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Detection of Neisseria gonorrhoeae by PCR using orf1 gene as target

Nucleic acid amplification tests have the ability to specifically amplify small quantities of DNA and hence have been used successfully in the diagnosis of STDs. An in-house polymerase chain reaction (PCR) method was developed and evaluated for the detection of Neisseria gonorrhoeae DNA in the urogenital specimens collected (with consent) from patients visiting an STD clinic in India.

The primers (forward primer 5'-CAACATTTCCGATTTGCA-3' and reverse primer 5'-GTTATACAGCTGGCTGTTAA-3') amplify the 221-480 bp region of orf1 gene. Clinical isolates (n = 40) of Neisseria gonorrhoeae were recovered from urethral or cervical swabs by inoculation onto modified Thayer-Martin medium and identified by Gram stain, colony morphology, positive oxidase, and rapid carbohydrate utilisation test. For PCR the clinical samples (n = 489) were centrifuged (30 minutes, 14 000 g) and the cell pellet was lysed with 50 mM TRIS-HCl (pH 7.5) 1% Triton X-100, 1 mM EDTA, 250 µg of proteinase K per ml at 37°C for 1 hour, boiled for 10 minutes, and centrifuged. Eight µl of lysate was used for amplification (40 cycles) under standard conditions. Each cycle consisted of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. The amplified PCR product (10 µl) was analysed by electrophoresis in a 2% agarose gel and characterised by sequencing.

An amplified product of 260 base pairs (bp) of orf1 gene was observed with all N. gonorrhoeae isolates but not when DNA from the other non-gonococcal strains (17 closely related Neisseria species, Corynebacterium, Chlamydia trachomatis, Candida, syphils, and members of Enterobacteriaceae) was used as template. For the 427 clinical swabs collected from men, 379 were positive and 46 were negative by both culture method and orf1-PCR assay. Urethral specimens from two men were culture negative but PCR positive for orf1 gene. Since these two samples tested PCR positive for cppB gene of N. gonorrhoeae they were considered true positives. Thus, a total of 381 men (89%) were classified as true positives based on the PCR assay (table 1). Of the 62 women tested, 52 were true positives, and five were true negatives as they gave concordant results irrespective of the site of collection and the diagnostic method used (table 1). Four culture negative specimens tested positive by the PCR assays using primers specific to orf1 as well as cppB gene and were, therefore, considered positive. One culture negative specimen was positive by the orf1-PCR assay for its endocervical specimen but negative for urethral specimens. For the cppB gene amplification, the specimen yielded a negative result for both the sites. This was therefore classified as true negative. The sensitivity, specificity, positive predictive value, negative predictive value for the PCR method described here would be 100%, 98%, 99.7%, and 100% respectively. The gold standard has been reported as having a sensitivity of 85–95%.

The high specificity and sensitivity (25 fg DNA per assay, equivalent to 10 cells) coupled with low cost and rapidity of the in-house PCR assay described here can serve as a promising diagnostic method for the detection of gonococci directly from clinical swab samples.

Acknowledgements

We thank Dr Krishna Ray and staff at the STD unit, Safdarjung Hospital, New Delhi, for providing the clinical specimens. We extend our appreciation to Professor J W Lipsall, Prince of Wales Hospital, Australia, for providing us with different Neisseria species. One of us (UC) is grateful to CSIR for the award of a senior research fellowship.

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References


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Nevirapine + efavirenz based salvage therapy in heavily pretreated HIV infected patients

The emergence of protease inhibitors (PIs) and multiple drug therapy for HIV infection has greatly decreased mortality in countries where these medications are available. Unfortunately, many patients eventually develop viral resistance to treatment because of HIV virus mutations. As clinicians await development of new drugs to combat resistant virus, innovative strategies with existing drugs may be particularly valuable. Patients having failed regimens containing nucleoside reverse transcriptase inhibitors (NRTIs) and PIs face limited options for future therapy. A regimen containing the two potent non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (NVP) and efavirenz (EFV), could provide an effective alternative, since both can be conveniently dosed once daily and have demonstrated efficacy in patients with high viral loads.

