



## Department of Anatomical Pathology

*Head:* **Dr Melanie Louw**

### Diagnostic services

The department provides a comprehensive diagnostic histopathology service to the Steve Biko Academic Hospital, as well as Tshwane District Hospital, the Kalafong Hospital and all the Tshwane Metro clinics. In addition, the service was extended to the State hospitals and clinics in Mpumalanga, portions of the Limpopo province and North West province.

As a referral centre, the department offers a consultation service to private pathology laboratories in Pretoria, Polokwane laboratories, and 1 Military Hospital. Technical support is provided to the Department of Forensic Pathology, University of Pretoria, and the veterinary service of the Pretoria Zoo.

More than 23,000 histology cases and 38,000 cytopathology cases are seen annually. Since participation in the national Department of Health's screening programme for cervical carcinoma, the number of cervical cytopathology smears increased dramatically. During 2008/09 the cytology department extended their services which include the focal point automated screening facilities. The number of autopsies performed during the review period remained constant.

There were at least three outreach programmes to Mpumalanga and the Limpopo provinces where training sessions were offered in cytopathology. One outreach programme was held with Momentum Life claim assessors.

### Research projects

#### Gastric carcinoma

*Researchers* Dr M Louw, Prof L Dreyer, Prof SW van der Merwe, Prof EJ van Rensburg

This is an ongoing collaboration between the gastroenterology unit and the Department of Human Genetics of the University of Pretoria and the Vreij University of Amsterdam, The Netherlands. The study evaluates the genetic aspects of a low and high risk gastric carcinoma population.

#### Osteosarcomas

*Researchers:* Dr M Louw, Prof SC Boy

Expression of CD31 and hepatocyte growth factor in osteosarcomas was studied. Results are being finalised.

#### Placental pathology

*Researchers:* Dr M Kgoebane-Maseko, Dr C Campaini

In this ongoing collaboration with the Department of Gynaecology and Obstetrics, University of Pretoria, the pathological features of placentas of patients with amniotic fluid infection syndrome are being evaluated.

#### Cervical carcinomas

*Researchers:* Prof L Dreyer, Dr JE Dinkel, Dr GH Davel, Dr M Louw, Dr C Campaini, Dr P Bloem

This ongoing study into cervical carcinomas is conducted in collaboration with the Department of Gynaecology and Obstetrics, University of Pretoria.

## Teaching and training

### Undergraduate

The department is responsible for the teaching of anatomical pathology to approximately 1,300 candidates, including students in medicine, dentistry, physiotherapy, occupational therapy and radiography. Contact time with students is in excess of 200 hours per annum including lectures, autopsy seminars and practicals.

### Postgraduate

There are currently seven registrars in Anatomical Pathology, which includes one registrar from Forensic Pathology and one registrar from Oral Pathology.

Pathology forms an integral part of the study for most of the MMed disciplines in the Faculty of Medicine. As part of the training in pathology the department offers approximately 200 interdepartmental discussions on a wide range of topics throughout the year. Fifty-nine MMed candidates in 17 different disciplines were examined in anatomical pathology as part of their MMed studies during the reporting period.

Training of medical technologists also takes place in the department.

## Department of Chemical Pathology

*Acting head:* **Dr Nicky Oosthuizen**

### Diagnostic services

For the period under review, referrals from other NHLS laboratories averaged 3,500 tests per month and total tests processed amounted to approximately 980,000. Two new automated chemistry and immunochemistry systems - Dx C880i analysers - were installed. The planned transfer of all automated testing from the six analysers in operation to only two analysers, represents significant consolidation with its associated benefits. Method validation commenced in September 2008 and will be completed early in the new financial year. Once as the transfer of tests is complete, automation of pre-analytical processes will commence with the installation of the Automate 800. Due to discontinuation of the CZE 2000 and Paragon gel electrophoresis product lines, all electrophoretic methods were transferred to the Sebia Hydrasys semi-automated gel electrophoresis system. The outcome of the annual SANAS accreditation inspection in November was retention of current accreditation status with relatively few non-conformances.

### Research projects

#### **Validation of the Accutrend Plus Lactate meter**

*Researchers:* Dr NM Oosthuizen, Dr M Funk

*Funding:* University of Pretoria, Dr M Funk

The aim is to evaluate analytical performance of the Accutrend Plus Lactate meter in order to decide on its medical acceptability as a point-of-care testing device in settings such as HIV clinics and critical care units. Performance characteristics such as precision, analytical range and accuracy will be assessed by performing linearity, replication, method comparison and interference experiments. Blood samples will be obtained from laboratory personnel, hospital patients in critical care and marathon runners.

#### **Molecular genetic analysis of pseudohypoparathyroidism using PCR-based techniques**

*Researchers:* Dr NM Oosthuizen, Dr M Bester, Prof L van Rensburg, Miss P Yu

*Funding:* University of Pretoria

The aim of the study is to characterise genetic/epigenetic changes in the *GNAS* gene of a patient with clinical features of pseudohypoparathyroidism type 1b. PCR-based techniques using methylation-sensitive restriction enzymes and bisulfite deamination described in the literature, will be employed. If testing yields evidence of familial disease, family members will be screened using the same methods.

