PORTFOLIO OF EVIDENCE

Submitted to Fulfill Requirements for Assessment for
the ACS Certificate of Attainment

XXXXXXXXX

Modality: Clinical Microbiology

May 2005

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Summary

My enthusiasm for microbiology developed during my studies at the University XXXXXXXXXX for a Higher National Diploma in Applied Biology, graduating in 1995, and then for a Bachelor of Science in Biological Sciences, graduating in 1997. It was then a privilege to be able to expand upon my passion for microbiology and research by studying at XXXXXXXXXX University for a Doctor of Philosophy, awarded in 2003. I was subsequently appointed as a non-trainee Clinical Scientist Grade A in the microbiology section of the Department of Infection at XXXXXXXXXXX NHS Foundation Trust in July 2001, and was upgraded to Clinical Scientist Grade B in September 2003 after the award of my PhD. During my employment I have been responsible for providing a specialist molecular mycobacterial diagnostic service and expanding the repertoire of microbiological molecular diagnostic assays in the department by research, development and implementation of new diagnostic molecular assays. I have continually participated in training programmes and courses and undertaken professional development in microbiology and virology to expand my expertise, become fully developed in this role, and to meet the required standards for Clinical Microbiologists.

Portfolio

The supporting evidence in this portfolio is cross-referenced to the required competences for the ACS Certificate of Attainment shown in Appendix 1 using the following key:

S = Scientific
CL = Clinical
T = Technical
R = Research & Development
CO = Communication
P = Problem Solving
M = Management

For example, the reference [S2b.1g/2b.1p] relates to the third area in the Scientific competency (experience of searching for knowledge, critical appraisal of information and integration into the knowledge base).

1 Pre-employment training and experience

1.1 Undergraduate studies

1 During the period September 1992 to July 1995 I studied for a Higher National Diploma in Applied Biology at the University XXXXXXXXX, graduating with mainly distinction and merit grades for modules studied. These modules included Human Health, Disease Prevention and Treatment, Introductory Microbiology, Molecular and Cellular Bioscience and ecological/environmental fields.[S3a.1p, T3a.2p] I then went on to study for a Bachelor of Science in Biological Sciences from September 1995 to July 1997, also at the University XXXXXXXXX, graduating with an upper second with honours classification. Modules studied included Medical Microbiology and Public Health, which included training in the microbiology and epidemiology of infection, Applied Food Microbiology and Food Safety, Gene Expression and Manipulation and biodegradation/biodeterioration/biotechnology fields.[S3a.1p, T3a.2p]

2 During the final year of both of these courses, I undertook microbiological research projects. The HND project was entitled “An Investigation of the Antimicrobial Sensitivity of Various *Bacillus* Species” (see Appendix 2) and the BSc project entitled “The Antimicrobial Efficiencies of Facial Washes” (see Appendix 3). Both of these projects involved submission of a thesis, an oral presentation of the results and a *viva voce* defence of the work. Completion of these projects enabled me to gain experience of project management, including problem

solving and consumables requisitioning, technical microbiology skills, including bacterial isolation, antibiotic assays and sampling techniques, and the ability to communicate the results of a study.[S2b.1g/2b.1p, R2b.1p, CO1b.5p]

1.2 Postgraduate studies

3 During the period September 1997 to December 2000 I studied for a Doctor of Philosophy at XXXXXXXXX University under the supervision of XXXXXXXXX of the Department of Life Sciences. The project was undertaken in collaboration with XXXXXXXXX of the Department of Civil Engineering at XXXXXXXXX University XXXXXXXXX, and was funded by the Engineering and Physical Sciences Research Council. The overall aim of this project was to determine the safety of effluents from two laboratory-scale bioreactors operated to decolourise a synthetic textile effluent containing a reactive azo dye. Safety, in terms of potential detrimental effects on human health, was assessed by measuring the toxicity and genotoxicity of the decolourised effluent using bacterial bioluminescent and differential kill assays respectively. The microflora of each bioreactor, which were operated by another PhD student at XXXXXXXXX University XXXXXXXXX, was studied in order to elucidate the interrelations between toxicity and/or genotoxicity, decolourisation efficacy and the microbial population. Using both traditional culturing and biochemical techniques, and molecular techniques such as Fluorescent *in situ* Hybridisation, Denaturing Gradient Gel Electrophoresis and sequencing of the 16S rRNA gene for identification, I established that both the diversity and predominant species of the microflora differed due to the design of the bioreactors and the parameters under which they were operated. In September 2003 I was awarded a PhD for successful defence of a thesis entitled "Microbial Degradation of Textile Dyes to Safe End-Products" (see Appendix 4).

4 An abstract of this work was submitted and a poster presented at the Environment Agency and International Water Association joint Young Researchers Conference in March 2000 (see Appendix 5). I also co-authored a chapter with XXXXXXXXX for a book reviewing the subject of wastewater decolourisation, which is intended for publishing through the Society for Dyers and Colourists (see Appendix 6). The results of my project were also published in the Journal of Biotechnology (2003 **101**:49-56. See Appendix 7). Completion of this project enabled me to further develop my skills in project management, including method

development and troubleshooting, the technical skills required for bacterial isolation and identification from complex matrices, a thorough understanding of the principles behind and technical proficiency in many molecular techniques,[S3a.1p, S2b.1g/2b.1p, T3a.2p, M-] and the ability to critically appraise the literature and present the results of the study in many different media.[CO2b.1p/1b.5p] In addition, I also completed an MSc module entitled Research Methods which included the critical appraisal of a journal article and presentation of a fictional grant application, which enhanced my skills in the critical appraisal of the literature.[R2b.1p]

5 During this project, I established a number of collaborative links with research institutes and industrial partners. I worked as a visiting researcher at the XXXXXXXXX XXXXXXXXX in June 1999 and at the XXXXXXXXX in Oxford from April to July in 2000 to gain experience in the molecular techniques employed in this project. I also visited the laboratories of XXXXXXXXX in Cardiff in July 1998 to learn how to use a pre-production toxicity assay which was validated with assistance from data produced during my PhD project, and provided consultancy work for the use of this instrument after its launch. In addition, I assisted XXXXXXXXX in a voluntary capacity by helping to host meetings of the XXXXXXXXX. All of these experiences have enabled me to develop my technical competency in molecular microbiological assays and my communication skills with scientific professionals and the general public.[CO1b.4g] For the duration of my studies, I was also employed as a part-time lecturer. I demonstrated practical sessions to undergraduate students, in subjects such as medical microbiology, biochemistry, environmental microbiology and biodegradation and biodeterioration, and was responsible for teaching practical microbiology techniques, the care of cultures from experiments and the assessment and grading of laboratory reports. This enables me to improve both my communication skills and project management skills.[M-]

2 Post-employment training and experience

6 In July 2001 I was appointed as a non-trainee Clinical Scientist Grade A (SL01) in the microbiology section of the Department of Infection at XXXXXXXXX NHS Foundation Trust. The main responsibilities of this position are the provision of a specialist molecular diagnostic service and the research, development and implementation of new molecular diagnostic assays in the current diagnostic service. In September 2003, my position was

upgraded to Clinical Scientist Grade B (SM01) after I was awarded my PhD. Throughout the period of my employment I have undertaken a training programme that included relevant courses in order to meet the required standards for Clinical Scientists in clinical microbiology. Relevant details are given in the following sections.

2.1 Pre-registration training

2.1.1 Health & Safety and Quality Control

7 I have undertaken several tutorial sessions with the Health & Safety (H&S) Manager of the Department of Infection concerning H&S legislation and practice (see Appendix 8).[T3a.2p, M2c.2g/3a.3g/2c.2p] During these tutorials, I gained an understanding of the legislation for safe laboratory practice, such as the Health & Safety at Work Act (1974) and Reporting of Injuries, Diseases and Dangerous Occurrences Regulations (1995), and for the categorisation of dangerous pathogens according to the 4th edition of the "Categorisation of Biological Agents According to Hazard and Categories of Containment" Advisory Committee on Dangerous Pathogens regulations (1995).[CL3a.2p] Knowledge of control measures was also acquired, such as the local Codes of Practice and Standard Operating Procedures (SOPs) employed in both containment levels (CL) 2 and 3, and the local immunisation policy and the role of the Occupational Health Department in this. An understanding of waste management procedures was gained, such as the categorisation and disposal of waste, and disinfection and sterilisation procedures such as autoclaving. Hazard and risk assessment legislation and procedures were comprehensively covered, such as the Control of Substances Hazardous to Health (2002), biological, chemical, sharps, electrical and ionising radiation hazards, and the safe handling of clinical material and cultures at both CL2 and CL3.

8 I also attended a Chartered Institute of Environmental Health (CIEH) risk assessment course, and produced a risk assessment for processing specimens containing transmissible spongiform encephalopathy viruses within the department, which will be verified by the CIEH for certification. In addition, I assisted with the review of molecular mycobacterial SOPs during the last Clinical Pathology Accreditation (CPA) (UK) assessment, including a review of H&S practices, and assisted in the completion of the last departmental Safety Audit.

9 I have also received training in the principles of Quality Control (QC) and audit.[CL3a.2p, T2c.2g] The Department of Infection is CPA Accredited, and as such employs internal QC

measures and participates in external QC programmes, such as the UK National External Quality Assurance Schemes for microbiology, virology and antibiotic assays, and Quality Control for Molecular Diagnostics programmes for molecular biology. In addition to departmental training, I have also attended conferences and meetings covering QC principles, particularly with reference to molecular techniques (e.g. PCR Applications in Biomedical Sciences at Staffordshire University).[T2c.2g]

2.1.2 Microbiology and Virology

10 Diagnostic services are provided by the Department of Infection in two main areas, those of microbiology and virology. During my employment I have received training in both of these areas, in order to gain a comprehensive understanding of the investigations and clinical decisions involved with microbiological diagnostics.

11 The microbiology laboratory is divided into several sections according to the types of specimen processed or organisms isolated, including wounds, urines, stools, blood cultures, respiratory specimens, genitourinary swabs and swabs for methicillin resistant *Staphylococcus aureus* (MRSA). The laboratory also has CL3 facilities for isolation of hazard group 3 pathogens including *Mycobacterium tuberculosis* (MTB). I have received comprehensive training in all of these sections (see Appendix 9).[S3a.1g, T3a.2p] Knowledge of safe laboratory practices and familiarisation with the relevant SOPs specific to each section was gained (e.g. the use of needles especially in CL3), and of the relevant pathogens with reference to particular patient groups (e.g. paediatrics and immunocompromised persons).[CL3a.2p, T3a.2p]

12 I also acquired the ability to conduct the practical techniques required to process all specimen types, such as decontamination of respiratory specimens for mycobacterial culture, and then to culture specimens using solid and liquid media (e.g. the correct inoculum density for sensitivity testing), including specimens or cultures processed as part of the department's QC programmes.[T2b.4p, T2c.2g] Knowledge of the correct general or selective media for use when culturing different organisms and specimen types was acquired, such as the use of nalidixic acid to suppress the growth of Gram negative organisms or of Chocolate Blood Agar for culturing *Haemophilus influenzae*. In addition, I gained the ability to recognise and identify pathogenic organisms using colonial morphology, microscopy and biochemical reactions (including the API commercial kits). I also received training in the molecular assays

used in the diagnostic mycobacterial service.[T2b.4p] I also gained an understanding of the interpretation of both the assays (e.g. agreement of the morphology, microscopy, biochemical and antimicrobial susceptibility data) and of the clinical picture (e.g. *Streptococcus pneumoniae* in respiratory specimens from ITU patients), and of assay troubleshooting.[CL2b.1p] All of this training allowed me to use scientific and technical knowledge to produce a clinically significant diagnostic result.[CL2b.3p]

13 The virology laboratory is divided into several sections according to the techniques employed, including manual serology, automated serology, virus isolation and molecular biology. I have received comprehensive training in all of these sections (see Appendix 10).[S3a.1g, T3a.2p] Knowledge of safe laboratory practices and familiarisation with the relevant SOPs specific to each section was gained.[CL3a.2p, T3a.2p] An understanding of the clinically important viral pathogens was gained with reference to particular specimen types and patient groups, such as cytomegalovirus (CMV) in immunosuppressed patients (e.g. human immunodeficiency virus (HIV) positive patients, leukaemic and transplant patients, neonates and pregnant patients), HIV in African and East European (e.g. atypical strains and presentations) and UK patients (e.g. dual infections with hepatitis B (HBV) or hepatitis C (HCV) virus) and respiratory syncytial virus (RSV) in neonates.

