

These are exciting times for the laboratory diagnosis of bacterial STIs with a move to more sensitive and specific tests, which have been long awaited. The molecular approach offers significant opportunities but also many challenges in identifying organism specific targets at sites colonised by genetically related bacteria. In this issue I have concentrated primarily on providing information on a number of molecular tests for bacterial STIs including the launch of our new diagnostic service for *Mycoplasma genitalium*. On 12 October 2006 we published the GRASP report 2005

providing further information on the burden of antimicrobial resistant gonorrhoea in England & Wales. We are very proud of this surveillance programme which has informed and changed clinical practice, and is the mainstay of our gonococcal reference service.

In September 2006 it was my pleasure to accept a Police Commendation for work in STBRL that aided in the conviction in a child abuse case. During the presentation there were a number of citations of distressing cases of both physical and sexual abuse on children. As microbiologists we have an important role to play in helping in cases

where children are infected with sexually transmitted infections. However, there is much work to do to validate tests for use in children, inform guidelines for screening in abuse cases and develop new methods to ensure that information given is accurate and can withstand cross-examination in a court of law.

Finally, I have included information on two forthcoming meetings on Chlamydia in January 2007 and An update on bacterial STIs in March 2007.

Catherine Ison

Confirming the *C. trachomatis* status of referred rectal specimens

There are no licensed tests, nucleic acid amplification, (NAAT), or enzyme immunoassays, (EIA) for detecting *C. trachomatis* (CT) in rectal samples. This created a considerable problem for diagnostic laboratories following the alert and subsequent identification of an outbreak of lymphogranuloma venereum (LGV) presenting as acute proctitis in men who have sex with men (MSM). STBRL offer a free-of-charge service for genotyping of *C. trachomatis*, as part of the outbreak control, but only accept rectal specimens taken from symptomatic MSM patients, which are CT positive at the local referring laboratory, in order to ensure that resources are targeted appropriately.

This has posed ethical, legal and financial dilemmas and concerns regarding CPA accreditation for many primary diagnostic laboratories throughout the United Kingdom. On arrival at STBRL the positive CT status of all referred samples is confirmed, using a plasmid based in-house real-time PCR method. This has allowed us to ascertain the reliability of all laboratory methods for the detection of CT in rectal specimens, by examining the correlation between our real-time PCR results and the method used to determine the positive CT status of the specimen at the referring centre.

A total of 1566 rectal specimens were included in the analysis and STBRL were able to confirm the presence of CT DNA in 91.6% detected by culture, 93.5% by NAAT but only 41.5% referred after EIA testing. Although this is a highly selected sample and there is the possibility of some false negatives due to degradation of DNA during storage and transport, the confirmation

of CT in a large number of specimens initially tested by NAATs at the primary centre highlights the high specificity that can be achieved and provides some validation data for the use of NAATs with rectal specimens. This is in sharp contrast to specimens screened using EIA based tests where only 41.5% of specimens could be confirmed as CT positive. The low confirmatory rate of the EIA test, reflects its low specificity when testing rectal specimens.

The results of this study provide some validation data for use of CT NAATs with rectal specimens to support those primary diagnostic laboratories that are currently using NAATs, despite their unlicensed status. STBRL also hope to raise awareness that the CT EIA tests have very low specificity when screening rectal specimens and STBRL cannot support their use.

Sarah Alexander

NAATs for *Neisseria gonorrhoeae*

There is increasing interest in using NAATs for the detection of *N. gonorrhoeae*. This is primarily being driven by the extensive use of NAATs for *Chlamydia trachomatis*, both as part of the National Chlamydia Screening Programme and for GUM clinic patients, and by the availability of commercial assays which can detect both organisms in the same sample, often simultaneously. Gonococcal infection has been found predominantly among individuals at high risk, often concentrated in large cities, in contrast to chlamydial infection, which has been found to be widespread in the population, in approximately 10% of sexually active young people. The evidence

for an asymptomatic reservoir of gonorrhoea in the community is still being acquired and is most likely to be found in locations with higher incidence of infection among GUM patients.

NAATs for *N. gonorrhoeae* have, until recently, been less robust than those for *C. trachomatis* because of the close genetic relatedness of the species of *Neisseria* and the challenge of identifying a gonococcal specific DNA sequence for the primers. While there has been considerable improvement, there are still concerns that these tests are unsuitable for

continued on page 2

continued from page 1

pharyngeal and rectal samples (and are unlicensed for extra-genital sites) and further validation studies are needed. A specific concern for gonorrhoea is the need to retain viable cultures to allow surveillance of antimicrobial resistance and to detect possible emerging resistance to the cephalosporins.

