

STBRL has now completed its second year under my directorship and this has seen a considerable increase in the services we provide and our workload. In this newsletter we have highlighted areas of special interest, such as the identification of a new phenotype of *Neisseria gonorrhoeae*, the introduction of new molecular diagnostic tests for lymphogranuloma venereum (LGV) and genital ulcer disease (GUD), the serological diagnosis of syphilis as well as updates on existing projects.

One of our greatest challenges over the past 18 months has been the provision of a national diagnostic service for LGV. We have successfully established this service and have refined the techniques used (see below). However, a number of issues have emerged which need serious consideration.

- How to test rectal specimens for *C. trachomatis*?
- Is there an asymptomatic reservoir for LGV?
- Should all men who have sex with men be routinely screened for rectal chlamydia?
- Where are the resources to pay for this?

A strong evidence base will be needed to answer the laboratory and clinical issues raised here.

We have also begun to attract research projects which complement our reference service. Funding has been obtained to develop molecular typing methods for *C. trachomatis* from the Medical Research Council Sexual Health Call 2005 and a second term of funding awarded by the

European Union (DG-SANCO) for European Surveillance of Sexually Transmitted Infection (ESSTI), a project in collaboration with the Surveillance Section at the Centre for Infections.

We continue to receive a number of specimens from child or sexual abuse cases. We do recommend that positive results for gonorrhoea, chlamydia or syphilis be confirmed by referral to STBRL. It is important that such specimens are clearly marked and are accompanied by a Chain of Evidence form. If you require any advice regarding medico-legal samples please contact me.

Catherine Ison

Neisseria gonorrhoeae- Nal sens/Cip Res

We have been referred five isolates of *N. gonorrhoeae* from four different GUM clinics in England and Wales exhibiting resistance to the fluoroquinolones, that are susceptible to nalidixic acid. Resistance to the fluoroquinolones is often determined by the use of nalidixic acid discs or Etests, however these isolates would wrongly be determined to be susceptible. Laboratories should be aware of this unusual phenotype and consider screening for resistance by the use of ciprofloxacin or ofloxacin discs, Etests or the modified breakpoints for nalidixic acid (www.bsac.org).

The first report of this phenotype was published in 2005¹ and the mechanism of resistance in these strains is being studied. If any laboratories detect this particular phenotype STBRL are happy to receive any such isolate.

1. Ragnathan PL, Ison CA, Livermore DA. Nalidixic acid-susceptible, ciprofloxacin-resistant *Neisseria gonorrhoeae* strain in the UK. J Antimicrob Chemother. 2005;56:437

Iona Martin

RT- PCR for GUD

During the last year STBRL have implemented and validated a real-time PCR for the direct and simultaneous detection of *Treponema pallidum*, *Haemophilus ducreyi* and Herpes simplex virus (HSV), from genital and oral swabs. This multiplex PCR, originally developed at CDC Atlanta, amplifies DNA from the haemolytic cytotoxin of *H. ducreyi*, the 47Kd lipoprotein of *T. pallidum* and the glycoprotein D in HSV. The PCR also contains an internal control, which targets the human RNase P gene, which provides valuable information on the success of the extraction method and possible inhibition.

T. pallidum, *H. ducreyi* and HSV, are the three major causative agents of sexually acquired genital ulcer disease (GUD) within the UK and these organisms are notoriously difficult to culture *in vitro*. It is therefore hoped that this service will aid laboratories and GU services in the differential diagnosis of genital ulcerative disease and consequently the provision of appropriate therapy. Please be aware that STBRL are unable to perform these PCRs individually and therefore any swab taken from an ulcer site that is referred to the reference lab will be examined for all three agents. If further details are required please contact stbri@hpa.org.uk or phone 020 8327 6464.

Sarah Alexander

Laboratory diagnosis of syphilis

STBRL has been providing a referral and reference service for the serological diagnosis of syphilis for London and the South East since January 2005.

During this time we have noted that we have received two types of samples:

- Sera, which have been screened by a single test (usually EIA), and are referred to STBRL for confirmation by additional tests.
- Sera, which have been tested by two or more tests, and are referred to STBRL for confirmation as part of our reference service.

STBRL test all specimens by EIA (total antibody), EIA IgM, TPPA (*Treponema pallidum* particle agglutination) and RPR (Rapid Plasma Reagin), and turn around time for this panel of tests is 3-7 days, depending on the day it is received. If additional tests, repeat testing or confirmation by immuno-blotting (Inno-Lia), is required the testing may take up to 10 days.

The provision of a timely result for the patient is important, particularly for infectious syphilis, and the current system of referring samples for confirmation by a second test was presumably established when syphilis was uncommon.

However, syphilis is now causing numerous outbreaks across the UK. The inherent delay in sending batches of specimens for confirmation, together with the time delay in processing reports therefore results in a considerable time lag in the patient receiving their result. There is an urgent need to review critically ways to improve laboratory diagnosis of syphilis.

Catherine Ison

Did you get your report?

