Guidelines for the use and interpretation of nucleic acid detection tests for *Neisseria gonorrhoeae* in Australia

Summary

The Public Health Laboratory Network (PHLN) convened a workshop of Australian experts in Melbourne on 23 March 2005 to identify laboratory issues of relevance and suggest guidelines for use of nucleic acid detection tests (NADT) for diagnosis of gonorrhoea in Australia. The proceedings of that meeting were endorsed by the members of the PHLN and the Communicable Diseases Network of Australia.

Given the present state of knowledge and experience of conditions currently existing in Australia, the following recommendations were made:

Recommendation 1: Assays using detection of the *cppB* gene should not be used for either screening or supplemental assays.

Recommendation 2: All in-house screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.

Recommendation 3: All commercial screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.

Recommendation 4: If a sample is positive in a screening assay but a suitable supplemental assay is negative, then the result should be reported as negative.

Recommendation 5: Laboratories should ensure that the test combination they use would yield a positive predictive value of at least 90 per cent in a population with a prevalence of 1 per cent.

Recommendation 6: For the purposes of test evaluation, as distinct from diagnostic testing, true positives be defined by meeting one or more of the following criteria:

1) culture positive using contemporary isolation and identification techniques;

2) positive result on NADTs directed to targets on three separate genes that are known to have discriminatory capacity;

3) sequencing of a gene known to separate gonococcal from non-gonococcal species.

Recommendation 7: Inhibitor controls should be routinely included in all NADT

Recommendation 8: Cultures are the preferred test for samples from non-genital sites. If however it is necessary to perform a NADT, then more stringent criteria should be applied, and positive samples should meet the 'test evaluation' criteria for a 'true positive'.

Recommendation 9: In order to properly assess the routine diagnostic system in Australia, the following quality assurance samples should be distributed in addition to the routine samples currently used:

1. *cppB* negative *N. gonorrhoeae;*

2. non-gonococcal species known to cause false positive reactions: these should be dispatched both as a single species, as well as mixture with *N. gonorrhoeae.* In the latter circumstance, the non-gonococcal species should be present in 10-fold excess;

3. Urine samples: preferably a single patient sample, otherwise a spiked sample.

4. Validation panels should be made that include samples that are culture positive but PCR negative. True positive samples should also be made available.

In addition, a process should be established for full phenotypic and genotypic characterisation of unidentified species that yield false positive results in NADT for gonococci.

Recommendation 10: Strategies should be put in place to ensure that sufficient numbers of gonococcal isolates are obtained to allow reliable monitoring of antimicrobial resistance.

Recommendation 11: Public health practitioners need to define the relevant populations that need to be targeted and identify any that require enhanced surveillance.

Background

In recent years there has been a significant change in the approach to the diagnosis of gonorrhoea. Traditional culture methods for isolation of *Neisseria gonorrhoeae* have been increasingly replaced by nucleic acid detection tests, especially in remote areas of Australia. This has occurred for a number of reasons, in particular the increased robustness and the improved sensitivity for testing of patents outside hospital clinics. In general NADT have performed well, especially as reliable and sensitive tests for the exclusion of gonococcal infection.

However, after increasing experience with these tests, some problems have been identified with NADT. The most widely used commercial assay, the Cobas Amplicor, was found to produce a large number of false positive results1 and it was realised that the target sequence in the cytosine DNA methyltransferase (CMT) gene of *N. gonorrhoeae*was also present in some strains of other *Neisseria* species, including *N. cinerea* and *N. subflava.* These are normal flora in the upper respiratory tract, and may also be present in the genital tract. It is still not clear whether this is the sole mechanism of false positive results with this assay.

Subsequently, a number of supplemental assays have been developed and used in Australia. The initial ones were directed at targets in the 16S RNA and the *cppB* genes. Neither of these targets was, in themselves, fully specific but the combination of two assays substantially improved specificity. However, some clearly problematic results remained, with some false positives still occurring in genital tract and urine samples, and more commonly in results with throat swabs, irrespective of the assay combination used.

In 2002 the Public Health Laboratory Network was asked by the Communicable Disease Network of Australia to provide an opinion on the Cobas Amplicor test. PHLN recommended that it should only be used for urine or genital tract samples (or, cautiously, for normally sterile specimens, such as joint fluids) unless validated for other samples. They also recommended that a screening assay reactive on the Cobas Amplicor should not be reported as positive unless there was also at least one positive supplemental PCR assay or a positive culture. Furthermore, screening and supplemental assays should be directed at a different targets selected from *cppB,* 16S rRNA and CMT genes. Since 2002, a number of Australian laboratories have evaluated further a number of existing assays and devised alternative methods for improving the specificity of gonococcal NADT.