## Teaching and training

### Undergraduate

The department is involved in the MBChB undergraduate programme, presenting lectures to and examining students in homeostasis, abdomen and breast, disorders of childhood, genito-urinary tract conditions, traumatology and special activity blocks. The latter block comprises diagnostic laboratory medicine, imaging and evidence-based medicine modules for MBChB VI students.

### Postgraduate

The department participates in teaching and assessment of MMed students in family medicine, internal medicine, neurology, paediatrics, anaesthesiology, and nuclear medicine. Three chemical pathology registrars were

successful in examinations during 2008 (two in the final examination and one in the intermediary exam). Two clinical pathology registrars passed their part 1 examinations in October 2008. Three new clinical pathology registrars are currently doing their chemical pathology rotations. The department applied for and attained full accreditation status from the HPCSA for medical scientist intern training.

### Research output

Published publications: 4  
Conference presentations:  
    International: 1  
    National: 3

### Professional development

Postgraduate candidates graduated: 4 (1 MMed (Chemical Pathology), 3 MSc (all cum laude))  
Postgraduate candidates enrolled: 16 (5 BSc Hons; 7 MSc; 4 MMed (Chemical Pathology))

## Department of Haematology

*Head: Prof Roger Pool*

### Diagnostic services

The Department of Haematology renders tertiary laboratory and clinical services to the Steve Biko Academic Hospital, Tshwane District Hospital, Kalafong Hospital and surrounding clinics. Clinical services are organised under the umbrella of the departments of Internal Medicine and Paediatrics while laboratory services fall within the ambit of the Tshwane Academic Division of the NHLS. Laboratory services are offered from two separate sites viz. the Steve Biko Academic Hospital core laboratory and the Prinshof campus.

### Core laboratory

All tests with a rapid turnaround time which have an immediate impact on patient management are, performed at this site. In haematology, these consist mainly of full blood counts and coagulation profiles. Registrars from the department are always available in the core laboratory both to assist with the interpretation of results and to provide advice to clinicians regarding the work-up of patients with haematological disease. During the period under review, a new automated cell counter with automatic slide staining capabilities was acquired.

### Prinshof campus

PFA-100® was offered as a routine test primarily for the screening of von Willebrand disease. A high performance liquid chromatography instrument for the analysis of haemoglobin has been validated and will be employed as an initial screening tool for the evaluation of haemoglobinopathies. The laboratory will acquire a new platelet aggregometer which is expected to enhance the ability to investigate and diagnose a wide range of platelet function abnormalities. The building of a molecular laboratory was completed and most of the instrumentation required to perform a range of molecular pathology-based tests was acquired. The laboratory continued to participate successfully in the quality assurance programmes of the NHLS, Royal College of Pathologists of Australasia and UKNEQAS. The laboratory maintained its SANAS accreditation following a surveillance inspection in November 2008.

### Flow cytometry

This service is offered jointly with the Department of Immunology. Specimens are received from the Steve Biko Academic Hospital, Tshwane District Hospital, Kalafong Hospital as well as from laboratories in North West Province, Mpumalanga and Limpopo. The flow cytometry laboratory expanded with training being offered to consultants from other universities, to haematology and clinical pathology registrars from the University of Pretoria as well as to students doing their BSc(Hons) degrees.

### Clinical services

The adult haematology clinic continued to grow and provide a consultation service both to the Steve Biko Academic Hospital and to the wider medical community of Pretoria and the surrounding areas. The number of patients attending this clinic has increased to the extent that two clinics are now run in parallel on the same day allowing double the number of patients to be seen. A computer database of clinic patients has been compiled in order to facilitate patient follow-up as well as to make the extraction of statistical data easier. The clinic is in the process of writing up investigation, diagnosis and management protocols for all of the conditions seen. The clinic also serves as an important vehicle for the teaching of undergraduate medical students. The most common conditions seen in the clinic (excluding haematological malignancy) are immune thrombocytopenic purpura, aplastic anaemia, myelodysplasia, myeloproliferative disorders and nutritional deficiencies. Members of the department also provide valuable input into the paediatric haematology clinic.

### **Haemophilia clinic**

A new computer database was established for the haemophilia clinic, making follow-up of patients and extraction of statistical information much easier. The department, together with the departments of Orthopaedics, Paediatrics and Physiotherapy, continues to play a pivotal role in the management and follow-up of patients with haemophilia. A total of 237 visits were made to the clinic during the last year with the majority of patients having severe haemophilia A. A warfarin dosage service is provided for patients attending the cardiology clinic at the Steve Biko Academic Hospital as well as for patients on anti-coagulant therapy from surrounding clinics, old age homes and prisons.