14 I also observed and acquired the ability to conduct many assay types used in the laboratory, including specimens processed as part of the department's QC programmes.[T2b.4p, T2c.2g] The serological assays comprise many practical techniques to detect all antibody (Ab) and antigen (Ag) markers, such as Complement Fixation Tests for herpes simplex virus (HSV) and *Chlamydia psittaci* Ag, manually performed Enzyme Linked ImmunoSorbent Assays (ELISAs) for RSV and Influenza A Ag and CMV Ab, automated ELISAs for CMV, HIV and hepatitis virus Ab and Ag (AxSym) and for *Treponema pallidum* and varicella zoster virus (VZV) Ab (BEP 2000) and latex agglutination for VZV and rubella virus Ag confirmatory assays. Tissue culture for direct isolation of viruses and observation of a cytopathic effect (CPE) utilises Human Embryonic Lung (HEL) cells in tubes (e.g. isolation of HSV from eye swabs) and Vero cells in shell vials (e.g. isolation of adenovirus from eye swabs and enterovirus from respiratory samples). HEL cells are also used with chambered slides in the Direct Early Antigen Fluorescent Foci (DEAFF) test for CMV. Direct detection of viruses using electron microscopy is performed on faecal specimens (e.g. small round-structured

virus, astrovirus), urine (e.g. BK virus) and lesions (e.g. VZV, HSV). The molecular assays comprise many techniques such as PCR (e.g. enterovirus, HSV and VZV from CSF), real-time PCR (e.g. CMV and BK viral loads), microwell plate hybridisation (e.g. HCV), strand displacement amplification (e.g. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* screening) and sequencing (e.g. HIV resistance screening). I also gained an understanding of the interpretation of both the assays (e.g. compliance with validation criteria) and of the clinical picture (e.g. HSV and JC virus encephalitis), and of assay troubleshooting.[CL2b.1p] All of this training allowed me to use scientific and technical knowledge to produce a clinically significant diagnostic result.[CL2b.3p]

15 In addition to training in the laboratory, I also completed the 6-month Basic Virology course provided by the Institute of Biomedical Science (XXXXXXXXX Region) Virology Discussion Group in March 2004.[S3a.1g, T3a.2p] Topics covered during the course included epidemiology, pathogenesis, control and treatment and technical methods, and many viruses (e.g. hepatitis viruses, herpesviruses, respiratory viruses and retroviruses) and diseases (e.g. atypical pneumonia, gastroenteritis and intrauterine infections) were extensively discussed.

16 In addition to the practical aspects of microbiology, I also gained an understanding of the processes for requesting and reporting diagnostic assays within the laboratories. I attained knowledge of the procedures by which specimens are delivered to the department, the computer systems used to request tests and of the processes of inputting information into the Laboratory Information Management Systems (LIMS) in Specimen Reception. I also acquired the ability to correctly input results into the LIMS, and to file hardcopies. Knowledge was gained of the processes for data validation by senior Biomedical Scientists (BMSs) and of scrutiny performed by senior Clinical Scientists, Specialist Registrars (SpRs) and consultants before results are entered into the computer systems used to report diagnostic results. I am also competent in the use of the LIMS and other results reporting systems to retrieve other pathology indicators in order to apply relevant diagnostic procedures to the clinical situation (e.g. CXR in MTB investigations, FBC, pyrexia, C-reactive protein) and for data comparison in problem solving (e.g. MTB and *Neisseria gonorrhoeae* culture and molecular assay data).[S2c.1p, CL3a.1p, P2a.2g/2c.1g]

2.1.3 Clinical

17 During my employment I have participated in several programmes to increase my knowledge of the clinical impact of microbiological investigations and of the pathology of infections. Weekly seminars are conducted by the Infection SpRs, consultants and senior Clinical Scientists as part of the department's Postgraduate Education Programme, covering literature reviews, case studies and seminars on topics such as antibiotic resistance (e.g. resistance to cephalosporins in Gram negative bacteria, extended spectrum β-lactamases), disease processes (e.g. gonococcal endocarditis, herpesvirus replication), disease management (e.g. diagnosis and management of respiratory viruses in the immunocompromised patient, MRSA decontamination) and organisms (e.g. *Listeria monocytogenes*, enterovirus 71). External speakers are also invited (e.g. MRSA bacteraemia at XXXXXXXXX Hospital, immune responses in tuberculosis at XXXXXXXXX Hospital).[S3a.1p, S3a.1g] I have given a seminar on the basics of molecular techniques and the molecular mycobacterial assays used in the department, and am due to give one on the clinical impact of molecular mycobacterial diagnostics.[S3a.1p, CO1b.5p, CO2b.1p/1b.5p] In addition, I have accompanied the SpRs and consultants on ward rounds and have attended grand rounds and case presentations, which have increased my knowledge of the clinical impact of diagnostics especially in the context of problem solving.[CL3a.1p, P2b.1g]

18 I have also attended many seminars and courses at external institutions and national and international conferences covering aspects of clinical disease pathology and the technical background of microbiological diagnostics (see Appendix 11).[S3a.1p, CL3a.1p, T3a.2p, CO-] This knowledge has allowed me to relate the provision of diagnostic services in the department to the wider clinical picture, especially in the context of formulating a diagnostic strategy and problem solving.[S3a.2g, P2b.1g] In addition, individual reading and literature reviews have increased my knowledge of the pathology of infections, the technical aspects of diagnostic assays and of the evidence base for the gold-standard assays in microbiological diagnostics.[S3a.1g, S3a.2g, CL3a.1p, CL3a.2p, T3a.2p] I have also liaised with commercial companies on technical aspects of diagnostic assays, specifically on matters concerning application to the department's diagnostic service and problem solving.[T2b.4p, P2b.1g]

2.1.4 Epidemiology and Infection Control

19 I have undertaken several tutorial sessions with the Microbiology Consultant and Infection Control (IC) Doctor of the Department of Infection concerning the epidemiology of infection and the role of the IC team within the Trust (see Appendix 12).[CL3a.1p] During these tutorials I have gained an understanding of the nature of reservoirs of infection in a hospital setting, such as the patient or the environment. The types of infection encountered in hospitals, such as opportunistic, community-acquired and hospital-acquired (more recently termed healthcare-associated infection), were also discussed. Knowledge of the modes of transmission of infection was also acquired, such as airborne (e.g. *Staphylococcus aureus*), food and drink (e.g. *Salmonella* spp.), direct and indirect contact (e.g. *Klebsiella* spp. and *Acinetobacter* spp., blood-borne viruses) and arthropod-borne (e.g. malaria).

20 An understanding of the control of hospital infection was gained, such as knowledge of the epidemiology of the disease in order to break the chain of infection, decontamination, disinfection and sterilisation processes (e.g. centralisation of processes for accountability and trace-ability, single-use items wherever possible) and hospital hygiene (e.g. universal precautions). The organisation of IC and role of the team was also discussed, including the IC Nurse, Officer, Committee and Trust Board and the Communicable Disease Surveillance Centre, in addition to alert organisms (e.g. tuberculosis, VRE) and conditions (e.g. respiratory infections in the community, diarrhoea in paediatric wards). I have also gained knowledge of the principles of prevalence and incidence surveillance and audit, alert organism and condition surveillance (e.g. laboratory-based ward surveillance) and Clinical Governance.

2.1.5 Teaching and Management experience

21 As part of my personal development in project management and supervisory skills, I have attended training courses on these subjects provided by the Training & Development Department of XXXXXXXXX NHS Foundation Trust (see Appendix 11). These courses enabled me to apply PRINCE2 methodology to planning and delivering projects, and also to communicate more effectively in a supervisory role.[M-] During my employment I have been responsible for supervision of two projects conducted by MSc students studying within the department, involving a clarification of the aims and objectives of the projects, supervision of

the practical work undertaken, assistance with troubleshooting and assistance with the production of project theses.[R2b.1p, CO1b.4g, P2b.1g, M-]

22 I have been a member of the Molecular Diagnostics Group, comprising management and senior scientists from departments within pathology with an interest in molecular diagnostics, since I gained my position. My role is to represent the interests of and report on any service developments within the bacteriology laboratory to this group,[CO1b.2p] which was formed to implement a grant given to develop a pan-pathology molecular diagnostic service. In October 2003 I also took part in a laboratory demonstration day as part of an Infectious Disease module for students studying for an MSc in Medical Immunology. This demonstration involved the production and presentation of materials and visual aids to give an overview of tuberculosis and to demonstrate the techniques involved in the mycobacterial diagnostic service provided by the department, including the molecular assays.[CO1b.4g]

23 From April to December 2003 I was a member of the Clinical Laboratory Services Strategic Working Group, which was formed to inform the Trust's 2003 Future Service Strategy. The aim of this group was to consider future models for a 5- to 10-year plan of service delivery by Clinical Laboratory Services, comprising pathology services, the Information Technology Department and external stakeholders (e.g. Primary Care Trusts, affiliated Trusts and patient groups). In collaboration with another member of the group, I conducted a risk analysis of patient, specimen and information flow through a number of the Trust's care pathways, produced a Strengths, Weaknesses, Opportunities and Threats (SWOT) questionnaire that was completed by all of the departments within pathology and produced and presented an analysis of the SWOT survey to the group.[CO-, CO1b.4g]

2.2 Responsibilities

2.2.1 Service Provision

24 I am responsible for providing an 'as required' molecular mycobacterial diagnostic service.[T2b.4p] This service is provided by myself and competent BMSs in the department, for whom I have the responsibility for training.[CO1b.4g] The three commercially-available assays offered by the department for mycobacterial diagnostics are a real-time PCR assay for differentiation of *Mycobacterium tuberculosis* (MTB) from other mycobacteria, the determination of susceptibility of MTB to rifampicin using PCR and membrane hybridisation,

and the speciation of 13 clinically important mycobacteria by PCR and membrane hybridisation. All specimens that require mycobacterial diagnostics are examined by microscopy and cultured using standard solid and liquid media (including rapid automated liquid culturing), and outsourced for identification using biochemical techniques. Following departmental criteria and with reference to the specifications of the assays, I have determined the optimum patient groups and specimen types to be tested by molecular assays for more efficient and effective patient care. I advise the Infection SpRs, IC doctors and consultants and BMSs on the suitability of the specimen and technical limitations of the assays for individual cases to achieve the desired diagnostic information, taking into account the clinical situation.[S3a.2g, CL3a.1p, CL2b.3p]

25 Most of the requests for mycobacterial diagnostics are made through the Infection SpRs, although I also advise on the suitability of molecular diagnostics in cases from other departments within XXXXXXXXX NHS Foundation Trust or external laboratories as required.[CL3a.2p, CL2b.3p, CO1b.2p, CO1b.4g] I also advise on the availability of further types of diagnostic assays or of alternative facilities by outsourcing if required.[CL2b.1p, P-] For example, the XXXXXXXXX at XXXXXXXXX Hospital is the only alternative facility to offer the same assay for rifampicin susceptibility as XXXXXXXXX in London. Where there is a non-standard case of particular importance, I liaise with the relevant consultants to devise an investigation strategy, including development of the methods as necessary (e.g. extraction of DNA from paraffin-embedded tissue sections in a case of suspected non-pulmonary tuberculosis and a case of bacterial infective endocarditis).[CL2b.3p]

26 During my employment I have introduced a validation procedure for molecular mycobacterial assays, whereby the interpretation of data must be verified with another senior BMS or Clinical Scientist competent to perform the assays.[CL2b.1p] This is especially important when diagnosing mycobacterial diseases, as the consequences of information about the presence of these pathogens, their species and antimicrobial susceptibilities on the treatment and management of patients are extremely significant (e.g. the antimicrobials prescribed and length of course for quadruple therapy of tuberculosis are very different than those prescribed for shorter-course therapy of mycobacteria other than tuberculosis (MOTT), and rifampicin is one of the first line antimicrobials prescribed for tuberculosis therapy).[S2a.1p, CL3a.2p] The results of the assays are then reported to the Infection SpRs or IC doctors and consultants and entered

into the department LIMS. A constant liaison with the SpRs and consultants on the patient status have allowed me to gain an understanding of the effectiveness of these procedures, both in individual cases and of the application of molecular assays to mycobacterial diagnostics.[S2c.1p]

27 I have also introduced aspects of Quality Control (QC) management to the mycobacterial assays, including batch identification and validation testing of all reagents and participation in the Quality Control for Molecular Diagnostics proficiency programmes for MTB since 2001.[T2c.1p, M2c.2g/3a.3g/2c.2p] I am also responsible for stock checking and ordering for the mycobacterial assays, and have produced a batch identification database linked to order and expiry dates.[M2c.2g/3a.3g/2c.2p] In addition to QC and individual assay validation, I also monitor the correlation of the molecular assays with microscopy and culturing data, both for individual cases to identify diagnostic errors and to evaluate specificity levels of the tests used.[P2a.2g/2c.1g]

28 I also perform troubleshooting on the assays as required.[P2b.1g] The troubleshooting process may involve several steps dependant upon the nature and severity of any impact upon a patient diagnosis, including liaison with Infection SpRs or IC doctors and consultants as required, data analysis of the aberrant assay, performance of alternative assays that will give the required diagnostic information (e.g. speciation of a mycobacterium by membrane hybridisation where the MTB/MOTT differentiation real-time PCR has failed), liaison with the SpRs or consultants to advise on the error that occurred and it's meaning to the diagnosis, filing of the necessary error-logging documentation (e.g. form IR1) and a revision of the procedures as necessary (e.g. revision of the validation criteria for the MTB/MOTT differentiation real-time PCR when a 'grey zone' is identified).[T2b.4p, T2c.1p, CO1b.5p, P2b.1g]