In addition, it was recognised that the shift to NADT means that there may not be sufficient gonococcal cultures to provide enough isolates to properly monitor antimicrobial susceptibility testing. There are not currently any NADT–based methods for reliably determining antimicrobial resistance in gonococci. It is therefore essential that pragmatic strategies be implemented that guarantee that sufficient isolates, representative of the relevant Australian populations, are available for antimicrobial resistance surveillance in gonococci, despite the shift to NADT.

A meeting of laboratory experts (see Appendix 1 for attendees) was convened by the PHLN in Melbourne on 23 March 2005 to identify laboratory issues of relevance and suggest guidelines for use of NADT for diagnosis of gonorrhoea in Australia. A review of the currently available NADT for gonococci and their potential (or actual) roles as screening and/or supplemental assays was undertaken in order to establish estimates of the performance characteristics of different screening/supplemental combinations in symptomatic, asymptomatic, urban and remote area populations. The meeting also discussed the quality assurance and quality control requirements for these tests. Recommendations were also generated for the surveillance of antimicrobial susceptibility, with particular reference to strategies for obtaining the requisite number of isolates.

Recent developments in in-house NADT for *Neisseria gonorrhoeae* in Australia

Since 2002, a number of Australian laboratories have evaluated further a number of existing assays and devised alternative methods for improving the specificity of gonococcal NADT.

*cppB* gene based assays

Assays using *cppB* gene targets have been used widely as both screening and supplemental assays. In addition to the specificity problems mentioned above, there has been recent emergence of a *cppB*gene negative subtype of *N. gonorrhoeae.* As a result assays that target *cppB*gene may fail to detect up to 10 per cent of cases if used as a screening assay2 and may fail to confirm some true positive results in other screening assays.3

*porA* pseudogene assay

A real-time PCR directed at the *porA* pseudogene was developed in Queensland4 and was shown to reliably discriminate between gonococci and a large range of non- *Neisseriae* and non-gonococcal *Neisseriae.* It was equivalent in sensitivity to the Cobas Amplicor and more sensitive than culture, particularly in samples with low organism numbers. The additional positives using the *porA* pseudogene were mainly in throat or rectal swabs. All were shown to be true positives by discrepant analysis. A multi centre evaluation of this assay is continuing.

*opa* gene assay

A real-time PCR directed at a 90 bp target within a highly-conserved region of the *opa* gene, which is present in multiple copies has been developed in Melbourne.5 Like the porA pseudogene assay, it showed excellent discrimination between gonococci (including those that were negative in the *cppB* gene-based assay) and a large range of non- *Neisseriae* and non-gonococcal *Neisseriae.* When compared with a range of other NADT for gonococci, the *opa* gene assay was as sensitive, and with high specificity.

CMT gene assay

A group from Perth reported on their experience with a range of CMT and *cppB*gene targets (Harnett G, personal communication). The best combinations using nested PCRs showed a significant false positive rate of up to 10 per cent for genital tract samples and a very high false positive rate for throat swabs. Subsequently a more specific sequence was identified that showed excellent discrimination between gonococci and non-gonococcal *Neisseria* in a real time PCR. Evaluation of over 7,000 specimens demonstrated a sensitivity equivalent to the nested PCRs but with a specificity of 99.8 per cent. Some of the few false positives had a single base pair difference from *N. gonorrhoeae* that did not reduce the probe binding sufficiently to give negative result. Further refinements are underway.

Recent changes in commercial NADT in Australia

A number of commercial assays have been withdrawn because of problems noted only after extended use. The Roche 16S RNA assay and the Abbott LCR assay, both of which were previously used in Australia, were no longer available at the time of this meeting.

Strand displacement amplification

Becton Dickinson developed a strand displacement amplification assay, the SD ProbeTec, directed to a pilin gene target, which is claimed to have high sensitivity and specificity. However, published data indicate that this assay has a false positive rate of 67 per cent for weakly reactive samples and 3.3 per cent for strongly reactive samples.6 Other studies also suggest difficulties with this assay,7,8 if performed in isolation.

Transcription mediated amplification

The APTIMA Combo 2 uses transcription-mediated amplification (TMA) to amplify rRNA. It is claimed to have high sensitivity and specificity,9,10 though concerns were expressed by participants that the supplemental assay used in the published evaluation was an earlier version of the same assay, and therefore may not be a suitable as a discriminator because of the risk that it would yield the same false positives as the primary assay.