### **Research projects**

#### **The correlation between immunophenotyping, DNA ploidy and s-phase fraction as determined by flow cytometry in childhood B cell acute lymphoblastic leukaemia**

*Researcher:* Prof R Pool

Lymphoblasts lack specific morphological or cytochemical features which lead to great reliance being placed on immunophenotyping in order to make a definitive diagnosis. Immunophenotypes (pre-pre B, common, mature B) are well established predictors of prognosis in childhood acute lymphoblastic leukaemia. Less well defined are the prognostic values of the DNA index and the s-phase fraction as determined by flow cytometry. The s-phase fraction is defined as the percentage cells in a tumour which is in the phase of the cell cycle during which DNA is synthesised. The aim of the study is to determine the correlation between the s-phase as determined by DNA ploidy analysis and the immunophenotype as determined by flow cytometry.

#### **The reference range of HbA<sub>2</sub> levels in HIV-infected patients before and after treatment with highly active antiretroviral treatment**

*Researcher:* Dr YL Naidoo

*Funding:* Discovery Academic Fellowship

An increase in haemoglobin (Hb)A<sub>2</sub> has been reported in normal individuals as well as in acquired conditions such as hyperthyroidism, megaloblastic anaemia, HIV infection, patients on highly active antiretroviral treatment (HAART), rarely in erythroleukaemia and, more importantly, in inherited conditions. The aim of this study is to assess the effect that different regimens of HAART, used in the South African National Antiretroviral Guidelines, has on HbA<sub>2</sub> levels, to establish a reference range for HbA<sub>2</sub> based on the population demographics visiting voluntary counseling and testing clinics in South Africa and to determine what impact HbA<sub>2</sub> has on the final HbA<sub>2</sub> values in South Africa.

#### **The prevalence of secondary dysplastic features in HIV-infected patients as determined by flow cytometry**

*Researcher:* Dr A Delport

The purpose of this study is to evaluate whether the immunophenotypic features of secondary dysplasia in bone marrow aspirates of HIV-positive patients correlate with the immunophenotypic dysplastic features found in primary myelodysplastic syndromes as described in the literature. It therefore aims to investigate the expression of CD antigens on the cell surfaces of HIV-infected individuals. Very little research has been done on the immunophenotypic features of secondary dysplasia in HIV. New information is needed that could lead to a more accurate diagnosis, improved therapy and which would bring new advances into patient care. It is also necessary to evaluate the correlation of immunophenotypic dysplastic features with the morphological dysplasia seen in HIV.

#### **The influence of pregnancy on PFA-100 values**

*Researcher:* G Koele

Platelets which are produced in the bone marrow, play a role in primary haemostasis at sites of blood vessel injury. The PFA-100® instrument will be used to evaluate the platelet function in pregnant women in the first, second and third trimesters. Women between the ages of 15-35 of all ethnic groups are included in the study. The control group will consist of age-matched non-pregnant women. The ultimate goal of the study is to determine how, if at all, platelet function changes during various stages of pregnancy. The information obtained from this study may contribute to a greater understanding of pregnancy-associated thrombocytopathy.

#### **A comparison between serum ferritin and bone marrow iron as a measure of total body iron stores**

*Researcher:* E van Wyk

Anaemia of chronic disease is a condition in which there is impaired utilisation of iron, without iron deficiency necessarily being present. Anaemia of chronic disease may complicate interpretation of haematologic markers when iron deficiency coexists. The gold standard for the diagnosis of iron deficiency is considered to be the assessment of a bone marrow aspirate stained with Perls' stain. The primary aim of this project is to investigate the correlation between ferritin and bone marrow iron in patients suffering anaemia from chronic disease. It is often difficult to interpret ferritin levels in patients with HIV infection due to the presence of concomitant inflammation. The secondary aim is to determine whether relationships exist between ferritin, C-reactive protein and the CD4-count.

## Examining the value of mean platelet volume in establishing the cause of thrombocytopenia

Researcher: E van Wyngaardt

Thrombocytopenia is one of the most common causes of abnormal bleeding. Mean platelet volume (MPV) is a parameter which forms part of the routine full blood count which reflects the average size of platelets. The purpose of this study is to determine whether the MPV can be used to distinguish between central (bone marrow) and peripheral causes of thrombocytopenia. Thirty consecutive patients who have bone marrow aspirates for the investigation of thrombocytopenia will be enrolled in the study. Based on the number and maturity of megakaryocytes in the marrow, the causes of thrombocytopenia will be divided between i) decreased production and ii) peripheral destruction, consumption or sequestration (hypersplenism). An analysis will be undertaken to determine what relationship, if any, exists between the MPV and the aetiology of thrombocytopenia.