STBRL strive to reduce the turn around time for samples received. To this aim we try to monitor the receipt of samples and the sending out of reports. We also record all queries regarding STBRL referral reports not received by the sending laboratory and instigate a Caldicott breach. In a few of these incidents the mistake was due to data entry mistakes. For the majority of the other cases the reason for the report failing to arrive at the desired location was unclear.

The issue of 'lost' referral forms is of concern due to an increased turn around time (and therefore patient notification and treatment) and Caldicott implications.

We would like to highlight the need for all laboratories using STBRL to use the barcoded STBRL reference laboratory forms. If you require a referral form please call the laboratory on 0208 327 6464. Alternatively for urgent use 'sample' referral forms can be found on our webpage <http://www.hpa.org.uk/cfi/stbrl/>.

Bethany Marsden
Catherine Ison

LGV Update

Enhanced surveillance in the UK for lymphogranuloma venereum, LGV, was launched in October 2004 and to date (28/03/2006) STBRL have now diagnosed 341 cases from more than 1500 submitted samples, all of which have been in men. LGV is caused by three serovars of *C. trachomatis*, L1, L2 and L3, it is more invasive than other serovars of chlamydia and requires three weeks of treatment of doxycycline or azithromycin, so accurate diagnosis is essential for patient management. All cases have been of serovar L2 and patients predominantly have had rectal infections, typically presenting with acute proctitis and very few have had urethral infection with inguinal lymphadenopathy. The cases have been predominantly diagnosed from individuals in London (73%) and Brighton (12%), but the remaining 15% from 23 cities/towns in the UK, showing a widespread geographical spread. This is the largest number of LGV cases reported within Europe where the outbreak is being detected.

Detection of LGV serovars is now determined directly using a Real-Time PCR method, developed by colleagues in Amsterdam, which exploits a 36bp deletion in the *pmpH* gene. This method enables easier differentiation of LGV from non-LGV associated serovars of *C. trachomatis* than conventional RFLP-PCR methods. It is hoped that the implementation of this test into our

routine programme will not only enable STBRL to detect more LGV cases which may previously have been missed because of mixed *C. trachomatis* infections or low DNA loads, but will decrease our turn around time on submitted samples. Referring laboratories should be aware that with this change in methodology comes a change in reports with LGV positive specimens being reported as "Evidence of LGV specific DNA" rather than a serovar specific result.

Specimens for LGV must meet our referral policy criteria, available on: www.hpa.org.uk/cfi/stbrl/lgv_surveillance.htm. Briefly, specimens will be tested from patients with anorectal or inguinal lymphadenopathy symptoms, with a positive *C. trachomatis* NAAT result. Please complete our referral form when submitting samples. We do not accept serum because of the lack of specificity of the currently available tests.

Iona Martin
Sarah Alexander

Location	Cases of LGV
London	241
Brighton	41
Scotland	10
Wales	3
other 23 locations in England	46

Pip negative gonococci : prevalence

The enzyme proline iminopeptidase (*Pip*) is used widely in clinical microbiology as a marker for the detection of *N. gonorrhoeae*. Gonococcal isolates lacking the *Pip* enzyme have been shown to generate false negative results when using some commercial biochemical test kits². During 2004 STBRL screened all isolates submitted as part of GRASP, of which 4.52% were lacking *Pip* activity³. The highest prevalence was observed in Cambridge, Brighton and Bristol. Variation in prevalence was also observed within London, between the nine centres submitting isolates. A significant association was found between *Pip* negative isolates and men who have sex with men which may account for the difference in prevalence between sites.

The high prevalence and widespread distribution of *Pip* negative *N. gonorrhoeae* isolates is a cause for concern and may result in

gonococci being misidentified. Referral of these isolates is not necessary, but STBRL do recommend that laboratories still using biochemical test kits which rely solely on the presence of the *Pip* enzyme (e.g. Neisseria Preformed Enzyme test and Gonocheck II) ensure that all negative cultures examined, are confirmed using an alternative (preferable immunological) method.

2. Alexander, S. and Ison, C. An Evaluation of Commercial Kits for the identification of *Neisseria gonorrhoeae*. J. Med. Microbiol. 2005; 54:1-4.
3. Alexander SA, Martin IMC, Fenton K, Ison C. The prevalence of proline iminopeptidase negative *Neisseria gonorrhoeae* throughout England & Wales. Sex Transm Infect. (In press)

Sarah Alexander

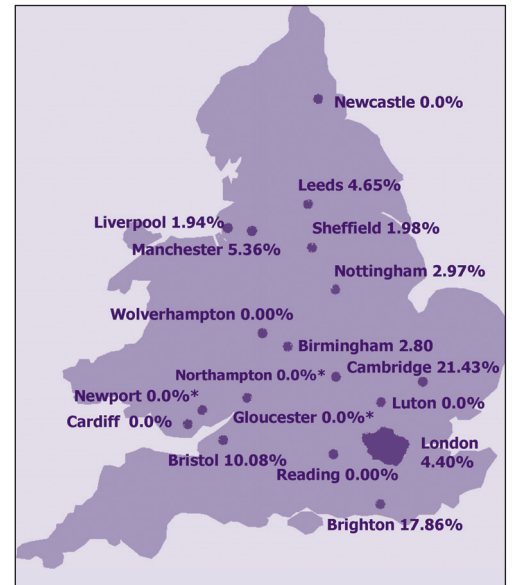


Figure: Distribution of *Pip* negative gonococci at GRASP sites in England & Wales.