Neither the SD ProbeTec nor the APTIMA Combo 2 has yet been properly evaluated in Australian populations.

Funding for gonococcal testing

The meeting also recognised that current funding for gonococcal nucleic acid testing does not allow for supplemental assays. There is a need to address this if supplemental assays are to become standard practice.

Antimicrobial susceptibility surveillance

Accurate and timely diagnosis, if accompanied by effective treatment, has been shown to make a significant contribution to the control of gonococcal disease.11 High and increasing rates of antimicrobial resistance in *N. gonorrhoeae* have reduced the treatment options and limited the effectiveness of gonococcal control programs in Australia.12

It is therefore most important that surveillance of antimicrobial resistance in gonococci, currently performed by the National Neisseria Network, continues. This requires examination of viable cultures of gonococci and sample numbers must be sufficiently large and be adequately representative of gonococci and of human populations. 12 Currently it is recommended that standard treatment schedules should be changed when the proportion of resistant isolates reaches 5 per cent. Therefore sample size should be sufficient to reliably detect proportional resistance rates around 5 per cent.

Workshop discussion and recommendations

The Workshop discussed the above issues and considered *inter alia* approaches recommended by the US Centers for Disease Control and Prevention (CDC) 14 for use of NADT in the USA. The CDC recommendations for suggested testing algorithms for NADT for gonococci may be paraphrased as follows:

(i) repeat testing of the same sample with the same assay;

(ii) repeat testing of the same sample with a different NADT (presumably with a different target sequence);

(iii) repeat testing of a repeat sample with the original NADT;

(iv) repeat testing of a repeat sample with a second NADT (presumably with a different target sequence).

The Working Group agreed that option (i) was not sufficiently stringent, while options (iii) and (iv) were impractical for Australian conditions and logistics.

Discussion was therefore focussed on option (ii) and the characteristics required for screening and supplemental NADT for gonococcal detection in Australia given the current state of knowledge. It was agreed that the terms 'screening assay' and 'supplemental assay' be used and that the term 'confirmatory assay' was misleading as no assay could truly confirm identity.

The Workshop noted that defects in both commercial and in-house assays became apparent despite initial favourable evaluations, and that in some instances those commercial NADT initially had US FDA approval. The defects became apparent only after more extensive testing of different gonococcal populations and as testing experience accumulated. This phenomenon is consistent with the known characteristics of *N. gonorrhoeae,* especially its capacity for genetic recombination through horizontal genetic exchange. 8,15–19 This capacity for continuing genetic diversification in gonococci means that regular and wide ranging appraisals of NADT are prudent. In the absence of these continuing appraisals at the present time, as well as the logistic problems referred to above, the participants agreed that option (ii) should be used as the basis for test algorithms for NADT for gonococci in Australia.

Screening assays

The requisite features of a screening NADT are that it should have a high sensitivity and thus a high negative predictive value. It is recognised that it has proved difficult to develop a single NADT assay that is both highly sensitive and highly specific, though tests are continuing to improve. Nevertheless it is desirable for screening NADT to be as specific as possible while retaining their sensitivity and negative predictive value.

In house assays directed at *cppB*targets should be used very cautiously as a screening or supplemental assay due to the presence of *cppB* gene negative strains in some Australian populations. 2 In addition, *cppB* targets are less specific than alternative targets, so that there is no imperative to maintain it for confirmatory purposes.

*Recommendation 1: Assays using detection of the cppB gene should not be used for either screening or supplemental assays.*

While the other in-house assays that have been described above are substantial improvements, workshop participants did not feel that there are as yet sufficient available data, local or otherwise, to recommend their use as a single assay. Therefore for the in-house NADT a supplementary assay should be performed on the original sample unless a positive culture has been obtained from the same site at the same time.

*Recommendation 2: All in-house screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.*

All of the commercial assays appear to have high sensitivity and a good negative predictive value. However the Amplicor and ProbeTec assays are known to have problems with false positives reactions, while current Australian data on the APTIMA Combo 2 NADT are inadequate to be confident of specificity. Therefore for the commercial NADT a supplementary assay should be performed on the original sample unless a positive culture has been obtained from the same site at the same time.