## Teaching and training

### Undergraduate

The department participates in one teaching block and two special activities for undergraduate students. The homeostasis block for MBChB II is medical students' first contact with haematology and consists of both theoretical and practical components. The lectures cover topics such as haematopoiesis, clinical and laboratory assessment of haematological disease, nutritional and haemolytic anaemias, bone marrow failure, haematological malignancy, disorders of haemostasis, thrombosis, haematological aspects of systemic disease, blood transfusion, cytogenetics and flow cytometry. This block also includes practical sessions which cover subjects such as the haemoglobin determinations, haematocrit, red cell indices and blood grouping.

The special activities block on haematological malignancy for MBChB III presents an overview of the haematological and lymphoid malignancies and is presented by means of case studies. Students are taught how to take histories and examine patients as well as how to perform the complete laboratory work-up for malignancy. They are exposed to various clinical and laboratory procedures which include bone marrow aspirates and biopsies, immunophenotyping, conventional cytogenetics, fluorescent *in situ* hybridisation and PCR.

The special activities block on laboratory medicine for MBChB IV covers topics such as the full blood count, nutritional and haemolytic anaemias, bleeding disorders, hypercoagulable states and blood transfusion. In this block students are taught how to work up a patient with haematological disease as well as cost-effective and rational use of laboratory resources.

### Postgraduate

A comprehensive practical and theoretical teaching programme has been put in place for registrars in haematology which seeks to cover the whole syllabus over a four-year period. Teaching of registrars takes place on a daily basis when peripheral blood films and bone marrow aspirates are reviewed around the teaching microscope. All consultants and registrars attend these informal training sessions at which diagnostic reports are formulated and fine-tuned. Registrars report on bone marrow aspirates independently but comments are always formulated in conjunction with a consultant who will authorise the final report. Special attention has been given to aspects of theoretical haematology, quality assurance and management and these are addressed in weekly lectures prepared for the department by registrars. These lectures are designed to stimulate registrars to read about theoretical aspects of haematology in preparation for the final MMed examination. Registrars in haematology are expected to attend and pass courses in research methodology, molecular biology and physiology within the first 18 months of registering for the MMed. course. They are also encouraged to obtain the Diploma in Clinical Blood Transfusion which is offered by the University of the Free State. Registrars also receive informal training in the haematology clinic and the flow cytometry laboratory and are encouraged to interact with their clinical colleagues in providing interpretation of laboratory tests and by participating in the management of patients with haematological disease. Fortnightly journal club meetings are held with the Department of Haematology, MEDUNSA, in which registrars have an opportunity to review recent trends in the international haematology literature. The MMed course in clinical pathology was recently re-introduced by the university. This is a five-year course in which students are required to spend a minimum of 18 months in haematology. These students enter into the postgraduate programme of the department and participate in service delivery, teaching and research. Registrars also participate in the practical training of both medical technologists and undergraduate medical students. Registrars in the Department of Paediatrics and Internal Medicine rotate through the Department of Haematology in preparation for their intermediate and final examinations.

### Medical technologists

There is continuous interaction between medical technologists and pathologists with regard to routine laboratory tests. In an attempt to promote professional development, a programme of ongoing training has been instituted using quality control morphology slides as a departure point for morphological teaching. All medical staff are expected to participate in this initiative.

## Outreach programme

The programme, jointly devised by the departments of Haematology, Microbiology and Medical Virology, is

presented three times a year, once in Polokwane, once in Nelspruit and once at the Institute of Pathology. A minimum number of 10 medical technologists attend each programme which lasts five hours and consists of presentations from the various departments. In haematology, there is a short introduction followed by an overview of benign and malignant haematological conditions. A short lecture on the haematological changes associated with HIV infection is presented.

### Honours

Dr YL Naidoo was awarded a Discovery Foundation Scholarship worth R537,000 which will be used to visit a centre of excellence in the UK. Dr Naidoo was accepted to do an MSc course in haemoglobinopathies at University College, London.

Dr S van den Berg was awarded a prize by Bio-Rad for the best poster presented at the 48<sup>th</sup> Congress of the Federation of South African Societies of Pathology.

### Research output

Publications published: 1  
Conference presentations: 5

### Professional development

Haematology registrars qualified: 2  
Haematology registrars enrolled: 6  
Clinical pathology registrars enrolled: 5  
Medical technologists qualified: 1  
Student medical technologists enrolled: 3

## Department of Immunology incorporating the MRC Unit for Inflammation and Immunity

Head: **Prof Ronald Anderson**

### Diagnostic services

During the past year the department has continued with its programme of automation and expansion of the repertoire of serodiagnostic services offered to clients, especially serodiagnosis of autoimmune diseases. This has proved to be a particularly successful initiative, which has resulted in significant improvements in turnaround times and increased referrals from other regions. Other service-rendering activities which have been improved through introduction of new technologies and/or expansion of the range of services offered include tissue-typing and immunophenotyping of leukaemias.

### Research programmes

The major research programmes progressed extremely well during the past year. These are infectious diseases research and research focused on the immunopathogenesis and therapy of acute and chronic inflammatory diseases of non-infective origin.