2.2.2 Service Development

29 During my employment I have introduced two new assays to the molecular mycobacterial diagnostic service, the MTB/MOTT differentiation real-time PCR and mycobacterial speciation (genotyping) by membrane hybridisation.[P-] The genotyping assay was introduced to the service after a need for a more rapid speciation assay was identified, as the IC procedures, patient management and antimycobacterial agents used differ in cases of infection with MTB or MOTT.[S2a.1p] The existing method of identifying mycobacterial

isolates was by biochemical techniques at an external laboratory. I conducted an analysis of clinical data for patients who had been diagnosed with an MOTT infection over a 20-month period, using parameters including in/outpatient status, direct microscopy result, quantity of growth, HIV status, mycobacterial species and time taken to achieve identification, to determine the clinical impact of a rapid speciation assay.[R2b.1p, P2a.2g/2c.1g]

30 After a literature review, two commercially-available assays were considered, and a comparison of the specifications, ease-of-use, 'hands-on' time requirements, costs per specimen, species identified and compliance with the *In Vitro* Directive was made, in addition to a Health & Safety Risk Assessment.[S2b.1g/2b.1p, S2b.1g, S3a.2g, R2b.1p, M2c.2g/3a.3g/2c.2p] The assays were performed prospectively in parallel in order to reduce operator bias, and the data were compared to the identification by the external laboratory.[R2b.1p] In September 2003, I presented the final report on the evaluation including a recommendation to the management of the department, which was accepted for immediate implementation (see Appendix 13).[R2b.1p, CO1b.5p] I was responsible for writing an SOP for this assay to departmental standards and formats, and for training of all BMSs involved in the provision of the molecular mycobacterial service.[T3a.2p, CO1b.4g]

31 The real-time PCR assay was introduced to replace the existing MTB/MOTT differentiation assay used in the department since 1997, which was based on PCR and microwell plate hybridisation, due to supply difficulties and a high percentage of failure of the assay kit controls which persisted after I had performed troubleshooting of the assay and liaised with the manufacturer's technical support department. After a literature review, I decided that the most suitable assay for the department in terms of clinical need and technical applicability was a real-time PCR assay.[S3a.2g]

32 The evaluation of this assay involved a comparison of the specifications, ease-of-use, 'hands-on' time requirements and costs per specimen of the real-time and microwell plate assays, in addition to a Health & Safety Risk Assessment and a comparison of the advantages and disadvantages of continuing with or replacement of the existing assay.[S2b.1g/2b.1p, S2b.1g, R2b.1p, M2c.2g/3a.3g/2c.2p] The assays were then performed in a parallel blinded trial on random specimens in order to reduce specimen and operator bias, and the data were analysed by comparison to the gold standard of mycobacterial culture and control failure and inhibition rates were determined.[R2b.1p] In June 2004, I presented the final report on the evaluation

including a recommendation to the management of the department, which was accepted for immediate implementation (see Appendix 14).[R2b.1p, CO1b.5p] I was responsible for writing the SOP for this assay, adhering to departmental standards and formats, and for the training of all BMSs for the molecular mycobacterial service.[T3a.2p, CO1b.4g]

33 An additional major service development with which I have been involved was the introduction of a molecular assay for primary screening of samples for *Neisseria gonorrhoeae* (GC). Before 2003, the existing method of detection of GC in genitourinary screening was by culturing of swab specimens and latex agglutination or biochemical tests. After I had appraised the literature on molecular assays for GC testing,[R2b.1p] a small trial was conducted in May 2002 on the use of the BDProbeTec™ ET (BDPT), a semiautomated system using strand displacement amplification, for detection of GC in comparison to culture. The BDPT had been recently introduced to screen swab and urine specimens for *Chlamydia trachomatis* (CT) to precede the Department of Health's Chlamydia Screening Programme, which was rolled out nationally in April 2003. Based upon the positive results of this trial, a large-scale trial of detection of GC by simultaneous testing for CT and GC on the BDPT was conducted between September 2002 and January 2003.

34 I conducted an analysis of the data by comparison to culture, using parameters including patient age and gender, specimen type, clinical presentation such as discharge and urethritis and microscopy for the presence of pus cells and Gram negative intracellular diplococci, including an assessment of the effect of factors such as prevalence on the sensitivity of the assay.[S2b.1g, R2b.1p] This analysis involved liaison with several sources for information, including the Department of Genitourinary Medicine (GUM) for clinical diagnoses, the microbiology section of the Department of Infection who perform GC culturing and the virology section of Infection who perform the BDPT assays.[CO1b.2p] In March 2003, I presented the final report on the trial to the management of both departments in a GUM/Infection Liaison meeting, where the decision was taken to implement screening of genitourinary specimens for GC by simultaneous detection of GC and CT on the BDPT (see Appendix 15).[R2b.1p, CO1b.5p] I also submitted this work as a paper to the Journal of Clinical Pathology (see Appendix 16).[R2b.1p, CO2b.1p/1b.5p] This paper was not accepted in its original format, and is currently undergoing revision. As part of further research to this work, I am

currently involved in a project to design an in-house real-time PCR method for confirmation of BDPT positives.

35 I am also a co-applicant on a service development grant awarded by the XXXXXXXXX Charity aimed at developing a diagnostic assay for the simultaneous detection of *Treponema pallidum* and *Haemophilus ducreyi* in genital ulcers. After I had appraised the literature on molecular detection of both *T. pallidum* and *H. ducreyi*, a project plan was developed and a grant proposal written by the Clinical Scientist for Molecular Diagnostics in Pathology and consultant physicians from the Departments of Infection and Genitourinary Medicine.[R2b.1p, CO2b.1p/1b.5p] In conjunction with the other Clinical Scientist in the group, I have produced a study datasheet for use in the clinics, assisted with method development (e.g. choosing an appropriate swab type) and performed experimental work.[R2b.1p] Preliminary data from clinic swab specimens has now been produced.

2.2.3 Research & Development

36 I am responsible for research and development of new molecular assays relevant to the department's delivery plans for implementation into the diagnostic service. My projects have included identification of unknown organisms from blood culture broths and continuous ambulatory peritoneal dialysis (CAPD) specimens using the 16S and 23S rRNA genes, speciation of *Candida* sp. isolates from blood cultures using the 18S rRNA gene, determination of methicillin resistance in isolates by detection of the *mecA* gene, identification of unknown isolates by sequencing of the 16S and 23S rRNA genes, typing of *Salmonella enterica* serotype *enteritidis* by Repetitive Sequence PCR (rep-PCR), typing of MRSA by rep-PCR, typing of vancomycin resistant *Enterococcus faecium* (VRE) by Multi Locus Sequence Typing (MLST) and detection of norovirus from faecal specimens using the RNA polymerase gene.

37 Each of these projects have involved a critical appraisal of the literature,[R2b.1p] in order to gain an understanding of the application of the assay in a clinical diagnostic setting and for integration of the most advanced techniques into the department's knowledge base.[S2b.1g/2b.1p] All projects have required the development of the experimental protocol for scientifically rigorous assay validation. Some projects have built on techniques from previous successful in-house projects but have required development of the methodology for application to different

specimen types. Other techniques required extensive modifications to make them compatible with existing working practices and applicable to a diagnostic setting.[R2b.1p] A range of microbiological and molecular biological techniques have been employed in these projects, such as culturing of both known and unknown organisms, DNA/RNA extraction from both bacteria and norovirus particles, DNA/RNA amplification by PCR and product detection using agarose gel electrophoresis, real-time PCR, membrane hybridisation and sequencing.[R2b.1p] During development of the norovirus assay, I had the opportunity to visit the Enteric Virus Unit in the XXXXXXXXXXXXXXXXXXXX at XXXXXXXXXXXXXXX. I had the opportunity to discuss the methods used in the assay for detection of norovirus from specimens sent from all over the country to this laboratory, which gave me a valuable insight into both the workings of a reference laboratory and the development of a clinically useful diagnostic norovirus assay.

38 Every assay has been developed with all of the appropriate controls, and tested for specificity and sensitivity before implementation.[T2c.1p] It is vital to ensure that each assay under development is properly validated and quality controlled, as these assays contribute to clinical decisions about patient and hospital management when employed as diagnostic tools (e.g. antibiotic therapy will be affected in a case of identification of resistance such as the possession of the *mecA* gene in MRSA, and wards may be closed to new admissions by IC doctors and nurses in a case where norovirus is detected).[S2a.1p] I have performed troubleshooting experiments when necessary, often employing flow diagrams describing the physical, chemical and biological parameters involved with each step of the assay.[T2b.4p, P2b.1g] A critical appraisal of the results of experiments, with reference to my knowledge of the technique and the assay controls applied, has often lead to further questions and experiments that have elucidated these questions (e.g. identification of a misclassified *Enterococcus faecium* as an *E. avium* by biochemical techniques during VRE MLST).[R2b.1p]

39 An example of project development by personal initiative is given by a case study concerning the identification of a blood culture isolate as a novel strain of *Francisella tularensis* (see Appendix 17).[P-] I sequenced the DNA from a blood culture isolate that was proving difficult to identify using the 23S rRNA gene, and checked the resulting consensus sequence against the public access databases (e.g. GenBank) using the Basic Local Alignment Search Tool (BLAST) algorithm. The best match was to a non-speciated *Francisella* sp.

described as a newly recognised 'Francisella-like organism', although matches with more nucleotide differences were found with the subspecies variants *F. tularensis* subsp. *holoarctica* and *F. tularensis* subsp. *novicida*. It was suggested the identification might be *F. philomiragia*, as the organism was oxidase positive. Upon searching the databases, I found that there were no 23S rRNA sequences available for *F. philomiragia* but the 16S rRNA gene had been sequenced. Therefore, I conducted a literature survey to identify the best primers to use for amplifying the 16S rRNA gene and developed and optimised the amplification protocol. After checking the 16S rRNA gene sequence against the databases, the best match was to *F. tularensis* and the subspecies variant *F. tularensis* subsp. *novicida*, with matches containing more nucleotide differences to both *F. philomiragia* and the non-speciated *Francisella* sp. previously indicated. The isolate was referred to HPA XXXX, HPA XXXX, and the XXXXXXXX in Germany, where it was concluded that it was a non-speciated *Francisella* sp. phylogenetically positioned between *F. tularensis* subsp. *holoarctica* and *F. tularensis* subsp. *novicida*.

40 Many of the projects noted above have involved my participation in collaborations with external agencies. For example, the genotyping of *Salmonella enterica* serotype *enteritidis* was conducted in collaboration with a visiting researcher from a hospital laboratory in Zagreb, Croatia, who supplied a number of *S. enterica* isolates. I liaised with the laboratory on the results of troubleshooting experiments I performed on some of these isolates that could not be typed by this method, such as purity plating, serotyping and biochemical identification, where it was found that many isolates were not pure cultures of *S. enterica*. In addition, collaborations are currently on-going with researchers from both academia (e.g. for identification of organisms in pulmonary disease by sequencing of the 16S and 23S rRNA genes) and industry (e.g. for commercial production of the membrane macroarray for identification of organisms by the 23S rRNA gene).[CO-, CO1b.5p]

2.3 Future projects and professional development

41 In addition to continually maintaining and developing the molecular mycobacterial diagnostic service, I will continue my research and service development activities with a goal towards implementing the most successful and applicable into the diagnostic service of the Department of Infection. Such projects include molecular detection of MRSA, the typing of

VRE by MLST, and also to extend the MLST project to include MRSA as part of an array of typing methodologies such as the use of the SSC*mec* type. I am also working towards implementing the detection of norovirus into the service, and plan to refine the methodology to provide an easy-to-use, rapid assay using a one-step multiplex RT-PCR. I will also continue to identify difficult organisms from the microbiology diagnostic laboratory using the combination of membrane macroarray and sequencing that has proven successful in the past.

42 I have been a member of the Association of Clinical Microbiologists from 2002, of the Society of General Microbiology from 1997 to 2001 and of the Society for Applied Microbiology from 1997. Attendance at seminars and conferences provided by these and other organisations has enabled me to continually develop my knowledge of microbiology, particularly with reference to the healthcare setting, and will continue to do so in the future. I will also maintain my links with the Institute of Biomedical Science (XXXX Region) Virology Discussion Group, and plan to complete the Virology Update course in the near future. I would also like to study towards part one membership of the Royal College of Pathologists.

Appendix 1: Competences required for applicants to attain state registration as Clinical Scientists

The competences listed in this appendix are cross-referenced to the relevant paragraphs of the portfolio of evidence.

