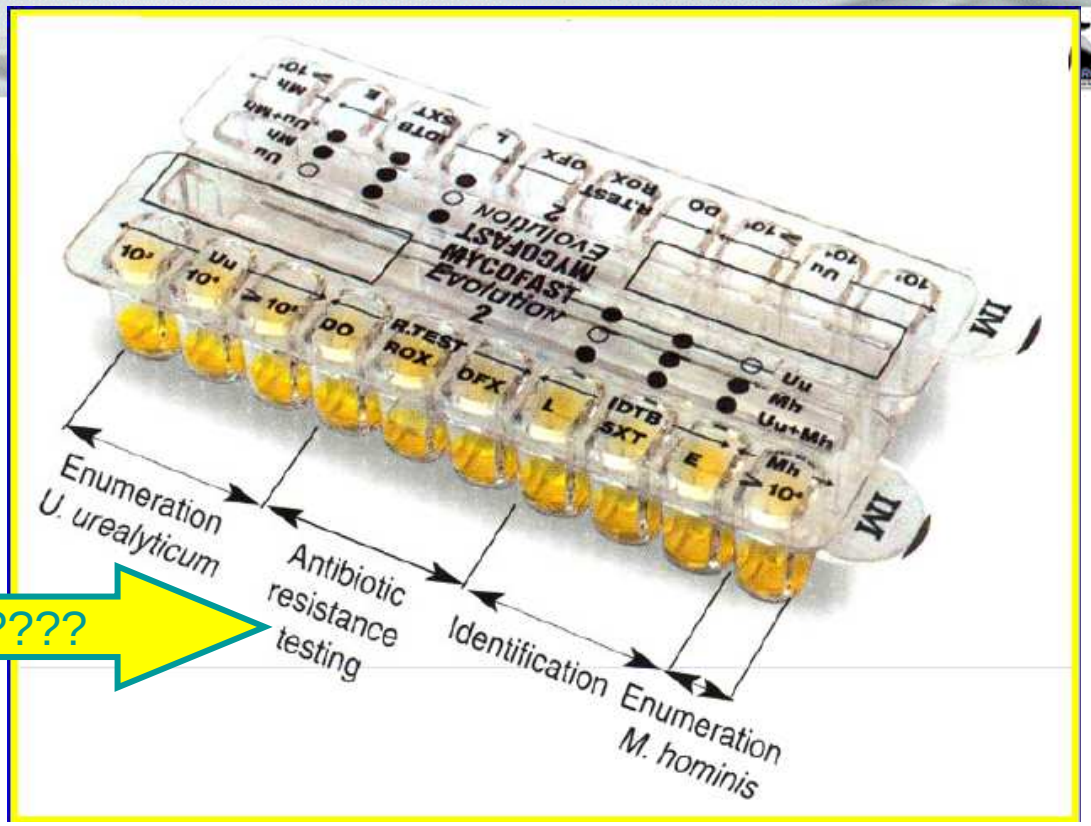


Osservazione del viraggio dell'indicatore presente nel brodo



Semina in microgallerie contenenti substrati e antibiotici in forma liofila e incubate a 37℃ 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. Fairley[1,2]

[1]Melbourne Sexual Health Centre, The Alfred Hospital, [2]School of Population Health and [3]Department of Obstetrics and Gynaecology, University of Melbourne, and [4]Department 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 ($P \leq .01$); *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 ($P \leq .001$). 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. Marrazzo[1] and David H. Martin[2]

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

S102 • CID 2007:44 (Suppl 3) • Marrazzo and Martin

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.

Mycoplasma genitalium as a Contributor to the Multiple Etiologies of Cervicitis in Women Attending Sexually Transmitted Disease Clinics

Charlotte Gaydos, DRPH, Nancy E. Maldeis, PHD,† Andrew Hardick, MS,* Justin Hardick, MS,* and Thomas C. Quinn, MD*‡*

Sexually Transmitted Diseases • Volume 36, Number 10, October 2009

l Diseases Association. Unauthorized reproduction of this article is prohibi

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 Gaydos[1,*], **Nancy E. Maldeis**[2], **Andrew Hardick**[1], **Justin Hardick**[1], and **Thomas C. Quinn**[1,3]

Sex Transm Infect. 2009 October ; 85(6): 438–440.

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.

Has the Time Come to Systematically Test for *Mycoplasma genitalium*?