#### Infectious diseases research

Tuberculosis, HIV/AIDS, and severe pneumococcal disease are the major research focus areas. Tuberculosis research is focused on the potassium ( $K^+$ ) transporters of *Mycobacterium tuberculosis*, especially their role in bacterial virulence, and potential to serve as novel targets for drugs/vaccines. The primary objective of HIV/AIDS research is to establish the effects of HIV infection on regional T-lymphocyte colonisation and macrophage activation in the gastrointestinal tract, as well as the effects of antiretroviral therapy on reconstitution of various sub-populations of T-lymphocytes, macrophage activation status, and privileged viral reservoirs, especially in relation to anatomical site and drug resistance. Novel insights into HIV immunopathogenesis of this type are essential for discerning approaches to drug and vaccine design. Pneumococcal diseases research continues to focus on pneumolysin, the major protein virulence factor of this microbial pathogen, by using strategies which target the production and/or cytotoxic and pro-inflammatory activities of the toxin.

#### Inflammatory diseases of non-infective origin

In the case of acute/chronic inflammatory diseases of non-infective origin, efforts are targeted primarily at identifying the mechanisms which initiate mobilisation of calcium during receptor-mediated activation of inflammatory cells, particularly the neutrophil, as well as those which restore  $Ca^{2+}$  homeostasis to the cells. This research has identified several novel  $Ca^{2+}$ -handling-based targets for anti-inflammatory therapy. The second component in this research programme involves identification of the mechanisms by which heavy metals of

environmental/industrial significance (cobalt, manganese, palladium, platinum, palladium) trigger and/or potentiate harmful inflammatory responses.

### Teaching and training

#### Undergraduate

The Department of Immunology provides teaching/training in basic/applied immunology to student medical technicians/technologists, undergraduate medical and dental students, as well as to students enrolled in various BSc courses.

#### Postgraduate

Training is offered at the BSc Hons, MSc and PhD levels, while registrars/clinical assistants from other departments (Medical Microbiology/Virology, Haematology), as well as clinical pathology registrars, rotate through the department's research and service laboratories. It is also the policy of the department to provide access to both equipment and supervision to researchers from other departments and academic institutions.

#### Honours

Prof S Cassol was awarded a substantial European Union research grant for HIV-1 drug resistance surveillance.

Following intensive external review, the MRC Unit for Inflammation with Prof R Anderson as director was granted a fifth and final five-year cycle commencing on 1 May 2009.

The oral presentation by Prof R Anderson and colleagues entitled 'Montelukast inhibits neutrophil pro-inflammatory activity by a cyclic AMP-dependent mechanism' was awarded the prize for the best overall presentation at the Combined Congress of the South African Thoracic and Critical Care Societies, held in Cape Town in August 2008.

#### Research output

Published publications: 18

National conference presentations: 3

#### Professional development

Postgraduate candidate graduated: 1 (MSc)

## Department of Medical Microbiology

**Head: Prof Anwar Hoosen**

### Diagnostic services

The Department of Medical Microbiology provides a comprehensive 24-hour diagnostic laboratory service to the following healthcare facilities: Steve Biko Academic Hospital, Kalafong Hospital, Tshwane District Hospital, Mamelodi Hospital and various Tshwane Metro clinics.

New tests that have been introduced in the past year include the quantitative PCT assay, *Clostridium difficile* toxin assay on the Vidas system, and the Hain's Life Sciences PCR test for identification and first line anti-mycobacterial susceptibility testing. The latter provides a more sensitive and rapid test for the diagnosis and susceptibility testing for tuberculosis.

The Microbiology laboratory serves as an enhanced surveillance site for NICD providing up-to-date data on resistance profiles of important organisms and unusual isolates. The laboratory detected an increase in *C. difficile* toxin identification towards the end of 2008. The hospital administration and the Infection Control Committee were informed and the policy for control of such an outbreak was implemented with the help of the department.

### Research

The Department of Medical Microbiology is currently conducting research focusing on three areas. The tuberculosis research programme includes projects for the development of rapid diagnostic tests and characterisation of *Mycobacterium* species. The antibiotic resistance profiles of mycobacteria are also being analysed using conventional and newer techniques. The department is also participating in two trials which are evaluating molecular-based real-time PCR assays for the rapid identification of tuberculosis directly from patients' specimens. The second focus area is that of antibiotic resistance. This programme investigates the prevalence of antibiotic resistance genes in pathogens of clinical importance. The third is the newly established sexually transmitted

infections research programme which investigates the rapid identification and characterisation of sexually transmitted organisms.