*Recommendation 3: All commercial screening assays that are positive should also be positive on a reliable supplemental assay before a positive result is reported.*

Desirable characteristics of a supplemental assay

Any supplemental assay must be as sensitive as the screening assay and must be directed at a target on a different gene that is known to have discriminatory value in identifying gonococcus. While it may be possible to use two targets on the same gene, there is insufficient data currently to support this proposition. In particular, the propensity for genetic exchange among *Neisseria* species raises the possibility of exchange of a large section of the gene that may include both of these targets.

Currently the acceptable assays are directed at various targets in the CMT gene, the pilin gene, 16S RNA gene, the *opa* gene and the *porA* pseudogene. Laboratories need to assess the value of particular targets either based on their own data or the data in the literature, noting again that the assessment should be on-going due to the propensity for genetic exchange in gonococcal populations.

*Recommendation 4: If a sample is positive in a screening assay but a suitable supplemental assay is negative, then the result should be reported as negative.*

Evaluating test performance

The workshop participants recognised that to implement the recommendations it is necessary to define the acceptable performance criteria for test combinations. The reported result should have a positive predictive value (PPV) of at least 90 per cent in the population being tested, based on current US recommendations for chlamydia and gonorrhoea testing. 14 However, calculating the PPV depends on disease prevalence, which is often difficult for laboratories to determine accurately. This may be because it is not known which population the patient belongs to, because the prevalence in the test population is unknown, because the test population contains mixed groups with differing prevalences and/or because there is a reasonable chance that an individual within that population may have a substantially different pretest probability from the average for that population. In view of these difficulties, it is preferred that the test PPV is calculated based on a prevalence of 1 per cent as that represents a low prevalence population. Therefore if the assay has a sensitivity of 99 per cent, then a specificity of 99.9 per cent is required to yield a PPV of >90 per cent. Getting a good negative predictive value in populations (or patients) with a pretest probability less than 1 per cent is extremely difficult and no test combination can be expected to yield a high PPV in that situation.

*Recommendation 5: Laboratories should ensure that the test combination they use would yield a positive predictive value of at least 90 per cent in a population with a prevalence of 1 per cent.*

For the purposes of test evaluation it is important that there is an accepted definition of genuine positive samples for comparing tests. Detection of *N. gonorrhoea* by culture using contemporary culture and identification methods is regarded as suitable confirmation that a positive NADT is genuine. While it is recognised that, in theory, one could get a false positive NADT accompanied by a genuine positive culture, this situation is unlikely and should become apparent during the process of continuing test evaluation.

Often culture is not suitable as a confirmatory test as either no culture has been performed, or it is less sensitive than the NADT being evaluated. In those circumstances other nucleic acid-based methods are required. Use of two separate targets may still yield false positive results, and therefore it should require detection of at least three separate targets that have discriminatory value in order to be acceptably confident that it is genuine. Alternatively, the identity may be confirmed by DNA sequencing. The propensity for genetic recombination in and between pathogenic and non-pathogenic *Neisseria* species has been referred to above. This raises theoretical concerns regarding sequencing for gonococcal confirmation, in that the sequenced gene may be residing in a non-gonococcal organism. There is now a considerable amount of literature on the use of sequencing for identification and typing of Neisseria species and, while misidentification due to genetic exchange remains a theoretical possibility, it is a highly unlikely, particularly if an internal gene such as CMT is sequenced.

*Recommendation 6: For the purposes of test evaluation, as distinct from diagnostic testing, true positives be defined by meeting one or more of the following criteria:*

*1) culture positive using contemporary isolation and identification techniques;*

*2) positive result on NADTs directed to targets on three separate genes that are known to have discriminatory capacity;*

*3) sequencing of a gene known to separate gonococcal from non-gonococcal species.*

Inhibitory samples

It is known that samples may contain substances that inhibit NADT and unless a suitable inhibitor control is included, the test may yield a false negative result.

*Recommendation 7: Inhibitor controls should be routinely included in all NADT.*

Testing of samples from extra-genital sites

As there is still limited data on the performance of NADT on these samples, and mixed *Neisseria* species are more likely, traditional cultures are the preferred test.

Interpretation of a negative result on NADT from extra-genital sites depends on sensitivity of the assay, and currently there is little data on this. Experience with genital tract samples strongly suggests that NADT will be more sensitive than culture. Currently we do not have sufficient Australian data, partly due to the lack of positive samples. The available sensitivity data needs to be reviewed before further recommendations can be made on this issue. Similarly there are unresolved concerns about specificity for non-genital samples.