Lisa E. Manhart, PhD

Sexually Transmitted Diseases • Volume 36, Number 10, October 2009

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 saggiare in modo sistematico tutte le donne sintomatiche

"Evidence for a role of *Mycoplasma genitalium* in pelvic inflammatory disease"
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Clinical Presentation of *Mycoplasma genitalium* Infection versus *Neisseria gonorrhoeae* Infection among Women with Pelvic Inflammatory Disease

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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 Moghaddam[2] *Sex Transm Infect* 2009;**85**:15–18. doi:10.1136/sti.2008.032730

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

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H Moi,[1] N Reinton,[2] A Moghaddam[2]

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

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

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

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

Tetracyclines and azithromycin are generally effective against *C. trachomatis* though sporadic reports of treatment failure have been reported with tetracyclines.[54] 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*[55–58] (IIa, B).

Diagnosi di infezione da *M. genitalium*

STD4D ACE Detection

Simultaneous identification from a single sample:

- ✓ *Mycoplasma hominis*
- ✓ *Mycoplasma genitalium*
- ✓ *Ureaplasma urealyticum*
- ✓ *Ureaplasma parvum*

STD4D ACE Detection

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 → gap (gliceraldeide-3-fosfato deidrogenasi)

Mycoplasma genitalium → gyrA (subunità A della DNA girasi)

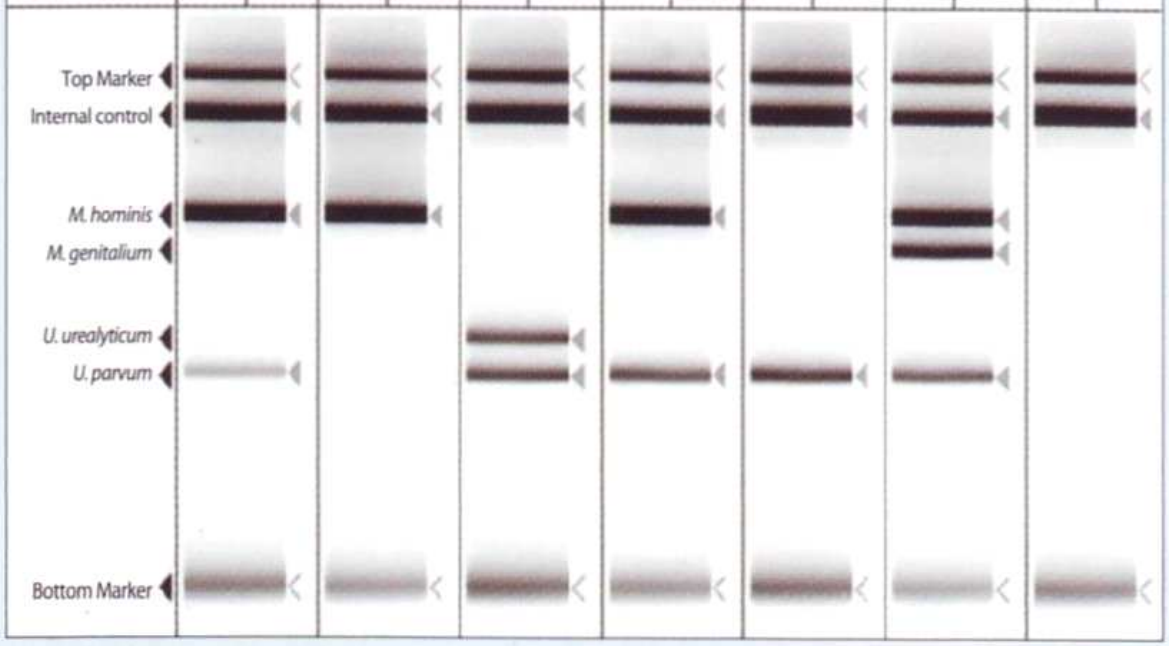
Ureaplasma urealyticum → ureasi

Ureaplasma parvum → ureasi

Automatic target identification

RISULTATI

Lane	L2		L3		L4		L5		L6		L7		L8	
Sample ID	1		2		3		4		5		6		7	
Internal control	+	101	+	105	+	105	+	105	+	103	+	110	+	103
M. hominis	+	105	+	110	−		+	107	−		+	113	−	
M. genitalium	−		−		−		−		−		+	114	−	
U. urealyticum	−		−		+	75	−		−		−		−	
U. parvum	+	32	−		+	79	+	73	+	86	+	75	−	
Unidentified	−		−		−		−		−		−		−	