### Tuberculosis research programme

*Collaborators:* Dr T Rossouw (ARV Clinic of the Tshwane District Hospital); Prof M Maeurer (Karolinska Institute, Sweden); Dr M van der Walt (Medical Research Council, Pretoria); Dr B Fourie (MEND-SA); Dr AM Dyrrol-Riise and Prof N Langeland (University of Bergen, Norway); Dr A Friedland and Prof H Viljoen (University of Nebraska-Lincoln)

#### Evaluation of a new immunochromatographic test

*Researchers:* Dr N Ismail, Ms D Pombo

A new rapid immunochromatographic test for the identification of *Mycobacterium tuberculosis* based on the MPT-64 antigen specific for tuberculosis has been evaluated. The test has proven to be highly sensitive and specific for the identification of *M. tuberculosis* from culture tubes when compared with the molecular probe (GenProbe) method currently used. The advantages over this molecular assay are: reduced cost, it is rapid (15 min) and does not require skilled staff or specialised equipment.

#### Evaluation and implementation of Hain Life Sciences' TBMDR Plus strips for analysis of mycobacteria growth indicator tube (MGIT)-positive cultures

*Researchers:* Ms L Khathi, Mr I Manenzhe, Ms C Kekana, Ms B Onwuegbuna, Ms D Pombo, Ms K Lindeque, Dr MM Kock, Dr N Ismail

The Hain test provides both *Mycobacterium tuberculosis* identification and screens the isoniazid and rifampicin coding genes for mutations in one easy assay, which proved to be rapid, specific and sensitive, compared to conventional culture techniques.

#### Diagnostic evaluation of a commercial real-time PCR assay for the detection of *Mycobacterium tuberculosis* from sputum

*Researchers:* Ms H Said, Ms C Veldsman, Mr S Omar, Mr E Makgotlho, Ms C Kekana, Ms B Onwuegbuna, Ms D Pombo, Ms K Lindeque, Dr MM Kock, Dr N Ismail, Prof AA Hoosen, Prof MM Ehlers

*Collaborators:* Dr U Eichenlaub, Dr F Karuse, Dr G Babitzki and Ms B Kanne (Roche Diagnostics, Germany); Mr A Cohen and Ms M Sorrill (Roche Diagnostics, South Africa)

The aim of this project was to evaluate the real-time PCR assay for the rapid identification of *Mycobacterium tuberculosis*, *M. kansasii* and *M. avium*. This assay provides results within 24 to 48 hours, ensuring that patients can commence with the correct treatment regimens within two to three days compared to conventional methods, which can take up to two months.

#### Current postgraduate student projects

- Prevalence of isoniazid and rifampicin resistance genes in *Mycobacterium tuberculosis* strains from HIV-positive patients  
*Researcher:* Ms C Veldsman  
*Funding:* NHLS Research Trust, Faculty of Health Sciences Research Committee
- Susceptibility testing and resistance mechanisms to second-line agents against *Mycobacterium tuberculosis* in Pretoria  
*Researcher:* Ms H Said  
*Funding:* NHLS Research Trust, Faculty of Health Sciences Research Committee
- Determination of the *Mycobacterium tuberculosis* genotypes circulating in a central Pretoria TB clinic using molecular methods  
*Researcher:* Ms A Bulane  
*Funding:* Faculty of Health Sciences Research Committee
- Molecular and immunological diagnosis of pleural tuberculosis in immuno-competent and immunocompromised patients presenting with pleural effusion  
*Researcher:* Mr S Omar  
*Funding:* Faculty of Health Sciences Research Committee
- Pyrazinamide resistance in *Mycobacterium tuberculosis*  
*Researcher:* Mr A Osman
- Evaluation of molecular TB diagnostic assays for the identification of non-tuberculosis mycobacterial strains  
*Researcher:* Ms L Khathi

- Diagnosis of TB meningitis using real-time PCR  
*Researcher:* Dr F Ismail

### Antibiotic resistance research programme

Research projects conducted in this research programme focus on the molecular identification and characterisation of emerging and re-emerging pathogens, with special emphasis on the detection of specific antibiotic resistance genes.

#### Prevalence of antibiotic resistance genes in *Enterobacter cloacae* and *Klebsiella pneumoniae*

*Researchers:* Ms A Bulane, Ms N Maningi, Prof MM Ehlers, Dr MM Kock

A multiplex PCR assay was used to detect the CTX-M, SHV and TEM genes responsible for extended spectrum beta-lactamase (ESBL) production in *Enterobacter* and *Klebsiella pneumoniae*. The prevalence of ESBL genes in *Enterobacter* species was 56% (54/97) and in *K. pneumoniae* 89% (87/97). Molecular techniques are useful for the surveillance and monitoring of these circulating and continuously evolving resistance genes.

#### Prevalence of antibiotic resistance genes in *Acinetobacter baumannii* isolated from clinical specimens

*Researchers:* Mr A Bellomo, Dr MM Kock, Prof MM Ehlers

*Acinetobacter baumannii* is an important cause of nosocomial infections in hospitals, with most cases occurring in immunocompromised patients. The prevalence of the two main gene families responsible for resistance in *A. baumannii*, namely metallo-beta-lactamase and the carbapenem-hydrolysing-class-D- $\beta$ -lactamase genes were investigated. The OXA-51 gene was found in 82% of the isolates. It is evident that there is an urgent need for further surveillance and monitoring of *A. baumannii* due to the high prevalence of antibiotic resistance genes in this clinical setting.