*Recommendation 8: Cultures are the preferred test for samples from non-genital sites. If however it is necessary to perform a NADT, then more stringent criteria should be applied, and positive samples should meet the 'test evaluation' criteria for a 'true positive'.*

Quality assurance (QA)

Reference is made above for the need for ongoing assessment and evaluation of any NADT or NADT combination selected for use. A properly constituted and thoughtful approach to external QA would simplify compliance with this requirement. Australian laboratories mainly test samples from low prevalence populations and therefore encounter relatively few positive samples. Also gonococcal strains that pose difficulties may be uncommon or may emerge within restricted populations. In order to properly monitor test performance quality assurance samples should include a wide range of problem specimens or organisms.

*Recommendation 9: In order to properly assess the routine diagnostic system in Australia, the following quality assurance samples should be distributed in addition to the routine samples currently used:*

*1.*cppB *negative*N. gonorrhoeae;

*2. Non-gonococcal species known to cause false positive reactions: these should be dispatched both as a single species, as well as mixture with*N. gonorrhoeae. *In the latter circumstance, the non-gonococcal species should be present in 10-fold excess;*

*3. Urine samples: Preferably a single patient sample, otherwise a spiked sample;*

*4. Validation panels should be made that include samples that are culture positive but PCR negative. True positive samples should also be made available.*

In addition, a process should be established for full phenotypic and genotypic characterisation of unidentified species that yield false positive results in NADT for gonococci.

Antimicrobial resistance (AMR) surveillance requirements – isolate numbers

Although the issues involved are complex, it is presently accepted that a sample size should be large enough to detect a proportional resistant rate of 5 per cent. Statistically the sample size requirements for this threshold can be quite demanding e.g. up to 1,200 isolates per study period. While comprehensive and continuous sampling is important to maximise isolate numbers to increase the validity of the surveillance, use of other approaches such as discontinuous samples and a Lot Quality Assurance assessment is possible. The latter approach requires that a *minimum* of 200 isolates from each population group for each study period should be examined. If it is found that either no or a very low proportion of isolates are resistant, or alternatively a high proportion are resistant, the basic question to continue or discontinue with a standard treatment is answered. No further enhancement of surveillance is required. However if the detected resistance is in the critical range where a change in treatment may be contemplated, 'enhanced surveillance', i.e. an enlarged sample, is required until such time as the question of treatment alteration is resolved.

In order to maintain these minimum numbers, the capacity for high quality gonococcal culture methods should be maintained within all the relevant populations. 14 All isolates should have susceptibility testing performed in a laboratory that reports to the National Neisseria Network (NNN) or else those isolates should be referred to the NNN laboratory in the relevant.

Cultures should be routinely performed on swabs where there is ready access to a suitable laboratory. Also, patients who have had a positive NADT and have not been treated should have a culture performed if that was not done initially.

A strategy that may be used to achieve the appropriate numbers of isolates in areas where primary culture is not routinely performed is to undertake 'targeted culture' of populations where a significant yield of gonococci may be expected so that a significant cost/benefit for culture is obtained. Such situations include culture of males with urethral discharge, especially in clinic situations, or culture of urine samples collected for NADT and that are positive on this test. Data collected indicate that up to 70 per cent of urine samples positive on NADT will provide an isolate of GC if properly cultured within 24 hours of collection. 20

*Recommendation 10: Strategies should be put in place to ensure that sufficient numbers of gonococcal isolates are obtained to allow reliable monitoring of antimicrobial resistance.*

These targets need to be met within each population in which there is expected to be a difference in rates of resistance or in which the risk of emergence of resistance is different. For example, it is important to ensure that sufficient samples are obtained to detect resistance within both Indigenous and non-Indigenous communities throughout Australia. Identification of the relevant population groups should be undertaken by public health practitioners experienced in the epidemiology and control of sexually transmitted infections.

*Recommendation 11: Public health practitioners need to define the relevant populations that need to be targeted and identify any that require enhanced surveillance.*

References

1. Farrell DJ. Evaluation of AMPLICOR Neisseria gonorrhoeae PCR using c *ppB* nested PCR and 16S rRNA PCR. *J Clin Microbiol* 1999;37:386–390.

2. Lum G, Freeman K, Nguyen NL, Limnios EA, Tabrizi SN, Carter I, *et al.* A cluster of culture positive gonococcal infections but with false-negative *cppB* gene based PCR. *Sex Transm Infect* 2005;81:400–402.