A retrospective chart review at an urban HIV hospital clinic identified 13 patients who had initiated an NVP + EFV based salvage
regimen (table 1). Inclusion of these patient charts in this study was approved by a research ethics committee at Bellevue Hospital. All patients received NVP + EFV at standard doses. The lower limit of quantitation was determined at 50 HIV RNA copies/ml using Roche Amplicor HIV-1 Monitor (RNA) (Roche Diagnostics, Branchburg, NJ, USA). Median baseline values were: viral load 33 900 copies/ml (range 3100–750 000 copies/ml) and CD4+ count 190 cells/μl (range 2–440 cells/μl). After a median follow up of 11 months (range 3–18 months), 85% (11/13) had viral loads < 50 copies/ml. Considering previous treatment experience, 90% (9/10) of NNRTI naive patients had viral loads < 50 copies/ml and 67% (2/3) of NNRTI experienced patients had viral loads < 50 copies/ml. Effectiveness of the dual NNRTI combination in heavily treated patients is in contrast with a study by Jordan and colleagues who demonstrated a response to Bactrim, and a second case resolved with only didanosine (ddI) in 19/21 patients after 12 months. The most common adverse event was elevated liver function test results (more than three times upper limit of normal) in three patients. One case of liver toxicity was attributed to Bactrim, and a second case resolved following interruption of EFV (EFV rechallenge in this patient was successful). No specific cause of liver toxicity could be identified in the third case, suggesting a possible association with antiretroviral treatment. Other adverse events included anaemia. The patient with EFV induced hepatotoxicity also had anaemia and EFV related central nervous system disturbances. None of the patients discontinued therapy because of adverse events. The relatively low incidence of adverse events and the absence of NNRTI associated metabolic disorders make this dual NNRTI based regimen additionally appealing.

This retrospective analysis demonstrated the effectiveness of the combination of two NNRTIs (NVP and EFV) in heavily treated PI experienced patients, with no apparent increase in NNRTI related side effects. Since few new antiretrovirals with novel resistance profiles are forthcoming in the near future, this regimen may provide a much needed alternative in heavily pretreated patients.

Table 1 Baseline characteristics and outcome for NNRTI naive and experienced patients

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Regimen</th>
<th>Previous drugs</th>
<th>Baseline viral load (copies/ml)</th>
<th>Baseline CD4+ cell count (cells x109/l)</th>
<th>Months of follow-up</th>
<th>Last viral load (copies/ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>NVP+EFV+d4T</td>
<td>d4T, dT, RTV, IDV, ABC</td>
<td>37100</td>
<td>290</td>
<td>15</td>
<td>164000</td>
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<tr>
<td>2</td>
<td>NVP+EFV+d4T</td>
<td>NVP, CBV, IDV</td>
<td>436000</td>
<td>183</td>
<td>7</td>
<td>&lt;50</td>
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<tr>
<td>3</td>
<td>NVP+EFV+ABC</td>
<td>IDV, d4T, NVP, ddlC</td>
<td>27000</td>
<td>–</td>
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<td>4</td>
<td>NVP+EFV+d4T</td>
<td>CBV, NVP, SQV</td>
<td>151000</td>
<td>161</td>
<td>9</td>
<td>&lt;50</td>
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<tr>
<td>5</td>
<td>NVP+EFV+d4T</td>
<td>NVP, CBV</td>
<td>31900</td>
<td>392</td>
<td>18</td>
<td>&lt;5000</td>
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<td>6</td>
<td>NVP+EFV+d4T+ddl</td>
<td>d4T, ddl, NVP</td>
<td>5000</td>
<td>440</td>
<td>6</td>
<td>&lt;5000</td>
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<td>7</td>
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<td>9</td>
<td>NVP+EFV+d4T</td>
<td>IDV, ddl</td>
<td>35900</td>
<td>180</td>
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<td>&lt;50</td>
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<tr>
<td>10</td>
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<td>RTV, other PI</td>
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<td>190</td>
<td>14</td>
<td>&lt;50</td>
</tr>
<tr>
<td>11*</td>
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<td>DLV, RTV, d4T</td>
<td>8900</td>
<td>276</td>
<td>13</td>
<td>&lt;50</td>
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<tr>
<td>12*</td>
<td>NVP+EFV+RTV+IDV</td>
<td>SQV, NVP, ddl, DLV</td>
<td>9000</td>
<td>233</td>
<td>11</td>
<td>33000</td>
</tr>
</tbody>
</table>

*NNRTI experienced patients. 