#### Molecular characterisation of methicillin-resistant *Staphylococcus aureus* strains

*Researchers:* Mr PE Makgotlho, Dr MM Kock, Prof MM Ehlers

The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) as well as the specific genotypes of MRSA was investigated by several multiplex PCR assays. There are five SCCmec types, which differentiate between healthcare-associated MRSA and community-associated MRSA (CA-MRSA) strains. The CA-MRSA isolates from the Steve Biko Academic Hospital showed a prevalence of 100% for the Panton-Valentine leukocidin toxin gene. This toxin is an important virulence factor for necrotising pneumonia.

### Current postgraduate student projects

- Prevalence of carbapenemases in *Acinetobacter baumannii* in the Pretoria region  
*Researcher:* Ms JM Hughes
- Efficacy of Vitek to diagnose extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Enterobacter* spp  
*Researcher:* Mr I Manenzhe

### Sexually transmitted infections research programme

Research in this area focuses on the detection, identification and characterisation of sexually transmitted pathogens with special emphasis on *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Ureaplasma* species using molecular tools. The mycoplasmas have been poorly studied in the South African context and the research will thus have valuable clinical applications. The knowledge of the specific circulating *C. trachomatis* serotypes and their antimicrobial profiles in the Gauteng region will have an impact on the treatment of the affected patients. Treatment regimens are not specifically tailored for the South African context and valuable insight, both academically and clinically, will thus be gained after the completion of this first part of the *Chlamydia* research project.

The department has been chosen to be a collaborator with Family Health International (FHI) in the FemPrep trial, which will commence in July 2009. All the sexually transmitted infections (STIs) diagnostic analyses will be performed by the Department of Medical Microbiology. This will aid in capacity development within the department as well as establish valuable links and collaborations with international agencies that can be used for further STI studies.

### Current postgraduate student projects

- Detection and characterisation of *Ureaplasma* species in symptomatic and asymptomatic men  
*Researchers:* Ms N Maningi, Dr MM Kock  
*Funding:* MM Kock University of Pretoria Research Development grant

- Prevalence of *Trichomonas vaginalis* in asymptomatic and symptomatic men  
*Researcher:* Ms I Rukasha
- Prevalence of *Chlamydia trachomatis* in symptomatic men in Steve Biko Academic Hospital  
*Researcher:* Mr P Pilane

### Miscellaneous research projects

- Evaluation of a molecular assay to detect *Pneumocystis jirovecii* in broncho-alveolar lavage and induced sputum  
*Researcher:* Mr B Muhamed  
*Collaborators:* Prof D Denning, Dr G Morgan, Dr F Sadler, Dr M Hughes (Myconostica, UK)
- Detection of the *Clostridium difficile* binary toxin and toxin variant strains  
*Researcher:* Dr S van den Berg
- Causative aetiology of patients with serious respiratory infections  
*Researcher:* Dr S Mahlangu

### Teaching and training

#### Undergraduate

Teaching and training are provided to medical technologists, undergraduate medical and dental students, as well as to allied healthcare students in the disciplines of nursing, physiotherapy and dietetics. The teaching is over the six-year medical curriculum and the department co-ordinates the second part of a 2<sup>nd</sup> year course for medical and dental students as well as a year course for the allied healthcare students, which include students from dietetics, physiotherapy and nursing sciences.

#### Postgraduate

The Department of Medical Microbiology has been accredited by the HPCSA as a training facility for intern medical scientists specialising in microbiology. The department teaches, trains and develops BSc Hons, MSc, MMed and PhD students and presents courses to the departmental postgraduate students as well as to those from other departments.

Continuing professional development-accredited medical microbiology tutorials are held weekly and it is attended by clinicians and pathologists from the private sector and other academic departments. Departmental journal club and research forum meetings with presentations from internal and invited speakers are held weekly.

#### Honours

Mr AN Bellomo received the second prize for a poster presentation in the category 'Junior Researcher: Basic Sciences' at Faculty Day, Faculty of Health Sciences, University of Pretoria in August 2008.

#### Research output

Published publications: 15  
Conference presentations  
International: 5  
National: 3

#### Professional development

Postgraduate candidates enrolled: 22 (5 BSc Hons, 7 MSc, 4 PhD, 6 MMed (Microbiology))  
Postgraduate candidates graduated: 4 (3 BSc Hons, 1 MSc)

## Department of Medical Virology

*Head:* **Dr Lynne Webber**

#### Diagnostic services

The diagnostic virology laboratory offers the following services, namely: serology, direct detection, culture and isolation and molecular biology techniques.

The diagnostic laboratory was recently accredited by the South African National Accreditation System (SANAS) and this was also the first time that the viral cell culture division was assessed.