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

APPENDIX 1

This set of documents must be completed and returned in your portfolio.
Please complete the three header sections above on each page.
Refer to the Specific Competences document for guidance in completing this document.
Use typescript or black ink and block capitals for all sections.

EXPERIENCE: The candidate should be able to demonstrate that he/she has worked in an environment that has enabled the individual to receive training and gain experience relevant to the competences set out below.

SCIENTIFIC

HPC Standards of Proficiency Codes for Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
3a.1p	• understanding the science that underpins the specialty (modality) and the broader aspects of medicine and clinical practice	PARAGRAPHS 1, 4, 17 + 18
3a.1g	• demonstrating a strong base of knowledge appropriate to the specialty and to the investigations and therapeutic options available	PARAGRAPHS 11, 13, 15, 17 + 18
2b.1g 2b.1p	• experience of searching for knowledge, critical appraisal of information and integration into the knowledge base	PARAGRAPHS 2, 4, 30, 32 + 37
2b.1g	• ability to apply knowledge to problems associated with the routine provision, and development, of the service	PARAGRAPHS 30, 32 + 34
2a.1p	• ability to identify the clinical decision which the test/intervention will inform	PARAGRAPHS 26, 29 + 38
2c.1p	• ability to make judgements on the effectiveness of procedures	PARAGRAPHS 16 + 26
3a.2g	• application of the knowledge base to the specialty (modality) and to the range of procedures/investigations available	PARAGRAPHS 18, 24, 30 + 31

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

CLINICAL

IPC Standards of Proficiency Codes for Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
2b.1p	• ability to provide interpretation of data and a diagnostic (therapeutic) opinion, including any further action to be taken by the individual directly responsible for the care of the patient	PARAGRAPHS 12, 14, 25 + 26
3a.1p	• understanding of the wider clinical situation relevant to the patients presenting to his/her specialty	PARAGRAPHS 16, 17, 18, 19 + 24
2b.3p	• ability to develop/devise an investigation strategy taking into account the complete clinical picture	PARAGRAPHS 12, 14, 24 + 25
3a.2p	• understanding of the clinical applications of his/her specialty and the consequences of decisions made upon his/her actions/advice	PARAGRAPHS 25 + 26
3a.2p	• awareness of the evidence base that underpins the use of the procedures employed by the service	PARAGRAPHS 7, 9, 11, 13 + 18

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

TECHNICAL

IPC Standards of Proficiency Codes or Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
3a.2p	• understanding of the principles associated with a range of techniques employed in the modality	PARAGRAPHS 1, 4, 11, 13, 15 + 18
3a.2p	• knowledge of the standards of practice expected from these techniques	PARAGRAPHS 7, 11, 13, 30 + 32
2b.4p	• experience of performing these techniques	PARAGRAPHS 12, 14 + 24
2b.4p	• the ability to solve problems that might arise during the routine application of these techniques (troubleshooting)	PARAGRAPHS 18, 28 + 38
2c.2g	• understanding of the principles of quality control and quality assurance	PARAGRAPHS 9, 12 + 14
2c.1p	• experience of the use of quality control and quality assurance techniques including restorative action when performance deteriorates	PARAGRAPHS 27, 28 + 38

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

RESEARCH AND DEVELOPMENT

IPC Standards of Proficiency Codes r Clinical Scienti	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
2b.1p	• ability to read and critically appraise the literature	PARAGRAPHS 4, 29, 30, 32, 33, 35 + 37
2b.1p	• ability to develop the aims and objectives associated with a project	PARAGRAPHS 21, 30, 32 + 37
2b.1p	• ability to develop an experimental protocol to meet the aims and objectives in a way that provides reliable and robust data (i.e. free of bias)	PARAGRAPHS 21, 30, 32 + 35
2b.1p	• ability to perform the required experimental work ability to produce and present the results (including statistical analysis)	PARAGRAPHS 2, 30, 32, 34 + 37
2b.1p	• ability to critically appraise results in the light of existing knowledge and the hypothesis developed and to formulate further research questions	PARAGRAPHS 29, 30, 34 + 38
2b.1p	• ability to present data and provide a critical appraisal to an audience of peers – both spoken and written	PARAGRAPHS 30, 32 + 34

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

COMMUNICATION

IPC Standards of Proficiency Codes for Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
-	• ability to assess a situation and act accordingly when representing the specialty	PARAGRAPHS 18, 23 + 40
1b.2p	• ability to respond to enquiries regarding the service provided when dealing with clinical colleagues	PARAGRAPHS 22, 25 + 34
1b.4g	• ability to communicate with patients, carers and relatives, the public and other healthcare professionals as appropriate	PARAGRAPHS 5, 21, 22, 23, 24, 25, 30 + 32
1b.5p	• ability to communicate the outcome of problem solving and research and development activities	PARAGRAPHS 2, 17, 28, 30, 32, 34 + 40
2b.1p 1b.5p	• evidence of presentation of scientific material at meetings and in the literature	PARAGRAPHS 4, 17, 34+ 35

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

COMPETENCES REQUIRED FOR APPLICANTS TO ATTAIN STATE REGISTRATION AS CLINICAL SCIENTISTS					
MODALITY:	CLINICAL MICROBIOLOGY	**SUBMODALITY: (if applicable)**		**APPLICANT'S NAME:**	[redacted]

PROBLEM SOLVING

HPC Standards of Proficiency Codes for Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
2a.2g 2c.1g	• to assess a situation	PARAGRAPHS 16, 27 + 29
2b.1g	• determine the nature and severity of the problem	PARAGRAPHS 17, 18 + 21
2b.1g	• call upon the required knowledge and experience to deal with the problem	PARAGRAPHS 18, 21 + 28
2b.1g	• initiate resolution of the problem	PARAGRAPHS 21, 28 + 38
-	• demonstrate personal initiative	PARAGRAPHS 25, 29 + 39

MANAGEMENT

HPC Standards of Proficiency Codes for Clinical Scientists	AREA OF COMPETENCE	INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED
-	• to understand the principles of management	PARAGRAPHS 4, 5 + 21
2c.2g 3a.3g 2c.2p	• to understand the principles of quality assurance, audit, safety and accreditation relevant to a specific discipline	PARAGRAPHS 7, 27, 30 + 32

Note:
The above are the generic competences that must be met by all Clinical Scientists. These competences have also been mapped onto specific subjects. Copies of these can be obtained from the ACS Administrative Office and the website.

Created: 16 August 2002
Modified: 1 July 2003
Reference: DOC-ACS002-Generic Competences
Version: Rev-02 – Codes related to HPC Standards of Proficiency documents (g=generic; p=profession specific)

Appendix 2: Abstract of HND thesis

"An Investigation of the Antimicrobial Sensitivity of Various *Bacillus* Species"

Section 1. Abstract.

The aim of this experiment was to assess the sensitivity of three species of Bacillus to three types of antibiotic, with a view to determining which species were suitable as sensitive indicator organisms in specific antibiotic assays. The method employed to assess the sensitivities of the micro-organisms was a series of microplate Minimum Inhibitory Concentration (MIC) assays. The three species of Bacillus used were *Bacillus megaterium*, *Bacillus pumilis* and *Bacillus stearothermophilus*; the three antibiotics used were Kanamycin, tetracycline and Rifampicin.

The results of this experiment showed that, of the antibiotics tested, *Bacillus pumilis* was most sensitive to Rifampicin with an MIC value of 1.008 μg ml^{-1}, *Bacillus megaterium* was most sensitive to both Tetracycline and Rifampicin with equal MIC values of 0.63 μg ml^{-1}, and *Bacillus stearothermophilus* was most sensitive to Tetracycline with an MIC value of 0.033 μg ml^{-1}. The experiment concluded that the most suitable indicator organism for Kanamycin is *Bacillus stearothermophilus* with an MIC value of 0.25 μg ml^{-1}, for Tetracycline is *Bacillus stearothermophilus* with an MIC value of 0.033 μg ml^{-1}, and for Rifampicin is *Bacillus megaterium* with an MIC value of 0.63 μg ml^{-1}. Further studies of the antibiotics tested should use the corresponding indicator organism.

Appendix 3: Abstract of BSc honours thesis

"The Antimicrobial Efficiencies of Facial Washes"

Section 1. Abstract.

The efficacy of two antibacterial facial wash products was compared using Minimum Inhibitory Concentration (MIC) assays against *Staphylococcus aureus* NCIMB 6571 and *Staphylococcus saprophyticus* NCIMB 8711. The facial wash products tested were a brand name Biactol Antibacterial Face Wash, active ingredient Phenoxyisopropanol (2% (w/v)), and an own brand Boots Mediclear Antibacterial Facial Skin Wash, active ingredient Triclosan (0.2 % (w/v)). The MIC ranges determined for Biactol were 7.24-3.62 mol ml^{-1} against *S. aureus*, and 10.86-7.24 mol ml^{-1} against *S. saprophyticus*. The MIC ranges determined for Boots Mediclear were 0.622-0.414 mol ml^{-1} against *S. aureus*, and 0.622-0.414 mol ml^{-1} against *S. saprophyticus*.

In addition, the efficacy of an antibacterial facial wash was compared to a non-antibacterial soap using Facial Swab assays to determine the reductions of microorganisms caused by use of these products, selecting for a mixture of the microbial flora found naturally on the skin and all Staphylococcal species present. The facial wash product tested was Boots Antibacterial Facial Skin Wash, and the soap tested was Imperial Leather non-antibacterial toilet soap. It was concluded by statistical analysis of the data that, although there is a difference between the reductions observed for facial wash and soap, it is not significant enough to purport the bactericidal effects of one product over the other.

Appendix 4: Abstract of PhD thesis

"Microbial Degradation of Textile Dyes to Safe End-Products"

Abstract

Discharges from textile dyehouses often include large amounts of reactive azo dyes. These are recalcitrant to conventional wastewater treatment processes thus resulting in coloured waterways. Azo dyes can be decolourised biologically in bioreactors via fission of the azo (-N=N-) bonds to produce aromatic amines, but these are known to be toxic and/or genotoxic. This investigation concentrates on the toxicity and genotoxicity produced by decolourisation of a simulated textile effluent (STE) containing the reactive azo dye C.I. Reactive Black 5 in its hydrolysed form (RBOH). Two bioreactors were used, a batch-fill type Sequencing Batch Reactor (SBR) and continuous flow type Hybrid Anaerobic Baffled Reactor (HABR), operated under both anaerobic and anaerobic-aerobic conditions. The microflora of each bioreactor was studied in order to elucidate the interrelations between toxicity and/or genotoxicity, decolourisation efficacy and the microbial population.

Initially, the rapid bacterial toxicity and genotoxicity assays chosen were validated for their suitability before applying them to assessment of the SBR and HABR. High levels of decolourisation were observed in both bioreactors under all conditions (86-94%). Under anaerobic conditions, high levels of toxicity were observed in both bioreactors (EC_{50} values 0.24-0.46% of original concentration), whilst the toxicity was only significantly decreased (EC_{50} 16.89-17.80%) after anaerobic-aerobic operation in the HABR due to mineralisation of aromatic amines. No significant genotoxicity was observed.

Microflora numbers and composition were assessed using Fluorescent *in situ* Hybridisation (FISH) and Denaturing Gradient Gel Electrophoresis (DGGE). It was concluded that both the SBR and HABR operated under anaerobic conditions could support a relatively diverse, thriving, stable bacterial and archaeal population, but that increasing bacterial diversity was encouraged by the compartmentalisation of the HABR. Operation under anaerobic-aerobic conditions showed greatest divergence and appearance of unique species adapted to the aerobic environment in the HABR, due to the different conditions that could be provided by a compartmentalised bioreactor. The predominant species identified by culturing and sequencing under both anaerobic and anaerobic-aerobic conditions were *Clostridium* and *Bacteroides* spp. in the SBR and *Staphylococcus* and *Bacteroides* spp. in the HABR. The archaeal population of the SBR was predominated by *Methanosaeta* sp. under anaerobic-aerobic conditions, and

Actinomadura sp. was only observed in aerated samples. Several bacterial species were found to be unique to the aerobic sections of the HABR, and some were identified as *Nocardia* and *Rhodococcus* spp..