References

3 Pollard R. Factors predictive of durable HIV suppression in randomized double-blind trial with nevirapine (NVP), zidovudine (ZDV), and lamivudine (3TC) in treatment-naive (ART-naive) patients with advanced AIDS. 7th Conference on Retroviruses and Opportunistic Infections. San Francisco, USA, 30 Jan–2 Feb 2000 (abstract S17).

Accepted for publication 25 October 2001

Comparing cost effectiveness of screening women for Chlamydia trachomatis in systematic and opportunistic approaches

Screening women for asymptomatic Chlamydia trachomatis (CT) infections is indicated to prevent the spread of CT and the development of complications such as pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, tubal infertility, and neonatal pneumonia (major outcomes averted; MOA). Cost effectiveness presents an important aspect in the decision making regarding actual implementation. Recently, in this journal Van Valkengoed et al published a paper on the cost effectiveness of systematic screening among women in Amsterdam (Netherlands), using pharmacoeconomic modelling. Using the same model, results on the cost effectiveness of an opportunistic screening in the same city have also been published. Specific model assumptions differed in both publications. The aim of this letter is to compare cost effectiveness of systematic and opportunistic screening using similar model assumptions and correcting for potential biases.

Opportunistic screening was done during May 1996 to May 1997 in a pilot study. Women visiting the participating GPs were eligible for screening if they considered themselves heterosexually active, were aged 15–40 years, and did not visit their GP for sexually transmitted disease complaints (participation among women: 96% compared with 50% in the systematic screening). In this letter we report on the age group 15–30. Obviously, the effectiveness of this type of screening depends on the frequency of visiting the GP; 87% of Dutch women aged 15–30 visit the GP at least once per year. As in the systematic universal screening, testing was done with ligase chain reaction (LCR) on urine. Participating GPs in the opportunistic screening had an over-representation compared to the general Amsterdam situation of participants from Caribbean, Surinam and other (source: Statistics Amsterdam).

Parameters in the pharmacoeconomic model were kept similar to the previous paper in this journal, except for the probability of PID after asymptomatic infection. For this probability we applied 20% compared to 10% in the paper by Van Valkengoed et al. We even consider 20% as a very conservative estimate for the risk of PID in our model. Cost effectiveness was estimated as net costs per MOA in baseline analysis using assumptions

www.sextransinf.com
References


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Major improvements in cost effectiveness of screening women for Chlamydia trachomatis using pooled urine specimens and high performance testing

Screening of asymptomatic Chlamydia trachomatis (CT) infections is indicated to prevent the spread of CT and the development of secondary complications like pelvic inflammatory disease, ectopic pregnancy, and tubal infertility. Cost effectiveness presents an important aspect in the decision making regarding actual implementation. A recent paper in this journal by Van Valkengoed et al. addressed cost effectiveness, using an established pharmacoeconomic model, of a systematic screening programme for asymptomatic CT infections in women registered in general practices in Amsterdam, based on mailed home obtained urine specimens. The aim of this letter is to extend the application of the pharmacoeconomic model with regard to pooling and improved test performances (sensitivity and specificity).

We recently determined the sensitivity and specificity for two commercially available CT detection assays for urine specimens from asymptptomatically CT infected women and men. In total, 296th mailed home obtained urine specimens were tested for CT using both ligase chain reaction (LCR) and polymerase chain reaction (PCR) testing. We showed that for individual testing, the test sensitivity/specificity for LCR and PCR could be estimated at 78.6%/99.7% and 98.8%/99.9%, respectively. Furthermore, we recently showed by using individual urine samples (n = 650) and samples pooled by five (n = 130) that pooling has a relative sensitivity and specificity of 100%. Since only CT positive pools have to be analysed for the individual CT positive cases approximately 60% of the number of tests could be saved in our population with an estimated CT prevalence of 2–3%.

In the pharmacoeconomic model test performances of 85.0% sensitivity and 99.0% specificity were previously assumed. Furthermore, the model included population based estimates of CT prevalence, the costs of the programme, the health gain effects and the related monetary benefits. Health gain effects considered were averted pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, infertility, and neonatal pneumonia (major outcomes averted; MOA). Both direct and indirect costs and benefits were considered. We investigated the effects on baseline cost effectiveness of pooling and improvements in test performance.