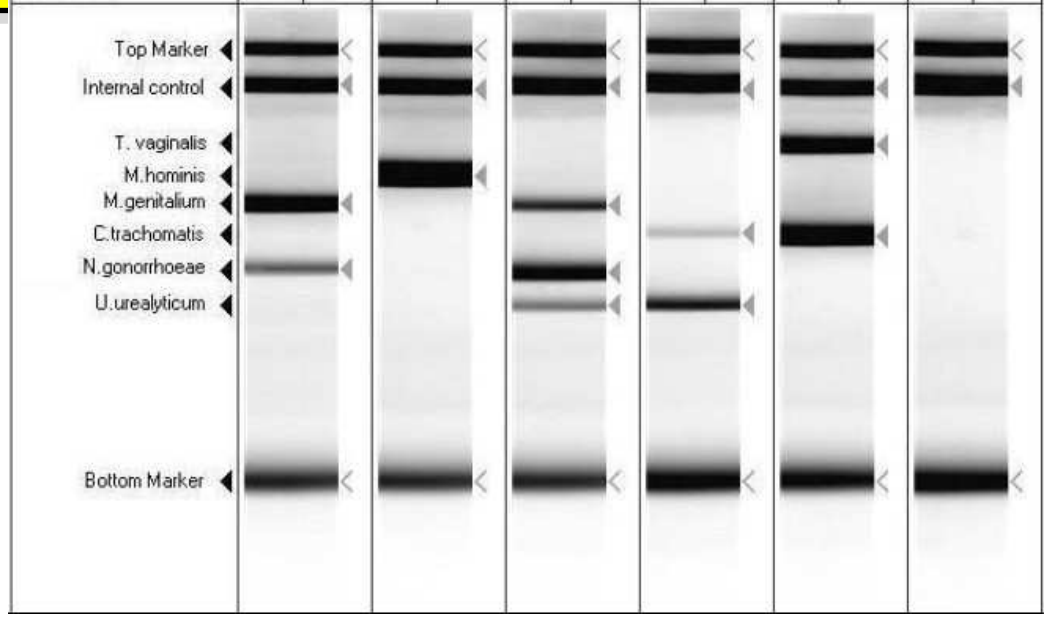
1~6: Clinical samples, 7: Negative control

STD6 ACE Detection

6 different STD pathogen from a single sample:

- ✓ *Trichomonas vaginalis*
- ✓ *Mycoplasma genitalium*
- ✓ *Mycoplasma hominis*
- ✓ *Chlamydia trachomatis*
- ✓ *Neisseria gonorrhoeae*
- ✓ *Ureaplasma urealyticum*

Lane	L2		L3		L4		L5		L6		L7	
Sample ID	B1:		C1:		D1:		E1:		F1:		G1:	
Internal control	+	102	+	103	+	103	+	107	+	101	+	102
T. vaginalis	−		−		−		−		+	107	−	
M.hominis	−		+	108	−		−		−		−	
M.genitalium	+	107	−		+	95	−		−		−	
C.trachomatis	−		−		−		+	28	+	110	−	
N.gonorrhoeae	+	67	−		+	111	−		−		−	
U.urealyticum	−		−		+	57	+	112	−		−	
Unidentified	−		−		−		−		−		−	



Confronto fra metodo Seegene e metodo DID su 171 campioni

Microrganismo	*M. hominis*		*U.urealyticum*		*U. parvum*
	STD4D	DID	STD4D	DID	STD4D
POSITIVI	6	2	6	11	7
TOTALE	STD4D vs DID 6-2		STD4D vs DID 13-11		

Clinical Study

Mycoplasma Genitalium Among Women With Nongonococcal, Nonchlamydial Pelvic Inflammatory Disease

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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.

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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

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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 *mgpa* 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 ($P \leqslant 0.01$; 95 % CI 0.88–0.98) and non-chlamydial-non-gonococcal urethritis ($P=0.0005$; 95 % CI 0.84–0.97).

