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'-CAACTATCCCGAGTGGCCA-3' and reverse primer 5'-GTTAATCAAGCTGTGGCGT-3') amplify the 221–480 bp region of orf1 gene. Clinical isolates (n = 40) of N 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, syphillis, and members of Enterobacteriaceae) was used as a 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). 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 daily7 and have demonstrated efficacy in patients with high viral loads.8

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 Amplirob HIV-1 Monitor (RNA) (Roche Diagnostics, Branchburg, NJ, USA). Median baseline values were: viral load 33,000 copies/ml (range 3100–750,000 copies/ml) and CD+ count 190 cells/μl (range 2–440 cells). 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 experienced patients had viral loads <50 copies/ml. Effectiveness of the dual NNRTI combination in heavily pretreated patients is in contrast with a study when there are only one or two NRTIs in the regimen (1). 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 x10^3/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+ddT</td>
<td>d4T, ddT, 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+ddT</td>
<td>NNRTI naive experienced</td>
<td>436000</td>
<td>183</td>
<td>7</td>
<td>&lt;50</td>
</tr>
<tr>
<td>3</td>
<td>NVP+EFV+ddT</td>
<td>d4T, d4T, NNRTI naive experienced</td>
<td>27000</td>
<td>–</td>
<td>13</td>
<td>&lt;50</td>
</tr>
<tr>
<td>4</td>
<td>NVP+EFV+ddT</td>
<td>CBV, NFV, SQV</td>
<td>151000</td>
<td>161</td>
<td>9</td>
<td>&lt;50</td>
</tr>
<tr>
<td>5</td>
<td>NVP+EFV+ddT</td>
<td>NFV, CBV</td>
<td>31900</td>
<td>392</td>
<td>18</td>
<td>&lt;50</td>
</tr>
<tr>
<td>6</td>
<td>NVP+EFV+ddT</td>
<td>d4T, d4T, NFV</td>
<td>50000</td>
<td>440</td>
<td>6</td>
<td>&lt;5000</td>
</tr>
<tr>
<td>7</td>
<td>NVP+EFV+RTV+IDV</td>
<td>IDV, NFV, IDV</td>
<td>750000</td>
<td>20</td>
<td>10</td>
<td>&lt;50</td>
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<tr>
<td>8</td>
<td>NVP+EFV+ddT</td>
<td>NFV, CBV</td>
<td>750000</td>
<td>2</td>
<td>11</td>
<td>&lt;50</td>
</tr>
<tr>
<td>9</td>
<td>NVP+EFV+ddT</td>
<td>NFV, ddD</td>
<td>750000</td>
<td>2</td>
<td>12</td>
<td>&lt;50</td>
</tr>
<tr>
<td>10</td>
<td>NVP+EFV+ddT</td>
<td>RTV, ddD</td>
<td>35900</td>
<td>180</td>
<td>3</td>
<td>&lt;5000</td>
</tr>
<tr>
<td>11</td>
<td>NVP+EFV+RTV+IDV</td>
<td>DLV, RTV, ddD</td>
<td>3100</td>
<td>190</td>
<td>14</td>
<td>&lt;50</td>
</tr>
<tr>
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<td>SQV, NFV, NVP, ddD</td>
<td>8900</td>
<td>276</td>
<td>13</td>
<td>&lt;50</td>
</tr>
<tr>
<td>13</td>
<td>NVP+EFV+RTV+IDV</td>
<td>SQV, ddT, ddD, EFV, IDV</td>
<td>3900</td>
<td>235</td>
<td>11</td>
<td>33000</td>
</tr>
</tbody>
</table>

*NNRTI experienced patients.

NVP = nevirapine, EFV = efavirenz, d4T = Stuvudine, ABC = abacavir, ddD = didanosine, RTV = ritonavir, IDV = indinavir, CBV = carbovir, SQV = saquinavir, DLV = delavirdine.

### 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 (ARV-) patients with advanced AIDS. 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, USA, 30 Jan–2 Feb 2000 (abstract 517).


7. Accepted for publication 25 October 2001.

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 and Surinam ethnicity with relatively high CT prevalence. To enhance valid comparison with the systematic screening, asymptomatic CT prevalence rates in the opportunistic screening were recalculated standardising for the distribution of the Amsterdam population over the ethnic groups of 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.
above and sensitivity analysis (PID risk at 10%, high performance testing and pooling).1,5

In the baseline analysis cost effectiveness is US$3300 per MOA for systematic screening of women aged 15–25 and $1400 for opportunistic screening of that same age group. Including sensitivity analysis, cost effectiveness of systematic screening ranges from $2000–$11 100 per MOA (see table 1). For opportunistic screening this range is $500–$4100 per MOA. For the age group of 15–30, cost effectiveness is estimated to be generally slightly less favourable.

We conclude that opportunistic instead of systematic screening reduces net costs per MOA up to 75% (age groups 15–25) and by approximately 50% (age groups 15–30) over a range of plausible assumptions. Opportunistic CT screening in Amsterdam is therefore more attractive than systematic screening from a pharmacoeconomic point of view. Obviously, pharmacoeconomics only present one aspect in decision making concerning CT screening, others being, for example, implementation issues and budgetary constraints.