Population based prevalence in the systematic screening was 2.2% for women aged 15–25 and 2.9% for women aged 15–25. Van Valkengoed et al. estimated baseline cost effectiveness for systematic screening in Amsterdam using LCR at net costs of US$11 100 for women aged 15–25 and US$15 800 per MOA for women aged 15–40 (table 1). High performance testing of 98.8% sensitivity and 99.9% specificity was estimated to reduce net cost per MOA by approximately 20%. Pooling urine specimens by five was estimated to reduce net costs per MOA by 75%. A total decrease of 67%
was estimated if both high performance testing and pooling are assumed (table 1). We conclude that with pooling and application of high performance testing major improvements in cost effectiveness of screening women for asymptomatic CT can be obtained.

**Acknowledgements**

This work was partly supported by ZON (Prevention Fund), grants 28-1181-1 and 28-2705. The authors acknowledge the assistance and cooperation of all researchers, physicians, nurses, and participants involved in the project on systematic screening for Chlamydia trachomatis in Amsterdam.

**References**


**Table 1: Demographic data and anti-HIV serology**

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<tr>
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<td>1</td>
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<tr>
<td>Ever</td>
<td>0</td>
<td>19</td>
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**Neuropsychiatric reaction induced by clarithromycin**

We read with interest the case report by Prime and French describing a patient with HIV infection who developed a severe neuropsychiatric reaction during clarithromycin, zidovudine, didanosine, and nevirapine treatment. We suggest that this reaction was caused by the clarithromycin and not the antiretrovirals. Indeed, central nervous system (CNS) symptoms are a known side effect of clarithromycin. CNS adverse effects, however, have also been reported with zidovudine and abacavir. So far with nevirapine neuropsychiatric side effects have not been described. For this reason we would like to report the case of a patient who developed CNS side effects shortly after starting nevirapine.

A 40 year old woman with HIV infection was initially treated with ritonavir, saquinavir, and stavudine. Because she developed lipoatrophy injection, and abnormal sexual intercourse (oral and anal sex) were also reviewed for each case.

For all 35 cases of sexual dysphoria, only two cases of anti-HIV seropositivity were detected. The prevalence was equal to 5.71%. These two cases were homosexual. The demographic data of HIV seronegative and HIV seropositive cases are shown in table 1.

Currently, the two major routes of transmission of HIV are blood borne and sexual propagation. Sexual propagation also includes the abnormal sexual behaviour such as oral and anal sex found in the “gay” population. Unique aspects of Thai culture have shaped the response of homosexual men and women to HIV infection in Thailand. Thailand is a relatively homogeneous society that has, by and large, felt vulnerable to AIDS, viewing it primarily as a Western phenomenon. This attitude has also been common in the gay community and has resulted in some homosexual men and women engaging in high risk behaviour.

In Thailand it has been argued that HIV infection is still a major health problem among homosexual men and women. The current HIV epidemic among young homosexual men and women is a major public health concern. Nevertheless, hardly any specific HIV education interventions have been designed for this population. In this study, the rather high rate of HIV infection among the homosexual men and women attending the hospital for further sex change surgery was detected. Compared with the rate in the general population in Thailand, this rate is five times higher. Therefore, this population is still a target group for HIV infection, and thus, proper control for this population is necessary.

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**Accepted for publication 16 November 2001**

**Explain the role of healthcare providers in addressing health concerns of homosexual men and women.**

Healthcare providers play a crucial role in addressing the health concerns of homosexual men and women. They can provide unbiased, quality care that recognizes the unique aspects of their lifestyle and helps them navigate the potential risks associated with sexual health. Providers can also educate patients about the importance of regular screening for sexually transmitted infections (STIs), such as Chlamydia trachomatis, and the role of antiretrovirals in managing HIV. Additionally, healthcare providers can address the psychosocial aspects of their patients’ lives, recognizing that the stigma and discrimination faced by this community can significantly impact their mental health. Effective communication and support are key components of care for these patients, helping to foster a safe and non-judgmental environment that encourages open discussion and timely intervention.
causing these symptoms. However, it is also possible that after stopping the clarithromycin the nevirapine levels decreased and that therefore potential nevirapine related side effects disappeared. We propose that in HIV clinical trials patients should be monitored more closely for any neuropsychiatric side effects and that if these side effects appear antiretroviral drug levels should be measured.