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Mycoplasma genitalium *Detected by Transcription-Mediated Amplification Is Associated With* Chlamydia trachomatis *in Adolescent Women*

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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 60 (18.2%), 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, 2.0; CI, 1.1–3.2). Dysuria (OR, 0.44; CI, 0.2–0.96) and use of hormonal contraception (OR, 0.55; CI, 0.3–1.0) 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.**

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.[1] 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 urethritis in men.[2] 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

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Antimicrobial Susceptibilities of *Mycoplasma genitalium* Strains Examined by Broth Dilution and Quantitative PCR▽

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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, sitafloxacin, and moxifloxacin were the most active drugs against *M. genitalium*, and their MIC_{90}s were 0.002, 0.008, 0.125, and 0.125 mg/liter, respectively.

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TABLE 1. MICs of *M. genitalium* as determined by conventional broth dilution and real-time quantitative PCR methods

Antimicrobial	Broth dilution				Quantitative TaqMan PCR			
	n[a]	MIC (mg/liter)			*n*	MIC (mg/liter)		
		Range	50%	90%		Range	50%	90%
STX	23	0.008–0.125	0.063	0.125	12	0.016–0.25	0.063	0.125
MXF	23	0.016–0.25	0.063	0.125	18	0.031–0.5	0.063	0.125
GAT	23	0.031–0.5	0.25	0.25	12	0.063–0.5	0.5	0.5
LVX	23	0.125–2	1	2	18	0.5–4	1	4
CIP	23	0.063–8	4	8	18	1–16	2	8
NOR	23	1–64	32	64	12	4–≥8	≥8	≥8
MIN	23	0.031–0.25	0.125	0.25		NT[b]	NT	NT
DOX	23	0.063–1	0.125	0.25	17	0.125–1	0.25	0.5
TET	23	0.063–2	0.125	0.5	17	0.125–4	0.5	2
AZM	23	0.0002–250	0.001	0.002	17	0.002–≥8	0.004	0.008
CLR	23	0.0005–128	0.004	0.008	17	0.016–≥16	0.032	0.063

[a] *n*, numbers of tested strains.
[b] NT, not tested.

PCR Multiplex in STD

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Nucleic Acid Amplification Tests for Diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* Rectal Infections▽

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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 20% 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.

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TABLE 2. Estimates of SDA, PCR, TMA, and culture sensitivities and specificities for detection of *N. gonorrhoeae* or *C. trachomatis* by reference standard

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

Detection of *Chlamydia trachomatis* and *Mycoplasma hominis, genitalium* and *Ureaplasma urealyticum* by Polymerase Chain Reaction in patients with sterile pyuria

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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 200 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 and were analyzed for routine urine examination and cultured on MacConkey agar, blood agar, and sabouraud agar to detect the presence of bacteria and *Candida*. The 200 samples (96 male, 104 female; aged ≥18 years) containing more than 10 leukocytes / HPF and negative for culture (showing no significant growth after 24 hr) 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 6 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), but 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 men.

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 those microorganisms in the urine samples of sterile pyuria patients.

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Table 2. **Microorganisms identified in the 200 studied specimens.**

Microorganisms	male		female		total		P value
	n	(%)	n	(%)	n	(%)	
C. trachomatis	11	(5.5)	9	(4.5)	20	(10.0)	0.509
U. urealyticum	1	(0.5)	9	(4.5)	10	(5.0)	0.008*
M. hominis	0	(0.0)	6	(3.0)	6	(3.0)	0.005*
M. genitalium	2	(1.0)	0	(0.0)	2	(1.0)	0.085

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Multiplex PCR Testing Detection of Higher-than-Expected Rates of Cervical *Mycoplasma*, *Ureaplasma*, and *Trichomonas* and Viral Agent Infections in Sexually Active Australian Women[▽]

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Knowing the prevalence of potential etiologic agents of nongonococcal and nonchlamydial 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*, *Ureaplasma 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* (6.1), *M. genitalium* (1.3), *M. hominis* (13.7), *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 2. Use of mPCRs for screening nongonococcal agents in cervical swabs