Acknowledgements
This work benefited from financial support by the Institute of Medical Technology Assessment (IMTA; Rotterdam, Netherlands) within the framework of the project “Guidelines and Cost-effectiveness for Sexually Transmitted Diseases.” The authors acknowledge the assistance and cooperation of all researchers, physicians, nurses, and participants involved in the projects on opportunistic and systematic screening for Chlamydia trachomatis in Amsterdam.

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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.1 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.2 In total, 2906 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 that pooling of 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 0.3%.

In the pharmacoeconomic model test performance of 85.0% sensitivity and 99.0% specificity were previously assumed.1 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 15–40 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).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 57%. 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.

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References

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Anti-HIV serology in patients with sexual dysphoria in screening test before sex change surgery

The health and behavioural issues of homosexual men and women have recently become a focus of research and interest. A well conceived framework within which to consider the uniqueness of problems faced by homosexual youths and the role of healthcare providers is needed.1 Significant physical morbidity occurs among homosexual men and women because healthcare providers are often unaware of their actual or potential health concerns. Physical health concerns mainly include HIV disease, hepatitis, and other sexually transmitted diseases. Health-care professionals, who are clinically competent in the care of homosexual men and women, should have the opportunity to reduce the risk of disease, while providing unbiased, quality care which recognises the unique problems of this population.2 In this study, we report the prevalence of HIV infection among the homosexual men and women who visited the pre-admission clinic, King Chulalongkorn Memorial Hospital, Bangkok, for further sex change surgery.

A prospective study on the data concerning anti-HIV test for 35 cases (33 homosexual men and two lesbian women) with sexual dysphoria who attended the pre-admission clinic, King Chulalongkorn Memorial Hospital, Bangkok, for further sex change surgery.

For all 35 cases of sexual dysphoria, only two cases of anti-HIV seropositivity were reviewed for each case. The demographic data about occupation, hormone injection, and abnormal sexual intercourse (as 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.2 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 invulnerable 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,3 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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Neuropsychiatric reaction induced by clarithromycin

We read with interest the case report by Prime and French1 describing a person with HIV infection who developed a severe neuropsychiatric reaction during clarithromycin, zidovudine, didanosine, and nevirapine treatment. The authors suggest that this reaction was caused by the clarithromycin and not the antiretrovirals. Indeed, central nervous system (CNS) symptoms are known side effect of clarithromycin.2 CNS adverse effects, however, have also been reported with zidovudine3 and efavirenz.4

So far with nevirapine neuropsychiatric side effects have not been decrease nevirapine, zidovudine, lamivudine, and stavudine. Because she developed lipodystrophy she was switched to nevirapine, lamivudine, and stavudine. Shortly after starting this treatment, she started to feel depressed and to experience bad dreams. Her CD4+ lymphocyte count was 727 × 10^9/l and her viral load was undetectable. She was living under stressful conditions (her husband was also living with HIV), but according to her there was no recent change in her life to explain this depression. The nevirapine was replaced by abacavir and from then on the CNS side effects rapidly disappeared.

A 40 year old woman with HIV infection was initially treated with ritonavir, saquinavir, and stavudine. Because she developed lipodystrophy she was switched to nevirapine, lamivudine, and stavudine. Shortly after starting this treatment, she started to feel depressed and to experience bad dreams. Her CD4+ lymphocyte count was 727 × 10^9/l and her viral load was undetectable. She was living under stressful conditions (her husband was also living with HIV), but according to her there was no recent change in her life to explain this depression. The nevirapine was replaced by abacavir and from then on the CNS side effects rapidly disappeared.

This case report strongly suggests that the nevirapine was responsible for the CNS symptoms.

CNS side effects related to antiviral treatment may be caused by high drug levels. Clarithromycin is known to increase nevirapine levels by about 26%.5 The fact that in the patient described by Prime and French the neuropsychiatric symptoms disappeared within 72 hours after stopping the clarithromycin suggests this drug was responsible for
immunological function was negative. An HIV-1 antibody test was positive, plasma HIV-1 RNA level had fallen to 25 000 copies/ml, CD4 count remained at 170 cells \( \times 10^3 \) but the eosinophil count had risen to 1.5 \( \times 10^3 \) (normal = 0.04–0.44 \( \times 10^3 \)). After 4 months of HAART the patient showed below the limit of detection, the CD4 count had risen to 230 cells \( \times 10^3 \), and the eosinophil count had further risen to 1.5 \( \times 10^3 \). 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 \( \times 10^3 \).

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 immuned induction by antiretroviral therapy, as demonstrated by recovery of partial CD4+ lymphocyte reactivation 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 meningsitis, 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

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.
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: pubsvc@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, Eßenerstraat 77, B-1740 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 Horsley, 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 Horsley, 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 Horsley, 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 Horsley, 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: zoubbere@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).