Appendix 5: Abstract of EA/IWA Young Researchers Conference poster

“Microbial Degradation of Textile Dyes to Safe End-Products”

Abstract for Young Researchers Conference Poster

Discharges from textile dyehouses often include large amounts of reactive azo dyes. These are recalcitrant to conventional wastewater treatment processes and thus are passed to natural watercourses and result in coloured waterways. Various methods of decolourisation, using physico-chemical or biological treatments, have been proved to work in specialised decolourisation facilities. Decolourisation of azo dyes relies on the fission of the azo (-N=N-) bonds within the molecules, yielding various aromatic amines which are known to be toxic and/or genotoxic. This investigation concentrates on the toxicity and genotoxicity of decolourised reactive azo dyes and naphthol compounds, assessed using the rapid bacterial bioassays Microtox® Acute Toxicity (Azur Environmental Ltd.) and *E. coli* Differential Kill assays respectively. Decolourisation assays were undertaken *in vitro* on the representative reactive azo textile dye of known structure CI Reactive Black 5 in both parent and hydrolysed forms, as it is known that reactive dyes undergo hydrolysis during the dyeing process. The dyes were decolourised biologically using the known decolourising bacterium *Enterococcus faecalis*, a decolourising environmental isolate identified as *Clostridium butyricum*, and chemically using sodium dithionite. In addition, two types of small-scale bioreactor were run to assess their ability to decolourise Reactive Black and the toxic/genotoxic potentials of the resultant effluents. The first was a batch process using a sequential batch reactor (SBR) run in both anaerobic and anaerobic-aerobic states, and the second was a continuous process using an anaerobic baffled reactor (ABR) with anaerobic and aerobic zones.

It was found that hydrolysis of the dye resulted in an increase in the toxic potential and a slight increase in the genotoxic potential (parent Reactive Black toxic EC_{50} value 27.48 mgL^{-1} and genotoxic CS value 1.505, hydrolysed Reactive Black toxic EC_{50} value 11.40 mgL^{-1} and genotoxic CS value 0.682). Decolourisation of both forms of the dye, biologically and chemically, resulted in an increase in toxic potential but a slight decrease in genotoxic potential (hydrolysed Reactive Black decolourised by *E. faecalis* toxic EC_{50} value 0.222 mgL^{-1} and genotoxic CS value 1.268). It was found that the toxic component of hydrolysed Reactive Black is the vinyl sulphone fraction (EC_{50} value 0.32 mgL^{-1}), whilst the diamino H-acid fraction and its commercial equivalent H-acid had lower toxic potentials (EC_{50} values >2.18 and 48.63 mgL^{-1} respectively).

The SBR showed that, under anaerobic conditions, approximately 91% decolourisation of Reactive Black was achieved, and that the resultant effluent had increased toxic potential (EC_{50} value 0.242% of original concentration) but decreased genotoxic potential (CS value 1.008) compared to the undecolourised dye (toxic EC_{50} value 12.53% of original concentration, genotoxic CS value 0.161). A short aeration step (30 min) reduced the toxicity slightly (EC_{50} value 0.336% of original concentration), had a negligible effect on genotoxic potential (CS value 0.977) and increased the decolourisation ability (94 - 95%) compared to a completely anaerobic process.

The ABR showed that the effluent from a completely anaerobic process exhibited 85% decolourisation, an increased toxic potential (EC_{50} value 0.455% of original concentration) and a decreased genotoxic potential (CS value 0.853) compared to the undecolourised dye (toxic EC_{50} value 4.74% of original concentration, genotoxic CS value 0.472). Addition of a further single aerobic cell reduced the toxic potential (EC_{50} value 3.0% of original concentration), and slightly decreased the decolourisation ability

(84%) compared to a completely anaerobic process. Subsequent addition of two further aerobic cells further decreased the toxic potential (EC_{50} value 17.3% of original concentration), produced a slight increase in genotoxic potential (CS value 0.655) and increased the decolourisation ability (88%).

Appendix 6: Contents of chapter for book on wastewater decolourisation

"Advances in microbial removal of colour from textile industry waste water"

Advances in microbial removal of colour from textile industry waste water.

xxxxxxxx and xxxxxxxx
Department of Life Sciences
xxxxxxxxxxxx University
xxxxxxxx
xxxxxxxxxxxx
Tel : xxxxxxxx, Fax: xxxxxxxx
Email : xxxxxxxxxxxx
Homepage : xxxxxxxxxxxx

Structure

Structure 1
1. Origin of the problem 2
2. Textile dyes in the environment 4
2.1 Textile dyes as pollutants 4
2.2 Decolourisation methods overview 5
3. Biological dye removal processes 6
3.1 Adsorption to organic materials 6
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Appendix 7: Abstract of publication in the Journal of Biotechnology

"The toxicity of textile reactive azo dyes after hydrolysis and decolourisation"

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Journal of Biotechnology 101 (2003) 49–56

JOURNAL OF Biotechnology

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The toxicity of textile reactive azo dyes after hydrolysis and decolourisation

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Received 11 December 2001; received in revised form 10 September 2002; accepted 24 September 2002

Abstract

The toxicity of C.I. Reactive Black 5 and three Procion dyes, as found in textile effluents, was determined using the bioluminescent bacterium *Vibrio fischeri*. Hydrolysed Reactive Black had a slightly greater toxicity than the parent form (EC_{50} 11.4±3.68 and 27.5±4.01 mg l^{-1}, respectively). A baffled bioreactor with anaerobic and aerobic compartments was used to decolourise hydrolysed Reactive Black 5 in a synthetic effluent. Decolourisation of hydrolysed Reactive Black resulted in an increased toxicity (EC_{50} 0.2±0.03 mg l^{-1}). Toxicity was not detectable when decolourised Reactive Black 5 was metabolised under aerobic conditions. No genotoxicity was detected after the decolourisation of either the parent or the hydrolysed reactive dyes, either in vitro or in the bioreactor. The toxicity and genotoxicity of decolourised C.I. Acid Orange 7 was due to the production of 1-amino-2-naphthol (EC_{50} 0.1±0.03 mg l^{-1}).

Keywords: Textile; Azo; Dyes; Decolourisation; Toxicity; Genotoxicity

1. Introduction

Reactive azo dyes occur in textile dyehouse wastewater in concentrations ranging from 5 to 1500 mg l^{-1} due to their poor fixation to fabrics (Pierce, 1994). Moreover, reactive azo dyes are not degraded by conventional aerobic sewage treatment plants as they are resistant to biological oxidative degradation (Easton, 1995). Decolourisation can be achieved using either anaerobic digestion, microbial generation of oxygen radicals, i.e. laccase activity of white-rot fungi (Schliephake et al., 2000) or expensive physico-chemical treatments (O'Neill et al., 2000). Although reactive textile dyes can be decolourised under anaerobic conditions due to reduction of the azo bond, the resultant aromatic amines resist further degradation and may be toxic or genotoxic (Sweeney et al., 1994). Toxicity could be eliminated through bacterial fission of the aromatic ring structure, a process that requires oxygen. Therefore, secondary

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PII: S0168-1656(02)00302-4

Appendix 8: Letter of support to demonstrate training in Laboratory Safety

[redacted]
NHS Foundation Trust
Department of Infection
Floor 5 North Wing

[redacted]
Tel [redacted]
Fax [redacted]
E-mail: [redacted]

5 November 2004

Dear Sir/Madam,

[redacted]

I have assessed [redacted] depth of knowledge and understanding for her requirements for her state registration for the following criteria for "Laboratory Safety":

To be aware of and understand current legislation and recommendations for safe laboratory practice	For example: COSHH Regs, 2002; Antiterrorism, Crime and Security Act, 2001; Categorisation of pathogens (ACDP), 1995. Local fire policy.
Recognition of hazards and assessment of risk.	Biological, chemical, physical, electrical and ionising. Handling clinical material. Manual handling and working with display screen equipment.
Control measures	Local policies, training, codes of practices, local immunisation practice, lone working procedures, standard operating procedures. Working at containment levels 2 and 3. Use of safety equipment and PPE.
Waste management	Disinfection and sterilisation. Categorisation of waste and methods for safe disposal.

A comprehensive understanding is required by all scientific staff working in the department.

Yours sincerely,

[redacted]

[redacted]
Training Manager

Portfolio-041112-[redacted]-HS

Appendix 9: Letter of support to demonstrate training in Bacteriology

[redacted] Hospital Trust

[redacted] Hospital
[redacted]
Tel [redacted]

Microbiology
Department of Infection.
Floor 5 North Wing

ext 3245
Fax: [redacted]
Email: [redacted]

Date: 27th April 2005.

To Whom it may Concern

Re: Microbiology Training of [redacted]

This is confirm that [redacted] has undergone a comprehensive "Training Programme" in the Microbiology section of the Department of Infection at [redacted] NHS Trust. The training was undertaken by fully experienced state registered Biomedical Scientists and overseen by the Diagnostic Laboratory Manager.

The training programme covered in depth the following areas:

- The preparation and use of culture media within the laboratory with special reference to incubation conditions and requirements of different groups of bacteria.
- The processing of various types of specimen (e.g. wound swabs, respiratory specimens, blood cultures, urine and faeces)
- The isolation, recognition and identification (including the use of commercial identification kits) of organisms from various body sites and their clinical significance (especially from special patient categories).
- A thorough knowledge of the range and use of antibiotics available. The setting up of antimicrobial susceptibility testing by disc diffusion and automated techniques.
- An overview of serological assays (e.g. latex agglutination)
- The use of automated equipment in the diagnosis of TB, bacteriuria and bacteraemia.

Yours sincerely

[redacted]
Diagnostic Service Manager, Microbiology.
Department of Infection.

H:\[redacted]letter.doc [redacted] 27.04.05

Appendix 10: Letter of support to demonstrate training in Virology

[redacted] Hospital NHS
NHS Trust

[redacted] Hospital
[redacted]
[redacted]
Tel: [redacted]

25/4/05
To Whom It May Concern:

Dear Sir/Madam,

Dr. [redacted] spent four weeks in the Virology section of the Department of Infection, [redacted] during Nov 2004.

She was able to observe the performance of a wide variety of procedures.

Several serological techniques were included,
e.g. enzyme immunoassays performed both manually and on automatic analysers, particle agglutination and complement fixation.
Automatic analysers were either random-access or microtitre-plate based.
[redacted] had the opportunity to try out some of these techniques under the supervision of the BMS2 in that section.

[redacted] also watched cell-culture being carried out and virus isolation tests being set up. She was able to observe cytopathic effects of some viruses in cell culture tubes and was shown some of the follow-up identification tests, e.g. fluorescent antibody.
She was shown, and had the opportunity of carrying out, direct fluorescent antibody detection of respiratory viruses in respiratory secretions.

[redacted] is already a skilled molecular biologist but while in virology she saw the way in which molecular techniques are now part of routine diagnostic virology. E.g. daily PCR for herpes simplex in genital specimens and for HSV, VZV and enteroviruses in CSF.

Thus she saw a good selection of assays in use for modern diagnostic virology and she had the opportunity to try out some of the techniques
She was able to ask about problems peculiar to particular techniques and the rationale for using one form of technology rather than another.
Viruses of clinical importance to different patient groups were discussed. In each section of the laboratory she was able to discuss with a senior BMS the testing algorithm for the viruses under investigation and to understand why different patient groups might require different testing strategies.

I believe she gained an insight into the day to day running of a virology section.

Yours sincerely,

[redacted]
Diagnostic Service Manager, Virology,
Department of Infection,
[redacted] Foundation Trust.

Appendix 11: Conferences, courses and seminars attended

Conferences, courses and seminars attended during 2001

- European Meeting on Molecular Diagnostics
  Scheveningen, The Netherlands, October 2001
- King's College London Training & Development – Searching the Medline database
  King's College London, London, November 2001
- Staffordshire University meeting – PCR Applications in Biomedical Sciences
  Staffordshire University, Stoke-on-Trent, November 2001 (4 CPD points)
- Human Genome Mapping Project Resource Centre – Introduction to Bioinformatics
  Cambridgeshire, December 2001

Conferences, courses and seminars attended during 2002

- PHLS meeting – TB molecular diagnosis and DNA fingerprinting
  King's College Hospital (Dulwich), London, January 2002 (5 CPD points)
- Applied Biosystems meeting – Enabling Discovery: Genotyping in the Genomic Era
  Cheshire, March 2002
- Institute of Biomedical Science XXXX Region, Virology Discussion Group Scientific Meetings – The Definitive Laboratory Guide to EBV Testing
  St. Mary's Hospital, London, March 2002 (0.2 CPD points)
- XXXX Department of Infection seminar – BacT/ALERT 3D
  XXXXXXXX Hospital, London, March 2002 (0.1 CPD point)
- Beckman Coulter and Promega Genetic Analysis Seminar series
  Buckinghamshire, May 2002
- Pharmacia Investigator Support Initiative workshop
  London, May 2002
- XXXX Department of Infection Postgraduate Education programme – TB: Return of the White Plague
  XXXXXXXX Hospital, London, July 2002
- ACM Scientific Meeting – Applications of molecular technology
  CPHL Colindale, London, October 2002 (4 CPD points)
- artus GmBH 1st Hamburg PCR Symposium
  Hamburg, Germany, November 2002

Conferences, courses and seminars attended during 2003

- XXXX Training & Development – Introduction to Access
  XXXXXXXX Hospital, London, April 2003
- 2nd Nucleic Acid Quantification Meeting
  St. Bartholomew's Hospital, London, May 2003
- Roche Diagnostics Ltd. – Molecular Diagnostics User Group Meeting
  Hertfordshire, June 2003 (0.9 CPD points)
- XXXX Department of Infection Postgraduate Education programme – Mechanisms of cavitation in TB
  XXXXXXXX Hospital, London, July 2003
- Roche Diagnostics Ltd. – Amplicor Mycobacterium Tuberculosis Assay training