Microorganism(s)	mPCR	No. (%) detected	
		Total cervical swabs (n = 233)	Total women (n = 175)
Mollicutes	VDL06		
U. parvum		112 (48.0)	93 (53.1)
U. urealyticum		6 (2.6)	6 (3.4)
M. hominis		15 (6.4)	13 (7.4)
M. genitalium		3 (1.3)	3 (1.7)
U. parvum + *M. hominis*		15 (6.4)	13 (7.4)
U. urealyticum + *M. hominis*		2 (0.9)	2 (1.1)
U. parvum + *U. urealyticum*		6 (2.6)	5 (2.9)
Viruses	VDL05		
CMV		14 (6.0)	11 (6.3)
EV		5 (2.1)	5 (2.8)
EBV		6 (2.6)	6 (3.4)
HSV-1		6 (2.6)	6 (3.4)
HSV-2		2 (0.8)	2 (1.1)
VZV		10 (4.3)	9 (5.1)
VZV + HSV-2		1 (0.4%)	1 (0.6)
Adenovirus species	VDL09		
A, B, C, D, E			0
Other agents	VDL07		
C. trachomatis		1 (0.4)	1 (0.6)
T. vaginalis		8 (3.4)	7 (4)
T. pallidum		0	0
Group B streptococci		1 (0.4)	1 (0.6)

Table 1. CDC Recommendations for STI Screening in Pregnancy

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

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

Majeroni, 2007

MMWR
Morbidity and Mortality Weekly Report
www.cdc.gov/mmwr

Recommendations and Reports | November 19, 2010 / Vol. 59 / No. RR-10

Prevention of Perinatal Group B Streptococcal Disease

Revised Guidelines from CDC, 2010

Continuing Education Examination available at http://www.cdc.gov/mmwr/cme/conted.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)

Test	Swab for NAAT and culture		No. positive by NAAT/ No. positive by culture	NAAT sensitivity†	No. negative by NAAT/ No. negative by culture	NAAT specificity†
	Timing	Type				
NAAT performed on nonenriched samples						
IDI-Strep§	IP	VR	140/149	94.0%	626/653	95.9%
IDI-Strep¶	IP	V	35/56	62.5%	252/259	97.3%
IDI-Strep**	AP/IP	VR	59/68	86.8%	157/165	95.2%
GeneXpert††	IP	VR	23/24	95.8%	20/31	64.5%
IDI-Strep§§	AP/IP	VR	149/188	79.3%	575/603	95.4%
GeneXpert§§	AP/IP	VR	173/190	91.1%	570/594	96.0%
BD GeneOhm¶¶	AP	V	64/83	77.1%	99/117	84.6%
BD GeneOhm***	IP	VR	49/54	90.7%	121/124	97.6%
GeneXpert†††	AP/IP	V	135/137	98.5%	723/726	99.6%
IDI-Strep§§§	IP	VR	38/42	90.5%	148/154	96.1%
NAAT performed on enriched¶¶¶ samples						
BD GeneOhm****	AP	VR	49/53	92.5%	136/147	92.5%
BD GeneOhm††††	AP	VR	§§§§	100.0%	§§§§	99.3%
BD GeneOhm††††	AP	VR	§§§§	92.5%	§§§§	99.3%
BD GeneOhm¶¶¶¶	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-

MAJOR ARTICLE

Diagnostic Accuracy of a Rapid Real-Time Polymerase Chain Reaction Assay for Universal Intrapartum Group B Streptococcus Screening

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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 (58.3%), whereas the negative predictive value was imperfect (92.1%).

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.

C.I.D. (2009); 49 (3): 417.423

Intrapartum Screening *GeneXpert vs.* Culture

INTRAPARTUM		CULTURE		
		-	+	TOTAL
PCR *Gene-Xpert*	-	723	2	725
	+	3	135	138
	TOTAL	756	137	863
	Invalid	*41*	*1*	*42*
	Error	*53*	*5*	*63*
	TOTAL	825	143	968

SEN	SPE	PPV	NPV
98.5	99.6	97.8	99.7

10.8% without PCR results

ORIGINAL ARTICLE

PRENATAL GROUP B *STREPTOCOCCUS* TEST USING REAL-TIME POLYMERASE CHAIN REACTION

Chi-Feng Wei, Bo-Ching She, Hung-Shuo Liang, Qing-Dong Ling[1], Chen-Yuan Tsai[2], Chih-Wei Yen[3], Hsueh-Yin Wu[2], Ming-Song Tsai[4]*

Department of Obstetrics and Gynecology, Cathay General Hospital, Si-Jhih, [1]Cathay Medical Research Institute, Taipei, Departments of [2]Laboratory Medicine and [3]Pediatrics, Cathay General Hospital, Si-Jhih, and [4]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

SMFM Meeting Papers www.AJOG.org

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

Rapid test vs intrapartum culture

	Intrapartum		
	Positive	**Negative**	**Total**
Rapid			
Positive	23	11	34
Negative	1	20	21
Total	24	31	55

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"

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