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**Immune reconstitution eosinophilia due to schistosomiasis**

A 21 year old black African heterosexual woman, formerly resident in south east Africa, presented in early October 2000 on arrival in the United Kingdom. Examination revealed hepatosplenomegaly. Investigation showed Hb = 10.2 g/dl, WBC = 2.9 × 10^9/L, neutrophils = 1.5, lymphocytes = 0.8, and eosinophils = 0.3 × 10^9/L. Liver ultrasound and electrolytes were normal. Liver function tests showed alkaline phosphatase = 1704 (normal = 45–122) U/l, and alanine transaminase = 65 (normal = 7–63) U/l. Blood urea and electrolytes were normal. A liver biopsy showed portal fibrosis with piecemeal necrosis and periportal lymphocytes. A chest radiograph showed micronodular abdominal lymphadenopathy and no ascites. Heptosplenomegaly; there was no intra-abdominal lymphadenopathy and no ascites. Computer tomography of the neck revealed lymphadenitis.

On 1 December 2000, four weeks after starting HAART the HIV RNA level had fallen to 25 000 copies/ml, CD4 count remained at 170 cells × 10^6/L but the eosinophil count had risen to 0.8 × 10^9/L (normal = 0.04–0.44 × 10^9/L). After 4 months of HAART the HIV level was below the limits of detection, the CD4 count had risen to 230 cells × 10^6/L and the eosinophil count had further risen to 1.3 × 10^9/L. At this time the patient agreed to treatment of schistosomiasis with praziquantel (40 mg/kg in two divided doses in 1 day). Following praziquantel the eosinophil count fell to 0.5 × 10^9/L. The development of eosinophilia in this HIV infected patient with schistosomiasis occurred in the context of a falling HIV RNA level and an increase of CD4 count, indicating partial immune reconstitution.

Partial restoration of cell mediated immune induction by antiretroviral therapy, as demonstrated by recovery of partial CD4+ T lymphocyte reaction to memory antigens, may cause development of an inflammatory response; in this case eosinophilia, in patients latently affected with opportunistic and non-opportunistic pathogens. Reactivation of mycobacterial lymphadenitis, cryptococcal meningitis, and cytomegalovirus retinitis and pneumonitis have been described. The case described here suggests that development of an eosinophilia to schistosomiasis should be added to the list of immune reconstitution phenomena.

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We agree with the authors’ statement that systematic screening of all women aged 15–40 years for asymptomatic Chlamydia trachomatis infection is not cost effective, especially when the prevalence of infection in Amsterdam is low (2.2%–2.8%). Not all countries have achieved such low levels. Even in England and Wales where the prevalence of the infection is higher it is not cost effective to screen all women. However, computer modelling performed for the chief medical officer’s expert advisory group on Chlamydia trachomatis in the United Kingdom and other countries has shown that it is cost effective to screen populations where the prevalence is 3%–6.

The Chlamydia Pilot Study, which was conducted in Wirral and Portsmouth in 1999–2000, detected a prevalence of chlamydial infection of approximately 10% in women aged between 16 and 25. There is, therefore, a strong argument for screening this age group in the United Kingdom at the present time and not above 25 years as prevalence above this age is low.

One must be careful when extrapolating data from a different country with a different population. However, it would be wise to consider that in the future in the United Kingdom, when screening is established, the prevalence may fall and the cost effectiveness may be reduced. Although it is not cost effective to screen men, as there are only minor sequelae to be prevented, one shouldn’t forget that they are the major reservoir of infection. We should aim not to reinforce existing inequalities by sparing them their share of responsibility for sexual health. Screening men as well will not only decrease the prevalence but also reduce the psychosocial impact of screening for genital chlamydia in women.

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Correspondence to: Dr Meena Gupta, m.hernon@nhs.uk

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The development of eosinophilia in this HIV infected patient with schistosomiasis occurred in the context of a falling HIV RNA level and an increase of CD4 count, indicating partial immune reconstitution.

Partial restoration of cell mediated immune induction by antiretroviral therapy, as demonstrated by recovery of partial CD4+ T lymphocyte reaction to memory antigens, may cause development of an inflammatory response; in this case eosinophilia, in patients latently affected with opportunistic and non-opportunistic pathogens. Reactivation of mycobacterial lymphadenitis, cryptococcal meningitis, and cytomegalovirus retinitis and pneumonitis have been described. The case described here suggests that development of an eosinophilia to schistosomiasis should be added to the list of immune reconstitution phenomena.