Gonorrhoea Surveillance Update

GRASP (Gonococcal Resistance to Antimicrobials Surveillance Programme) 2005 collection period was completed in September and the 26 sentinel sites collected just under 2000 isolates. Susceptibility testing is now completed at STBRL, and is being analysed with the demographic and behavioural data from the GUM clinics in preparation for the annual report. We are now preparing for GRASP 2006. We would like to thank all the GRASP study sites for their cooperation and participation once again last year.

GRASP 2004 saw a continued rise in the incidence of resistance to ciprofloxacin (MIC $\geq 1\text{mg/l}$), from 9% in 2003 to 14.1% in 2004, with marked regional variation. This is despite the change to the national treatment guidelines recommending the use of 3rd

generation cephalosporins. GRASP documented that 70% of gonococcal infections were treated with a cephalosporin in 2004 and 22% with a fluoroquinolone. Ciprofloxacin resistant gonococci are now significantly associated with men who have sex with men (MSM), which has been a significant change from 2000-2003 when it was heterosexually associated.

No worldwide verified reports of resistance to ceftriaxone or cefixime exist at present. The full GRASP 2004 report and previous years can be downloaded from: www.hpa.org.uk/infections/topics_az/hiv_and_sti/sti-gonorrhoea/epidemiology/grasp.htm.

ESSTI (European Surveillance of Sexually Transmitted Infections, <http://www.essti.org/>) is a collaboration between countries in

Western Europe. In 2004 we coordinated the first ever sentinel surveillance study to collect and test gonococcal isolates using the same methodology, involving 12 different countries. Over 1000 gonococcal isolates were tested between STBRL and colleagues at the Danish Neisseria reference laboratory at the Statens Serum Institut in Copenhagen. Very high levels of resistance to ciprofloxacin (prevalence of 30.9%), penicillin (21.3%) and azithromycin (8.2%) were determined, with considerable variation within the 12 countries. These data have implications for European treatment guidelines for gonorrhoea and for clinicians treating patients who are likely to have contracted gonorrhoea in Western Europe.

Iona Martin

Molecular typing

STBRL offers molecular typing for *N. gonorrhoeae*, for outbreaks and medico-legal purposes, to aid the differentiation of samples from linked and unlinked sources and identification of clusters.

The molecular epidemiology of gonorrhoea in South Wales is being investigated in collaboration with colleagues at CDSC Wales where the incidence is rising compared to the national decrease in cases. We are typing gonococcal isolates from consecutive patients using a highly discriminatory molecular typing method, NG-MAST (*N. gonorrhoeae* Multi Antigen Sequence Typing). Typing, demographic and behavioural data will be analysed to identify characteristics of any transmission clusters to aid intervention work and partner notification to maximum effect.

For medico legal cases involving gonorrhoea transmission we routinely use NG-MAST in STBRL, where we have matched samples, as it is one of the most discriminatory sequence based techniques for *N. gonorrhoeae*. If you would like more details please contact STBRL.

Molecular typing methods for *T. pallidum* and *C. trachomatis* are currently in development and validation in STBRL and collaborations are being established to test the usefulness in controlling on-going outbreaks such as syphilis and LGV.

Iona Martin

Molecular detection of *Mycoplasma genitalium*

STBRL is currently validating assays for the molecular detection of *M. genitalium* in clinical samples by Real-Time PCR. Methods are directed to the MgPa gene (Jensen *et al.* 2004) and confirmed by in house RT-PCR. It is hoped this service will be available in the near future.

Vicki Chalker

If you have any questions please contact us

Catherine Ison (Director)

Catherine.ison@hpa.org.uk
Tel: 0208 327 6462

All services provided by STBRL

Reena Dattani (PA and Office Manager)

Reena.Dattani@hpa.org.uk
Tel: 0208 327 6464

Enquiries

Sarah Alexander

Sarah.alexander@hpa.org.uk
Tel: 0208 327 6772

Molecular diagnostics
Gonococcal reference work
LGV

Iona Martin

iona.martin@hpa.org.uk
Tel: 0208 327 6771

Molecular typing
GRASP
LGV

Vicki Chalker

Vicki.chalker@hpa.org.uk
Tel: 020 8327 6776

Mycoplasma genitalium

Bethany Marsden

Bethany.marsden@hpa.org.uk
Tel: 020 8327 7328

Syphilis serology

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

Other members of the STBRL team are:

Daksha Hathi (Diagnostics), Hemanti Patel (Syphilis serology), Elisabeth Maclure (GRASP), Uchi Ugoji (LGV), Tahir Ali (Chlamydia typing), Michelle Cole (ESSTI), and Linda Durbin. Tony McNiff (MOLIS), Marlette Vigille (Quality), Leah Desouza Thomas (GRASP co-ordinator), and Emma Savage (ESSTI co-ordinator) also work with STBRL.