XXXXXXXX Hospital, London, July 2003

- XXXX Department of Infection seminar – Antibiotic Resistance Detection
  XXXXXXXX Hospital, London, November 2003 (1 CPD point)
- XXXX Department of Infection seminar – β-lactamases and β-lactam resistance
  XXXXXXXX Hospital, London, November 2003 (1 CPD point)

Conferences, courses and seminars attended during 2004

- XXXX Training & Development – So you want to be a Supervisor?
  XXXXXXXX Hospital, London, March 2004
- XXXX Training & Development – Advanced Presentation Skills
  XXXXXXXX Hospital, London, March 2004
- Institute of Biomedical Science (XXXX Region) Virology Discussion Group – Basic Virology course
  XXXXXXXX Hospital, London, September 2003 – March 2004 (36 CPD points)
- Oxoid Infection Control Seminar
  Warwickshire, June 2004 (7 CPD points)
- XXXX Training & Development – Introduction to Project Management
  XXXXXXXX Hospital, London, August 2004
- Roche Diagnostics Ltd. meeting – Molecular Technologies and MRSA
  XXXXXXXX Hospital, London, September 2004
- ACM Scientific Meeting – Hospital Acquired Infections
  CPHL Colindale, London, October 2004 (5 CPD points)

Conferences, courses and seminars attended during 2005

- XXXX Research & Development Department – Good Clinical Practice and the Medicines for Human Use (Clinical Trials) Regulations 2004
  XXXXXXXX Hospital, London, March 2005 (4 CPD points)
- XXXX Training & Development – Risk Assessment: Principles and Practice Certificate
  XXXXXXXX Hospital, London, April 2005
- Oxoid Infection Control Seminar Day (7 CPD points)
  Stratford-Upon-Avon, May 2005
- ACM Scientific Meeting – Vaccines and the Epidemiology of Infectious Disease
  CPHL Colindale, London, May 2005

Appendix 12: Letter of support to demonstrate training in Epidemiology

[redacted] Hospital NHS
NHS Trust

5th May 2005

[redacted] Hospital
[redacted]
[redacted]
Tel: [redacted]

Dear Sir/Madam

Re: Clinical Scientist State Registration training in the Epidemiology of Disease for [redacted]

I have conducted several tutorial sessions with Dr. [redacted] related to the epidemiological aspects of disease.

Topics covered in these sessions included:

1. The nature of reservoirs of infections
2. Types of infection (e.g. opportunistic, hospital-acquired)
3. Modes of transmission
4. The control of hospital infection (e.g. epidemiology, the role of the CDSC, significant organisms, disinfection/sterilisation, hospital hygiene)
5. The principles of surveillance and outbreak investigation

I can confirm that [redacted] has a good working knowledge of these topics, and has the ability to apply these processes to the clinical laboratory environment.

Yours faithfully

[redacted]

Professor [redacted]
Department of Infection

Appendix 13: Report of an evaluation of mycobacterial genotyping assays

“Proposal for internal speciation of mycobacteria using genotyping assays”

Proposal for internal speciation of mycobacteria using genotyping assays

Patient data analysis

Data for speciation of mycobacteria cultures from clinical specimens was analysed for the period 01.01.02-31.08.03. Parameters analysed included report destination and in/outpatient status, direct auramine result, quantity of growth, HIV status, mycobacterial species and time taken to achieve identification.

A total of 265 mycobacterial cultures from individual patients were outsourced for identification during the period analysed. Of these, initial cultures from 44 patients produced only light/scanty growth or a single colony, and therefore required subculture before outsourcing. The number of individual diagnoses (%) and their species were as follows: 17 (39) *M. tuberculosis*, 5 (11) *M. kansasii*, 5 (11) *M. chelonae*, 4 (9) MAI, 3 (7) *M. fortuitum*, 2 (5) *M. malmoense*, 2 (5) *M. xenopi* and 6 (14) unidentified environmental species. Only 7 of the patients with initial light/scanty cultures were known to be HIV positive, all of which were inpatients, with individual diagnoses comprising 3 MAI, 2 *M. tuberculosis*, 1 *M. kansasii* and 1 *M. xenopi*.

Table 1 shows details for the 44 patients with initial light/scanty cultures. The median time for speciation of these cultures was 4 weeks, with a range of 2-16 weeks. There were 27 inpatients in total, which were considered to be more time-critical for speciation and subsequent therapy, of which 7 were known HIV positive patients. The median time for speciation of cultures from inpatients (n = 27) was 1 week longer than that from outpatients (n = 17). Therefore, 27/265 (10%) patients with mycobacterial cultures outsourced for speciation during the period analysed would have benefited, in terms of time taken to achieve identification, from internal assessment using a rapid genotyping assay.

Genotyping assay kit information and speciation assay costs

Two genotyping assay kits have been evaluated in the Department of Infection, comprising INNO-LiPA Mycobacteria (Innogenetics, Belgium) and GenoType® Mycobacterium (Hain Lifescience, Germany). The main characteristics of the kits, including cost per test, assay time, inclusion of an amplification control and species identified by the assays, are shown in Table 2. The major factor in reducing identification time when using these kits is that tests can be performed on a single colony or equivalent in liquid culture, and so subculturing is not required. The most apparent differences between the kits are those of cost per test and species identified. At £33.83 per test, INNO-LiPA Mycobacteria is approximately three times the cost of GenoType® Mycobacterium. In addition, INNO-LiPA Mycobacteria does not identify *M. celatum*, *M. fortuitum*, *M. malmoense*, *M. peregrinum* or *M. phlei*, unlike GenoType® Mycobacterium. The 44 diagnoses comprising the patient data analysis included identifications of both *M. fortuitum* and *M. malmoense*. It should be noted that INNO-LiPA Mycobacteria V2 is now available at a cost of £700.00/20 tests (£35.00 per test), which includes probes for *M. fortuitum*, *M. malmoense*, *M. haemophilium*, *M. genavense*, *M. simiae*, *M. smegmatis* and *M. marinum*/*M. ulcerans* in addition those included in the original version. However, the V2 kit was not included in the evaluation.

The cost per test from outsourcing speciation of the mycobacterial cultures detailed in Table 1 is £50.00, although this cost includes both speciation and sensitivity testing. Outsourcing of mycobacterial speciation and sensitivity testing is currently through the XXXXXXXX Hospital, who are in the process of evaluating the INNO-LiPA Mycobacteria V2. Consideration of the implications of this on future Department of Infection mycobacterial speciation services, especially with regard to initial light/scanty cultures, should be made. Therefore, based upon time taken to achieve identification and cost per test, speciation of initial light/scanty cultures may be best achieved internally using a genotyping assay. Based upon applicability of the species included in

the assay and cost per test, the most appropriate genotyping assay for use currently in the Department of Infection is the GenoType® Mycobacterium.

Evaluation data for genotyping assay kits

The INNO-LiPA Mycobacteria and GenoType® Mycobacterium kits were evaluated prospectively on 8 cultures outsourced for identification. The INNO-LiPA Mycobacteria correctly identified 5/8 (63%) cultures. The remaining 3, identified as *M. fortuitum* (2) and *M. nonchromogenicum* (1) were not identified due the absence of probes to detect these species. The GenoType® Mycobacterium correctly identified 7/8 (88%) cultures, with the remaining culture (*M. nonchromogenicum*) not identified due the absence of probes for detection. It is of note that both kits identified one culture, assigned to the *M. avium*/*M. intracellulare* group by outsourcing, as *M. avium*. One culture identified as *M. fortuitum* by outsourcing was reported to be *M. peregrinum* by GenoType® Mycobacterium, which is similar to but phenotypically and genotypically distinct from *M. fortuitum*.

Therefore, based upon number of correct identifications, the most appropriate genotyping assay for use currently in the Department of Infection is the GenoType® Mycobacterium. The use of this assay would provide more definitive identifications for some species than those currently provided by outsourcing.

Table 1: Data for mycobacterial cultures outsourced for speciation producing only small amounts of growth.

Year	Report destination	Out/In patient	Lab. no.	Direct AFB[a]	Growth[b]	Medium	HIV status[c]	I.d.[d]	I.d. time (wks)[e]
2002	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	6
	XXXXX	In	XXXXX	+	Y	Kirschner	+	*M. tuberculosis*	4
	XXXXX	In	XXXXX	+	+	LJ	NK	*M. malmoense*	13
	XXXXX	In	XXXXX	-	+/-	LJ	+	MAI	4
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	3
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. kansaii*	2
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	3
	XXXXX	In	XXXXX	-	1 colony	LJ	NK	*M. fortuitum*	4
	XXXXX	Out	XXXXX	-	+	LJ	NK	*M. tuberculosis*	2
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. chelonae*	4
	XXXXX	In	XXXXX	-	1 colony	LJ	+	*M. kansaii*	5
	XXXXX	Out	XXXXX	-	Y	Kirschner	NK	*M. tuberculosis*	4
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. fortuitum*	4
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	5
	XXXXX	In	XXXXX	-	+/-	LJ	NK	MAI	5
	XXXXX	In	XXXXX	-	+/-	LJ	+	MAI	3
	XXXXX	In	XXXXX	-	1 colony	LJ	NK	*M. tuberculosis*	4
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	3
	XXXXX	In	XXXXX	-	+/-	LJ	+	MAI	3
	XXXXX	Out	XXXXX	-	+	LJ	NK	*M. xenopi*	5
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. kansaii*	3
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	3
	XXXXX	In	XXXXX	-	Y	MBact	+	*M. xenopi*	4
	XXXXX	Out	XXXXX	+	+/-	LJ	NK	*M. tuberculosis*	3
2003	XXXXX	Out	XXXXX	-	+/-	LJ	NK	Environmental	6
	XXXXX	In	XXXXX	-	Y	Kirschner	NK	*M. chelonae*	8
	XXXXX	In	XXXXX	-	+	LJ	NK	Environmental	8
	XXXXX	Out	XXXXX	-	+/-	Middlebrook	NK	*M. tuberculosis*	3
	XXXXX	In	XXXXX	-	1 colony	LJ	NK	*M. fortuitum*	5
	XXXXX	In	XXXXX	-	+/-	LJ	NK	Environmental	8
	XXXXX	Out	XXXXX	-	+/-	LJ	NK	*M. kansaii*	3
	XXXXX	In	XXXXX	-	1 colony	LJ	NK	Environmental	5
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. chelonae*	4
	XXXXX	In	XXXXX	-	+/-	LJ	NK	Environmental	6
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. kansaii*	4
	XXXXX	Out	XXXXX	-	1 colony	LJ	NK	*M. tuberculosis*	4
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	2
	XXXXX	Out	XXXXX	-	+	LJ	NK	*M. chelonae*	5
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	2
	XXXXX	In	XXXXX	-	+/-	LJ	NK	Environmental	6
	XXXXX	In	XXXXX	-	1 colony	LJ	+	*M. tuberculosis*	4
	XXXXX	Out	XXXXX	-	1 colony	LJ	NK	*M. chelonae*	4
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. malmoense*	16
	XXXXX	In	XXXXX	-	+/-	LJ	NK	*M. tuberculosis*	2

N.B. [a]- (no AFB per field), + (few AFB per field) [b]Y (growth in liquid medium) [c]NK (HIV status not known) [d]Environmental (unidentified *Mycobacterium* sp. not *M. tuberculosis*, *M. kansaii* or MAI) [e]time taken from initial growth of culture to receipt of identification [f]INNO-LiPA Rif TB performed to rule out *M. tuberculosis* [g]known MDRTB

Table 2: Main characteristics of INNO-LiPA Mycobacteria and GenoType® Mycobacterium genotyping kits

Characteristic	INNO-LiPA Mycobacteria	GenoType® Mycobacterium
Cost/kit (no. tests)[a]	£676.50 (20)	€186/£129.84 (12)
Cost/test[a]	£33.83	€15.50/£10.82
Species identified[b]	MTB, MK, MX, MG, MAIS, MA, MI, MS, MCH	MTB, MK, MX, MG, MA, MI, MS, MCH, MCE, MF, MM, MPE, MPH
Extraction provided	No	No
Amplification control	Yes	Yes
Assay time (h)[c]	5.5	5.5
'Hands-on' time (min)[d]	84	79

N.B. [a]GenoType® Mycobacterium costs based on exchange rate of £0.698/€ [b]MTB (*M. tuberculosis* complex), MK (*M. kansasii*), MX (*M. xenopi*), MG (*M. gordonae*), MAIS (*M. avium/M. intracellulare/M. scrofulaceum*, also reacts with *M. avium* complex, *M. malmoense* & *M. haemophilum*), MA (*M. avium*), MI (*M. intracellulare*), MS (*M. scrofulaceum*), MCH (*M. chelonae*), MCE (*M. celatum*), MF (*M. fortuitum*), MM (*M. malmoense*), MPE (*M. peregrinum*), MPH (*M. phlei*) [c]assay time based on INNO-LiPA Rif TB SOP extraction method [d]'hands-on' time excludes incubation procedures, etc.