3. Tapsall JW, Limnios EA, Nguyen NL, Carter I, Lum G, Freeman K, *et al.* Problematic *cppB*-gene based assays may contribute to failure to confirm BD ProbeTEC PCR for *Neisseria gonorrhoeae* with a *cppB*-gene based PCR. *J Clin Microbiol* 2005;43:2036–2037.

4. Whiley DM, Buda PJ, Bayliss J, Cover L, Bates J, Sloots TP. A new confirmatory *Neisseria gonorrhoeae* real-time PCR assay targeting the *porA* pseudogene. *Eur J Clin Microbiol Infect Dis* 2004;23:705–710.

5. Tabrizi SN, Chen S, Tapsall J, Garland S. Evaluation of *opa*-based real time PCR for detection of *N. gonorrhoeae. Sex Transm Dis*2005;32:199–202.

6. Culler EE, Caliendo AM, Nolte FS. Reproducibility of positive test results in the BDProbeTec ET system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae. J Clin Microbiol* 2003;41:3911–3914.

7. Palmer HM, Mallinson H, Wood RL, Herring RJ. Evaluation of the specificities of five DNA amplification methods for the detection of *Neisseria gonorrhoeae. J Clin Microbiol* 2003;41:835–837.

8. Koenig, MG, Kosha SL, Doty BL, Heath DG. Direct comparison of the BD ProbeTec ET system with in-house LightCycler PCR assays for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from clinical specimens. *J Clin Microbiol*2004;42:5751–5756.

9. Golden MR, Hughes JP, Cles LE, Crouse K, Gudgel K, Hu J, *et al.* APTIMA Combo 2 testing for *Neisseria gonorrhoeae*in a population of women with low prevalence of *N. gonorrhoeae*infection. *Clin Infectious Dis* 2004;39:1387–1390.

10. Gaydos CA, Quinn TC, Willis D, Weissfeld A, Hook EW, Martin DH, *et al.* Performance of the APTIMA Combo 2 assay for detection of *Chlamydia trachomatis*and *Neisseria gonorrhoeae*in female urine and endocervical swab specimens *J Clin Microbiol* 2003;41:304–309.

11. Miller PJ, Torzillo PJ, Hateley W. Impact of improved diagnosis and treatment on prevalence of gonorrhoea and chlamydial infection in remote aboriginal communities on Anangu Pitjantjatjara Lands. *Med J Aust* 1999;170:429–432.

12. Annual report of the Australian Gonococcal Surveillance Programme, 2004. *Commun Dis Intell* 2005;29:136–141.

13. World Health Organization. Surveillance standards for antimicrobial resistance. 2001. Available from <http://www.who.int/drugresistance/publications/WHO_CDS_CSR_DRS_2001_5/en/>

14. Johnson RE, Newhall WJ, John R, Papp JR, Knapp JS, Black CM, *et al.* Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections–2002 *MMWR Recomm Rep* 2002; 51(RR15);1–27.

15. Frosch M, Meyer TF. Transformation-mediated exchange of virulence determinants by co-cultivation of pathogenic *Neisseriae. FEMS Microbiol Lett* 1992;79:345–349.

16. Halter R, Pohlner J, Meyer TF, Abeck D, Johnson AP, Alexander FE, *et al.* Plasmid content and protein I serovar of non-penicillinase-producing gonococci isolated in Munich. *Epidemiol Infect* 1988;100:345–349.

17. Kroll JS, Wilks KE, Farrant JL, Langford PR. Natural genetic exchange between *Haemophilus* and *Neisseria:* intergeneric transfer of chromosomal genes between major human pathogens. *Proc Natl Acad Sci U S A* 1998;95:12381–12385.

18. Linz B, Schenker M, Zhu P, Achtman M. Frequent interspecific genetic exchange between commensal *Neisseriae*and *Neisseria meningitidis. Molecular Microbiol* 2000;36:1049–1058.

19. Rosson, S, de Gooyer E, Moore A, Morcom J, Davies JK. 1985. Site-specific recombination systems in gonococcal plasmids, p. In: Schooling GK ed. *The pathogenic Neisseria.* American Society for Microbiology, Washington, D.C. 1985;175–179.

20. Tapsall JW, Ray S, Limnios EA. Targeted culture for *N. gonorrhoeae* from FVU increases isolate numbers for gonococcal antimicrobial susceptibility monitoring: importance of time to culture and FVU physical characteristics. Abstract WP 010 16th Biennial meeting of the International Society for STD Research (ISSTDR), Amsterdam 10 –13 July 2005.

Appendix. List of attendees and relevant affiliations

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