**References**


Accepted for publication 13 December 2001

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**Cost effectiveness analysis of a population based screening programme for asymptomatic Chlamydia trachomatis infection in women**

With reference to the article by van Valkengoed et al, we would like to express our views.

**References**


Accepted for publication 13 December 2001
NOTICES

International Herpes Alliance and International Herpes Management Forum
The International Herpes Alliance has introduced a website (www.herpesalliance.org) from which can be downloaded patient information leaflets. Its sister organisation the International Herpes Management Forum (website: www.IHMF.org) has launched new guidelines on the management of herpesvirus infections in pregnancy at the 9th International Congress on Infectious Disease (ICID) in Buenos Aires.

Pan-American Health Organization, regional office of the World Health Organization
A catalogue of publications is available online (www.paho.org). The monthly journal of PAHO, the Pan American Journal of Public Health, is also available (subscriptions: pubsuc@tsp.sheridan.com).

Second International Conference on Sexual Health
To be held in Bangkok, Thailand on 23–28 February 2002
Further details: European Secretariat, Dr Richard Burack (tel: +44 (0) 20 8599 8029; email: siamcare@aol.com).

7th Congress of the European Society of Contraception, “Changing attitudes to contraception and reproductive health”
Genoa, Italy, 10–13 April 2002
Further details: ESC Central Office, Orgamed, Eissennestraat 77, B-1780 Temse, Belgium (tel: +32 2 582 08 52; fax: +32 2 582 55 15; email: orgamed@village.uunet.be).

MSSVD course in STIs and HIV, Module 1, Epidemiology of STIs and Bacterial Infections
Further details: Sue Bird, MSSVD STIs and HIV Course Secretariat, PO Box 77, East Horsham, KT24 5YP (tel: 01372 454210).

MSSVD course in STIs and HIV, Module 2, Sexual Health and Sexuality
At the Institute for Materials, 1 Carlton House Terrace, London, 26 April 2002.
Further details: Sue Bird, MSSVD STIs and HIV Course Secretariat, PO Box 77, East Horsham, KT24 5YP (tel: 01372 454210).

MSSVD course in STIs and HIV, Module 3, Viral Infections other than HIV
Further details: Sue Bird, MSSVD STIs and HIV Course Secretariat, PO Box 77, East Horsham, KT24 5YP (tel: 01372 454210).

MSSVD course in STIs and HIV, Module 4, HIV Infections
Further details: Sue Bird, MSSVD STIs and HIV Course Secretariat, PO Box 77, East Horsham, KT24 5YP (tel: 01372 454210).

10th International Symposium on Human Chlamydial Infection
16–21 June 2002, in Antalya, Turkey
The scientific programme will encompass the breadth of chlamydial research from clinical and epidemiological studies to molecular and cell biology of all species of Chlamydia. Further details: Professor A Demir Serter, Department of Clinical Microbiology and Infectious Diseases, Ege University, Faculty of Medicine, 35100 Bornova, Izmir, Turkey (fax: 90 232 343 71 30; email: ISHCIX@itsa.ucsf.edu).

10th International Congress on Behçet’s Disease
Berlin 27–29 June 2002
Further details: Professor Ch Zouboulis (email: zouubbere@zedat.fu-berlin.de).

20th World Congress of Dermatology
Paris, 1–5 July 2002
Further details: P Fournier, Colloquium, 12 rue de la Croix St Faubin, 75011 Paris, France (tel: +33 1 44 64 15 15; fax: +33 1 44 64 15 16; email: p.fournier@colloquium.fr; website: www.derm-wcd-2002.com).

18th Congress on Sexually Transmitted Infections
IUSTI–Europe 2002
12–14 September 2002, Hofburg Center, Vienna
Further details: Angelika Stary, M.D., c/o Administrative and Scientific Secretariat, Vienna Academy of Postgraduate Medical Education and Research, Aser Strasse 4, A–1090 Vienna, Austria (tel: +43 1 405 13 8513; fax: 43 1 407 82 74; email: iusti2002@medacad.org).