Table 3: Evaluation data for INNO-LiPA Mycobacteria and GenoType® Mycobacterium genotyping kits

Lab. no.	Direct AFB[a]	Medium	I.d.	INNO-LiPA Mycobacteria	GenoType® Mycobacterium
XXXXX	-	LJ	*M. fortuitum*	Unidentified	*M. fortuitum*
XXXXX	+	LJ	*M. tuberculosis*	*M. tuberculosis*	*M. tuberculosis*
XXXXX	-	LJ	*M. nonchromogenicum*	Unidentified	Unidentified
XXXXX	-	LJ	*M. gordonae*	*M. gordonae*	*M. gordonae*
XXXXX	-	LJ	*M. fortuitum*	Unidentified	*M. peregrinum*[b]
XXXXX	-	MBact	*M. xenopi*	*M. xenopi*	*M. xenopi*
XXXXX	++	LJ	*M. kansasii*	*M. kansasii*	*M. kansasii*
XXXXX	+++	LJ	MAI[c]	*M. avium*	*M. avium*

N.B. [a]- (no AFB per field), + (few AFB per field), ++ (moderate no. AFB per field), +++ (large no. AFB per field) [b]*M. peregrinum* is similar to but phenotypically and genotypically distinct from *M. fortuitum* [c]MAI (*M. avium*/*M. intracellulare*)

Appendix 14: Report of an evaluation of a real-time PCR assay for MTB

"Proposal for replacement of Amplicor with RealArt for molecular detection of *Mycobacterium tuberculosis* from specimens."

Proposal for replacement of Amplicor with RealArt for molecular detection of *Mycobacterium tuberculosis* from specimens.

Introduction

Many molecular diagnostic methods require a nucleic acid amplification step. Most additionally require a further post-amplification detection step e.g. agarose gel or hybridisation. Real-time assays combine amplification and detection and so require no post-amplification steps, and are consequently faster than many other molecular assays.
Real-time assays can use specific primers for amplification, and have the advantage of increased specificity also found with hybridisation via the use of a short dual-labelled probe. This probe is fluorigenic, and the amount of fluorescence in an amplification reaction is directly proportional to the amount of copied DNA. The instrument used measures the amount of fluorescence after each amplification cycle, and so DNA levels in the reactions are given in 'real time'.
The front-line molecular assay currently employed in the Department of Infection for detection of *M. tuberculosis* DNA from specimens is the Amplicor® *M. tuberculosis* (MTB) Test (Amplicor), which uses hybridisation as a post-amplification detection step. It is proposed that this assay be replaced with a real-time test, the RealArt™ *M. tuberculosis* TM PCR (RealArt).

Health & Safety considerations

The Amplicor assay requires extraction of DNA from clinical material, which takes place in Containment Level 3 (CL3). Specimens are heated to 60°C for 45 minutes. Amplification is also conducted in CL3, during which time both the extracts and the outside of the amplification tubes are heated to 94°C for 20 seconds a total of 43 times.
The RealArt assay also requires extraction of DNA from clinical material in CL3, during which specimens are heated to 95°C for 15 minutes. Studies on the viability of heat-treated *M. tuberculosis* cultures have shown that they are killed by heating to either 80°C for 20 minutes (Doig 2002) or 100°C for at least 5 minutes (Bemer-Melchior 1999). Amplification is conducted in the post-PCR Molecular Diagnostics Laboratory. To ensure that no *M. tuberculosis* cells become attached to the outside of the amplification tubes, tubes are kept in a sealed plastic bag whilst inside the cabinet, which is disposed of immediately prior to leaving CL3, and handled using clean gloves.

Results

Eighteen auramine smear positive and 1 smear negative clinical specimens were tested in parallel using the Amplicor and RealArt assays. Sixteen (84%) were respiratory specimens, 1 (5%) was an axilla fluid, 1 (5%) was a FNA and 1 (5%) was a pus.
Of the Amplicor assays, 12 (63%) were positive for *M. tuberculosis* DNA, 2 (11%) were negative and 5 (26%) assays were inhibited and excluded from analysis. This compares with inhibition of 4 of the previous 20 (20%) assays. It should be noted that 4/7 (57%) of the runs conducted during this evaluation were invalid due to failure of the positive control.
Of the RealArt assays, 16 (84%) were positive for *M. tuberculosis* DNA and 3 (16%) were negative. No inhibition was observed, which compares with the 0% inhibition rate from 30 sputum, BAL and bronchial secretion specimens shown in the RealArt kit insert (Artus 2003). None of the positive or negative controls in the 6 runs conducted during this evaluation failed.
Of the 19 specimens, 14 (74%) grew *M. tuberculosis*, 1 (5%) grew *M. chelonae* and 4 (21%) produced no growth in either solid or liquid culture.

Table 1 details the results of the Amplicor and RealArt assays along with mycobacterial growth for each specimen. Using the Amplicor result as the reference, the RealArt assay gave the correct result with 14/14 (100%) specimens. In cases where Amplicor was inhibited, the RealArt assay gave the correct result with 5/5 (100%) specimens using culture as the reference. This gives the RealArt

assay a sensitivity and specificity of 100% in this evaluation. There were 4 specimens from which no mycobacterial growth was observed, one that was positive by both Amplicor and RealArt, and one that was positive by RealArt and inhibited in the Amplicor assay. Both of these specimens were smear positive.

Table 1 Results of the Amplicor and RealArt assays for 19 clinical specimens

Lab. no.	Specimen type	Smear[a]	Amplicor result[b]	RealArt result	Growth
XXXXX	Sputum	+	-	-	*M. chelonae*
XXXXX	Sputum	++	+	+	*M. tuberculosis*
XXXXX	Sputum	+	+	+	No growth (>wk 12)
XXXXX	Sputum	+	+	+	*M. tuberculosis*
XXXXX	Sputum	+++	+	+	*M. tuberculosis*
XXXXX	Sputum	+	+	+	*M. tuberculosis*
XXXXX	Sputum	+	INH	+	*M. tuberculosis*
XXXXX	Sputum	+	+	+	*M. tuberculosis*
XXXXX	BAL	-	INH	-	Culture ongoing
XXXXX	Sputum	+	-	-	Culture ongoing
XXXXX	Sputum	+	+	+	*M. tuberculosis*
XXXXX	Axilla fluid	++	INH	+	*M. tuberculosis*
XXXXX	Lymph FNA	+	+	+	*M. tuberculosis*
XXXXX	Sputum	+	+	+	Culture ongoing
XXXXX	Sputum	++	+	+	*M. tuberculosis*
XXXXX	Sputum	+	+	+	*M. tuberculosis*
XXXXX	Pus	+	INH	+	*M. tuberculosis*
XXXXX	Sputum	++	INH	+	*M. tuberculosis*
XXXXX	Sputum	++	+	+	*M. tuberculosis*

[a]No. of AFB per field (-, <1; +, 1-9; ++, 10-99; +++, ≥100)
[b]INH, inhibited

Key comparisons, benefits and disadvantages

Table 2 details the comparisons between key parameters of the Amplicor and RealArt assays. There are a number benefits and disadvantages to replacement of Amplicor with RealArt, which are summarised below.

Benefits

- Reduction of total assay time

No post-amplification detection steps result in decreased assay time and a larger block of available time post-extraction.

- Fewer kits required

Only 2 kits are required for the RealArt assay as opposed to the 5 kits required by the Amplicor assay, which is logistically easier to manage and reduces delivery charges.

- Reduction of reagent costs

Mainly due to the requirement for fewer kits, the cost of the RealArt assay is almost £20 less per specimen.

- No observed run failures

Amplicor runs are often invalidated due to failure of the positive control (57% during this evaluation alone). No invalid runs were observed during this evaluation with the RealArt assay.

- No observed inhibition

Due to a more labour-intensive DNA extraction procedure, no inhibition was observed during this evaluation with the RealArt assay. Amplicor assays are inhibited ~20% of the time. Inhibited specimens require a clean-up procedure and repeat of the assay, which increases turn-around time and costs.

Disadvantages

- Increase in 'hands-on' time

Due to a more labour-intensive DNA extraction procedure, 'hands-on' time is increased by 17 minutes.

- Decrease in analytical sensitivity

The sensitivity of the RealArt assay is 2-fold lower than that of the Amplicor assay, although these assays are respectively 10- and 25-fold more sensitive than auramine smears.

Table 2 Comparison of key parameters of the Amplicor and RealArt assays

Parameter	Amplicor	RealArt
Cost per specimen (inc. controls)	£74.30	£56.62
CE marked kits	Yes	Yes
Validated specimen types	Sputum, BRW, BAL	Sputum, BAL, stomach fluid
Positive/negative/inhibition controls included?	Yes	Yes
Total assay time (h)	6.5	5
'Hands-on' time (min)	61	78
Analytical sensitivity (copies/reaction)	5	10

Conclusion

Based upon culture and Amplicor data, the RealArt assay achieved 100% sensitivity and specificity in this evaluation. RealArt outperformed Amplicor in terms of inhibition of specimens and failure of runs. Decreased assay time and reagent costs mean that the RealArt assay will serve as an excellent replacement as the front-line molecular assay for the detection of *M. tuberculosis* DNA from specimens.

References

Bemer-Melchior P, Drugeon HB. Inactivation of Mycobacterium tuberculosis for DNA typing analysis. *J Clin Microbiol* 1999;**37**:2350-1.

Doig C, Seagar AL, Watt B, *et al*. The efficacy of the heat killing of Mycobacterium tuberculosis. *J Clin Pathol* 2002;**55**:778-9.

Artus. RealArt™ *M. tuberculosis* TM PCR reagents: Quantitative in vitro diagnostics for use with the ABI PRISM® Sequence Detection Systems (Applied Biosystems). *User Manual* 2003 October.

Appendix 15: Report of a comparison of culture and a molecular assay for GC

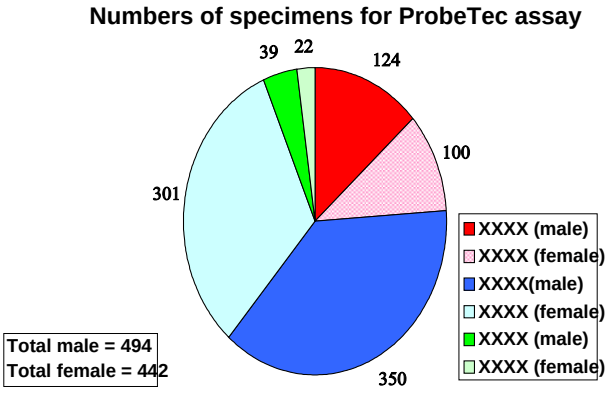
"Comparison of BDProbeTec ET with culture for detection of *Neisseria gonorrhoeae*"

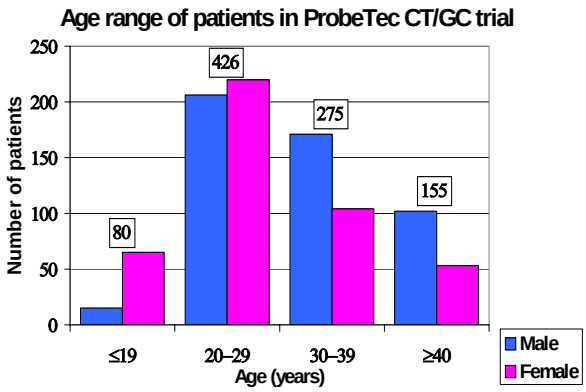
Comparison of BDProbeTec ET with culture for detection of *Neisseria gonorrhoeae*

Summary of trial data (Sep 2002 – Jan 2003)

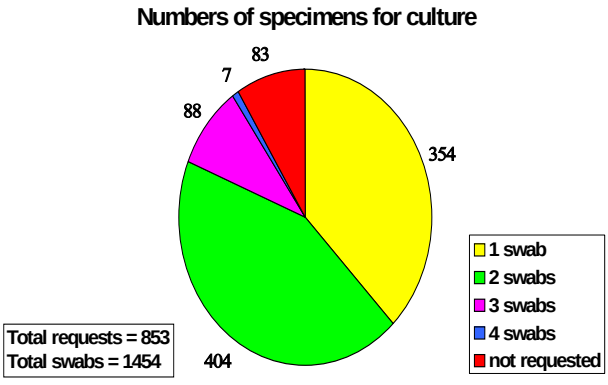
Demographics

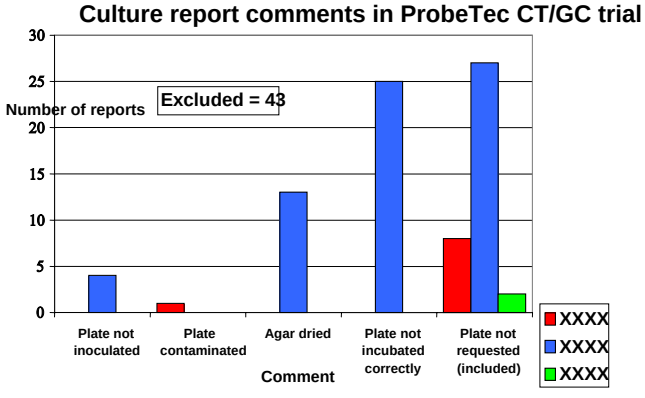
The ProbeTec™ ET (PT) combined assay for simultaneous detection of *C. trachomatis* (CT) and *N. gonorrhoeae* (GC) was used to compare to isolation of GC by culture. Of 494 male specimens tested on the PT, 489 (99.0%) were urine and 5 (1.0%) were urethral swabs, whilst of 442 female specimens, 24, 3 and 415 (5.4, 0.7 and 93.9%) were urine, urethral and cervical swabs respectively. The number of specimens from males and females from different clinics and the age range of the patients are shown in the figures below.