This is a time of considerable change for the laboratory diagnosis of gonorrhoea and many factors still need to be addressed and considered before we forge ahead with new approaches and so STBRL have issued the following recommendations:

- All GC NAATs should be repeated (supplementary test)
- GUM: GC NAATs confirmed by culture (BASHH guideline)
- Rectal/pharyngeal specimens should be cultured
- NAATs should be validated in different populations
- NAAT should have PPV of >90%
- Representative sample for antimicrobial susceptibility testing must be retained

Ison CA. GC NAATs is the time right?
Sex Transm Infect. 2006;**82**:141

Catherine Ison

GRASP 2006 update

The GRASP 2006 collection was carried out between June and August 2006 and 1793 samples were received. After disappointing retrieval rates in 2005, a choice of sending isolates on chocolate agar slopes or frozen in glycerol broth was offered to all 24 collaborating laboratories. Four centres chose to use slopes (190 isolates) and the remainder sent frozen isolates (1603) with a retrieval rate of 96% and 76% respectively. The overall retrieval rate was 79%, a significant improvement on 2005. Data collection and susceptibility testing is currently underway and it is hoped to produce the 2006 report in early 2007.

Elisabeth Maclure

GRASP Report 2005

(http://www.hpa.org.uk/infections/topics_az/hiv_and_sti/sti-gonorrhoea/epidemiology/grasp.htm)

The Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) published its sixth annual surveillance report in October. Currently in its seventh year, GRASP is a sentinel surveillance study which characterises antimicrobial susceptibility patterns for gonorrhoea in England and Wales.

The 2005 collection describes a disquieting situation with the increase in prevalence of certain types of resistant gonococcal isolates. While the clinical and behavioural characteristics of genitourinary medicine (GUM) patients has remained fairly constant over time, the numbers of gay men represented in the sample has increased.

The key points from the 2005 report are:

- Increase in ciprofloxacin resistant gonococci to 22% of total isolates.
- Increase in ciprofloxacin resistant gonorrhoea greatest in isolates from homosexual men (42%).

- Penicillin resistance was chromosomally-mediated (CMRNG) in 11% of isolates and strongly associated with infection in homosexual men and individuals with sexual contact abroad.
- Plasmid-mediated penicillin resistance (PPNG) decreased from 6% in 2004 to 4% in 2005.
- No isolates demonstrated decreased susceptibility to ceftriaxone, cefixime or spectinomycin.
- In 2005, 71% of patients were treated with a cephalosporin, in line with current treatment guidelines (www.bashh.org).
- Only 19% of GRASP patients were treated with a fluoroquinolone in 2005.
- Isolates from non-GUM clinic patients appeared more susceptible to antimicrobial agents, such as penicillin and ciprofloxacin, than those from GUM clinic patients.

Leah De Souza

Research update

***Chlamydia trachomatis* has been traditionally grouped into 14 serovars. Serovars A to C more commonly associated with trachoma and serovars D to K causing genital tract infections. Serovar L is sufficiently different enough that it has been categorised further into variants L1, L2 and L3 due to it causing lymphogranuloma venereum (LGV), an uncommon form of sexually transmitted disease.**

With the onset of molecular techniques, genotyping has overtaken serotyping for *C. trachomatis*. Analysis using restriction fragment length polymorphism of the PCR amplified *omp1* gene, has shown that there is an overall correlation of the genotypes with the serotypes and that RFLP analysis appears to be slightly more discriminatory than serotyping.

Even with the onset of genotyping, there is a lack of discrimination, which has left many important questions regarding epidemiology unanswered. For example, because the diversity of the chlamydial genotypes is unknown, this makes distinguishing between re-infection and new infections impossible, which in turn impacts decisions for clinical treatment.

We are in the process of comparing and analysing the sequenced chromosomes of serovars A, D and L2. Although the serovars appear to be virtually identical at the genomic level, we hope to determine if any genomic features can be exploited, as part of an investigation to determine if more discriminatory typing methods can be developed.

Tahir Ali

Molecular Detection of *Mycoplasma genitalium*

Mycoplasma genitalium is a sexually transmitted bacterium associated with nongonococcal urethritis, cervicitis, endometritis and pelvic inflammatory disease. *M. genitalium* was first isolated in 1980 from urethral specimens of two homosexual men with nongonococcal urethritis (Tully *et al.*, 1981). Testing for *M. genitalium* DNA has not previously been available within England and Wales in a clinical capacity.

STBRL has recently introduced real-time PCR for the molecular detection of *M. genitalium* and will be offering a reference service commencing in January 2007.

Molecular detection of *M. genitalium* in clinical samples is determined by real-time PCR directed against the MgPa adhesin gene (Jensen *et al.*, 2004) and confirmed by in house real-time PCR. It is anticipated that this service will primarily help with disease diagnosis and treatment whilst also

enhancing knowledge regarding awareness of the disease and prevalence.

Tully, *et al.*, 1981. A newly discovered mycoplasma in the human urogenital tract. Lancet i:1288-1291

Jensen *et al.*, 2004. Use of TaqMan5' nuclease real-time PCR for quantitative detection of *Mycoplasma genitalium* DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J. Clin Microbiol. 42(2): 683-92

Non-cultural detection of *N. gonorrhoeae* from clothing

In early 2005 STBRL received a gonococcal isolate from a seven year old child for confirmation for medico-legal purposes. A male relative of the child was suspected of sexually assaulting the child but refused to allow invasive specimens to be taken. STBRL were approached to attempt to establish the presence of infection in discharge present on boxer shorts obtained by the police from this man. In collaboration with the Forensic Science Service, molecular methods were able to detect gonococcal specific DNA. Subsequently molecular typing methods showed that both the isolate from the child and the DNA extracted from the shorts were indistinguishable by the NG-MAST method, sharing the same sequence type. On provision of this evidence the perpetrator changed his plea to guilty of sexual assault and was sentenced to two years.

I am indebted to the work of Dr Iona Martin, who developed NG-MAST and performed the molecular testing in this case, Dr Annette Nesbitt, the community paediatrician, Dr Greta Forster of the Haven Centre, Whitechapel and DC Kevin O'Loughlin for their assistance. The Police Commendation was given for novel work in establishing the presence of a sexually transmitted infection. However, this case was

not tested in a court of law, and raises many issues regarding a molecular approach to the diagnosis of sexually transmitted infection in a child, that need to be fully addressed before it can be successfully defended in a court of law.

Martin IMC *et al.* Non-cultural detection and molecular genotyping of *N. gonorrhoeae* from a piece of clothing. J Med Microbiol (accepted for publication)



Professor Cathy Ison, Dr Greta Forster and DC Kevin O'Loughlin

Clinical Specimens will be accepted from January 8th 2007

STBRL will accept specimens and DNA/whole nucleic acid extracts for *M. genitalium* from patients with clinical signs or known contact cases. Specimens accepted include extracted DNA, rectal and genital swabs or urine. Swabs should be sent in standard microbiological or chlamydial swab transport medium. Charcoal swabs will not be accepted. For other specimen types such as biopsies please contact (Stbrl@hpa.org.uk, 02083276464). The expected turn around time will be approximately 10 days. Samples received without any clinical information will be archived until sufficient information is provided. A specimen request form is available from (www.hpa.org.uk/cfi/stbrl/)

Please note, charges will be levied for this service.

For further information please contact Stbrl@hpa.org.uk, 02083276464, www.hpa.org.uk/cfi/stbrl/.

For queries regarding research specimens please contact Stbrl@hpa.org.uk in the first instance.

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Future meetings

British Association of Sexual Health & HIV (BASHH)
12 January 2007
Royal Society of Medicine - Bacterial Special Interest Group

13.00 Lunch 13.30-15.30pm

Can biology inform treatment regimens	Paddy Horner, Bristol
Diversity and innovations within the National Chlamydia Screening Programme	Lynsey Emmett, HPA, London
Immunological response to <i>Chlamydia trachomatis</i>	Hill Gaston, Cambridge
A new Chlamydia test: bridging the gap between diagnosis and treatment	Helen Lee, Cambridge

Everyone is welcome and there is no registration fee.

This meeting will be followed by the BASHH Annual General Meeting (16.00-17.00) and a session organised by the HIV Special Interest Group (17.30-19.00)
www.bashh.org

First notification

Update on Bacterial STIs organized by STBRL

Wednesday 20 March 2007

To be held at HPA Centre for Infections

Subjects to be covered:

Mycoplasma genitalium
Lymphogranuloma venereum
Syphilis
STIs and Forensic Science
Trends in STIs

Further details to be found on
<http://www.hpa.org.uk/cfi/stbri/default.htm>
or by calling 02083276464.

If you have any questions please contact us

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<http://www.hpa.org.uk/cfi/stbri/>

Other members of the STBRL team are:

Pamela Saunders and Daksha Hathi (Diagnostics), Hemanti Patel (Syphilis serology), John Anderson (GRASP), Tahir Ali (Chlamydia typing) and Michelle Cole (ESSTI). Tony McNiff (MOLIS), Marlette Vigille (Quality), Leah DeSouza-Thomas and Laura James (GRASP coordinators), Emma Savage (ESSTI coordinator) and Edmund Donovan (ESSTI administrator) also work with STBRL.