The number of urethral, cervical, rectal or throat swab specimens corresponding to the PT specimens requested for culture and the report comments on exclusion of culture data are shown in the figures below.





Of 936 total PT assays, 13 (1.4%) were excluded due to inhibition. Of 853 total culture requests, 43 (5.0%) were excluded due to culture problems mentioned in the report comments. After exclusion of both PT and culture data, there were 798 PT assays which had corresponding culture data.

Results

Of 923 PT assays, 76 (8.2%) were positive for CT only, 33 (3.6%) were positive for GC only and 15 (1.6%) were positive for both (a total of 91 (9.9%) CT positive and 48 (5.2%) GC positive). Of 810 culture requests, 44 (5.4%) isolated GC from one or more swabs. The amount of GC growth isolated from different swab samples and the specimen types with positive PT assays for GC are shown in the tables below.

Swab type	Amount of GC growth				**Positive/ total (%)**
	Scanty	Light	Moderate	Heavy	
Urethral	3	3	11	16	**33/850 (3.9)**
Cervical	1	3	4	6	**14/396 (3.5)**
Rectal	0	0	3	3	**6/95 (6.3)**
Throat	0	0	1	0	**1/113 (0.9)**

Specimen type	Gender		**Positive/ total (%)**
	Male	Female	
Urine	30/489	0/24	**30/513 (5.9)**
Urethral	0/5	0/3	**0/8 (0.0)**
Cervical	-	18/415	**18/415 (4.3)**

Of the 54 swabs that isolated GC, 1 (1.9%) was resistant to Penicillin and 4 (7.4%) were resistant to Ciprofloxacin. Of the PT specimens positive for GC, no other specimens were taken from the same patients at the time of testing. The tables below summarise the comparative data after resolution of discrepancies, and show the sensitivity, specificity, positive and negative predictive values in comparison to other studies identifying GC by PT and culture (Lovchik 1999, Akduman 2002).

	Culture +	Culture -	Total
PT +	38	5*	43
PT -	1	754	755
Total	39	759	**798**

* 1 of 5 assays showed weak positive

Sensitivity (%)	**97.4**	88.8-100
Specificity (%)	**99.3**	97.5-99.3
PPV	**0.88**	0.80-0.91
NPV	**1.00**	0.99-1.00

The 1 culture +/PT - specimen, a cervical swab, was from a patient with clinical presentations of GC. Of the 5 PT +/culture - specimens, 2 were cervical swabs from patients that did not have clinical presentations of GC and 2 were urines from male patients with clinical presentations of GC. The remaining specimen, which gave the weak positive response, was urine from a male patient who did not have clinical presentations of GC but had been diagnosed as GC positive previously. Taking the clinical picture into account, it appears likely that the false negativity rate of the PT is 1/755 (0.1%), whilst the false positivity is 3/43 (7.0%). The likely false positivity rate compares with other studies identifying GC by PT and culture (9.1-20.0%, Lovchik 1999; 15.1%, Akduman 2002), whilst the actual rate would need to be established after confirmatory testing.

Conclusions

- The sensitivity and specificity of the PT compare well with culture, with similar values shown in this study to other studies.
- All PT GC positive specimens should undergo confirmatory testing, possibly by both PT and culture.
- Further studies to assess the suitability of the specimen type assayed would need to be undertaken.

Appendix 16: Abstract of submission to the Journal of Clinical Pathology

"Evaluation of the BDProbeTec™ CT/NG combined assay for detection of *Neisseria gonorrhoeae* in urine and genital swab specimens"

Abstract

Aims. The performance of the BDProbeTec™ ET CT/NG Amplified DNA Assay (BDPT CT/NG assay), used for simultaneous detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG), was evaluated as a screening test for NG in first void urine (FVU) and swab specimens with comparison to culture.

Methods. The BDPT CT/NG assay was performed on 936 FVU and swab specimens. Culture for NG was performed on 853 sets of cervical, urethral, rectal and/or throat swabs. BDPT CT/NG data were compared with culture for 798 matched specimens; discrepancy analysis was performed by review of patient clinical information.

Results. The prevalence of NG infection in matched specimens detected by culture was 4.9%, and by BDPT CT/NG was 5.4%, either alone (3.9%) or as a coinfection with CT (1.5%). After resolution of discrepant data, the sensitivity, specificity and negative predictive values of the BDPT CT/NG assay for NG were 97.6, 99.6 and 99.9% respectively. The positive predictive value was higher when patient clinical information was taken into account (93.0%) than when it was not (88.4%). The false positivity rate was 7.0% (3/43).

Conclusions. The BDPT CT/NG assay is a highly sensitive and specific screening test for NG. However, because of the relatively low positive predictive value, positive results should be confirmed by retesting, preferably using a second test targeted at a different gene, and both culture data and clinical information should be taken into consideration.

Appendix 17: Case study – Identification of *Francisella tularensis* from blood culture

Identification of an unknown bacteraemia organism by sequencing of the 16S and 23S rRNA genes.

Case History

A 36-year old white female was admitted on 30th November 2003. She had been transferred from Belize where she had been rafting through caves inhabited by bats, and had trailed her hands in the water. She presented with fever, cough and a lesion on the right index finger that had developed shortly after rafting. She had been feeling sporadically unwell for 3 months, with 2 episodes of productive cough that had resolved with antibiotics, but no sweats or rigors. She had lived in Zambia for 13 years prior to August 2003, with a single sexual partner for 10 years and no previous illnesses or blood transfusions.

Laboratory Investigations at XXXXXXX Hospital

On admission, a full blood count showed severe lymphopaenia, with a CD4:CD8 ratio of 0.05, reduced haemoglobin and raised C-reactive protein (CRP). A chest X-ray showed a cavitating lesion with associated apical consolidation in the right apex of the lung, indicative of old tuberculosis infection. Herpes Simplex Virus (HSV) was detected in the finger lesion by electron microscopy, later confirmed by using Polymerase Chain Reaction (PCR) amplification.

On 4th December (day 5), a heavy growth of flucloxacillin sensitive *Staphylococcus aureus* was isolated from the finger lesion. Auramine staining of the sputum for acid-fast bacilli (AFB) was negative. *Candida albicans* was isolated from a high vaginal swab specimen. The patient was started on oral flucloxacillin, aciclovir and fluconazole.

On 8th December (day 9), virological serology showed that the patient was positive for Human Immunodeficiency Virus (HIV) antibodies.

On 13th December (day 14) 1 set of 8 blood culture bottles grew an unidentified Gram negative bacillus (GNB). A bone marrow specimen was negative for AFBs.

On 14th December (day 15), the patient's fevers persisted she became increasingly short of breath. A further 3 sets of blood culture bottles showed no growth, and 3 blood samples for mycobacterial culture showed no growth. The patient was started on septrin.

On 19th December (day 20), an induced sputum was seen to be positive for Candida hyphae and *Pneumocystis carinii* (PCP) by immunofluorescence, but negative for AFB by Ziehl-Neelsen staining.

On 21st December (day 22) the patient's fevers persisted. Tests showed a very high CRP of 206 and a HIV viral load of >500,000. There was no response to septrin, and so this was stopped.

On 24th December (day 25) the patient was admitted to the ICU, where she died. Post mortem tests on both the mediastinal lymph nodes and thyroid gland showed the

presence of GNB and PCP, and *Mycobacterium avium-intracellulare* and a fluconazole-resistant *Candida* sp. were eventually isolated. Two sets of 4 blood culture bottles grew a GNB after 3 days, which was an oxidase positive, nitrate positive, non-fermenter. This was tentatively identified by biochemical testing (API) as *Pasteurella haemolytica*, and was sensitive to gentamicin but resistant to septrin. The isolate was sent to the XXXXXXXXXXXX at XXXXXXXXXXX.

Identification by Sequencing at XXXXXXX Hospital

On 14th January 2004, I amplified and sequenced the 23S rRNA gene of the GNB isolate using primers matching highly conserved regions at the ends of the gene. Consensus matching of the sequence to the public access databases at the National Centre for Biotechnology Information (including GenBank, EMBL, DDBJ and PDB) using the Basic Local Alignment Search Tool (BLAST) algorithm showed that the best match (98% homology) was to a non-speciated *Francisella* sp. described as a newly recognised 'Francisella-like organism' (strain CYH-2002). Matches showing 96% homology were also observed to *F. tularensis* subsp. *novicida* and *F. tularensis* subsp. *holoarctica*, although these alignments showed a change in 6 successive nucleotides that were not consistent with any variant of *F. tularensis* on the public sequence databases. I have submitted the 23S rDNA sequence produced from this isolate to GenBank (Accession Number DQ054380). It was suggested that the organism may have been *F. philomiragia*, as it was oxidase positive.

Upon searching the databases, I found that there were no 23S rRNA sequences available for *F. philomiragia*, but that the 16S rRNA gene had been sequenced. After a literature search, I concluded that there were 2 sets of primers that were the optimal candidates for identification of unknown organisms by amplification of the 16S rRNA gene. These primers matched areas of the 16S rRNA gene with high sequence conservation bracketing regions of high sequence variation, and had been successfully used to identify unknown organisms in medical microbiology by sequencing or probing for a number of years. I developed and optimised the amplification protocol for these primer sets.

On 24th March 2004, I amplified and sequenced the 16S rRNA gene of the GNB isolate using both sets of primers. Consensus matching showed that the best matches (99% homology) were to *F. tularensis* and the subspecies variant *novicida*. Matches showing only 95% homology were seen with the type strain of *F. philomiragia* (ATCC 25015) and *Francisella* sp. strain CYH-2002 observed with 23S rRNA matches. I have submitted the 23S rDNA sequence produced from this isolate to GenBank (Accession Number DQ054381). It was concluded that the organism may have been a variant of *F. tularensis* and not *F. philomiragia*.

Confirmatory Identification Tests at Reference Laboratories and Other Centres

On 26th January, a report was received from HPA XXXXXX detailing tests that had been conducted to identify the isolate. Analysis of the Free Fatty Acids by Gas Liquid Chromatography identified the organism as *F. tularensis*. On repeat, the oxidase assay was shown to be very weak and therefore was considered negative. Sequencing of the 16S rRNA gene showed a match (99% homology) to *F. tularensis* subsp. *holoarctica*, and a *F. tularensis*-specific PCR performed at the XXXXXXXXXXXXXXXXXX

at HPA XXXXXXX was positive. It was concluded by HPA XXXXXXX that the isolate was *F. tularensis.*

The isolate was referred by HPA XXXXXXX to the XXXXXXXXXX in Germany, where culturing, biochemical, immunological and molecular biology assays, including sequencing of the 16S and 23S rRNA genes, were performed to identify the organism. Based upon the results of all of these assays, it was concluded that the isolate was a *Francisella* sp. that could not be assigned to any known species or subspecies, but that it was phylogenetically positioned between *F. tularensis* subsp. *holoarctica* and *F. tularensis* subsp. *novicida*.

Appendix 18: Qualification certificates

[redacted] UNIVERSITY OF [redacted]

[redacted]

has been awarded the

Higher National Diploma

in

Science (Applied Biology)

18th July 1995

[redacted]

[redacted]

Vice-Chancellor

BTEC

001/9334949/20161

[redacted] UNIVERSITY OF [redacted]

[redacted]

has been awarded the degree of

Bachelor of Science

with Second Class Honours (1st Division)

in

Biological Sciences

15th July 1997

[redacted]

[redacted]

Vice-Chancellor

001/9334949/44528

[redacted] University

[redacted]

has been awarded the degree of

DOCTOR OF PHILOSOPHY

in recognition of a programme of work entitled

'Microbial Degradation of Textile Dyes to Safe End-Products'

November 2003

[redacted]

Vice-Chancellor

[redacted]

[redacted] HA706690