HIV molecular testing services has increased overall and space is now required for additional equipment to cover the HIV-1 RNA viral load tests. Send-away tests still remain a significant component of the laboratory service and the main future priority is to minimise this drain from the services. Tests have been identified that will now be tested within this diagnostic laboratory. This will have a significant impact on revenue generated.

The staff shortage component has been addressed and students assist the technical staff in an extremely capable manner.

Strategies are being considered to start new tests, particularly within the molecular testing repertoire which is being used more extensively for diagnostic purposes. These tests and new services will be actively marketed.

Medical registrars and other staff members are also playing a large role in outreach programmes to assist clinics, primary healthcare units and other laboratory divisions.

### Research projects

- Evaluation of novel tests for diagnosing active pulmonary tuberculosis in HIV-infected individuals in South Africa (EDITH study)  
*Principal investigator:* Dr LM Webber  
*Study leader:* Dr H-F Kinkel (consultant)  
*Researchers:* Dr M van der Waalt (Tuberculosis Epidemiology and Intervention Research Unit, Medical Research Council(MRC)), Prof A Hoosen (Medical Microbiology), Dr T Rossouw (ARV clinic), Dr N Ismail (Medical Microbiology), Prof A Stoltz (Infectious Diseases Unit), Prof M Ehlers (Clinical Microbiology), Dr L van Rooyen (Ampath Laboratories), Prof J Verschoo (Tuberculosis research), Prof P Becker (MRC), Prof P Rheeder (Clinical Epidemiology Unit)
- Oral mucocoeles and ranulas in HIV-related salivary gland diseases as a prospective and pilot study  
*Principal investigator:* Dr K Syebele  
*Co-investigator:* Dr LM Webber  
*Funding:* University of Pretoria
- HIV and syphilis prevalence in a tertiary South African psychiatric hospital  
*Researcher:* Dr M Henning  
*Supervisor:* C Kruger (Department of Psychiatry); *co-supervisor:* Dr LM Webber  
*Funding:* School of Medicine, University of Pretoria
- Molecular characterisation of selected enteric viruses in human specimens and environmental samples (collaborative project with the Institute of Primate Research and the Kenya Medical Research Institute)  
*Principal investigators:* Prof MB Taylor (SA), Dr JM Mathiu (Kenya)  
*Researchers:* Dr W van Zyl, Dr M Wolfaardt, Mr M Magwalivha, Mr R Tshuma (SA); Mr N Kiulia (Kenya)  
*Funding:* National Research Foundation/NEPAD
- Enteric viruses: water and foodborne transmission (collaborative project with the Enteric Virus Group, Department of Microbiology, University of Barcelona, Spain)  
*Principal investigators:* Prof MB Taylor (SA), Prof A Bosch (Spain)  
*Researchers:* Dr W van Zyl, Dr M Wolfaardt, Mr M Magwalivha, Mr R Netshikweta (SA); Dr R Pinto (Spain)  
*Funding:* National Research Foundation Spain/South Africa Research Partnership programme)
- Development and application of cost-effective techniques for the sensitive detection of enteric viruses in surface and treated drinking water samples  
*Principal investigator:* Prof MB Taylor  
*Researchers:* Dr W van Zyl, Dr M Wolfaardt, Mr M Magwalivha, Mr R Netshikweta  
*Funding:* Rand Water, Sedibeng Water and Midvaal Water
- A quantitative investigation into the link between irrigation water quality and food safety  
*Project leader:* Prof TJ Britz (Department of Food Science, University of Stellenbosch)  
*Group leaders:* Dr E Buys (Department of Food Science, University of Pretoria), Dr J Barnes (Department of Community Health, University of Stellenbosch), Dr S Gunnar (Department of Food Science, University of Stellenbosch), Dr N Potgieter (Department of Microbiology, University of Venda), Prof MB Taylor  
*Funding:* Water Research Commission
- Assessment and optimisation of a real-time polymerase chain reaction for the sensitive and rapid detection of hepatitis A virus and human caliciviruses in food and water sources  
*Principal investigator:* Prof MB Taylor  
*Study leader:* Dr K Richter

*Researcher:* Mr R Netshikweta (MSc student)

*Funding:* NHLS Research Trust

Hepatitis A virus and noroviruses are the most important food- and waterborne viruses. In this project molecular-based techniques are to be optimised for the detection of these viruses in water sources and on fresh produce.

- The molecular characterisation of adenoviruses from human specimens and environmental samples  
*Principal investigator:* Prof MB Taylor  
*Study leader/researcher:* Mr M Magwalivha (MSc student)  
*Funding:* NHLS Research Trust
- Hepatitis B virus (HBV) genotype distribution among HBV serological patterns in AIDS patients and HIV-negative people in a Gauteng population  
*Principal investigator:* Prof DJ Martin  
*Study leader:* Dr S Mayaphi  
*Collaborators:* Prof J Mphahlele, Dr T Rossouw, Dr D Masemola  
*Funding:* NHLS Research Trust, Poliomyelitis Research Foundation
- Development of a multiplex RT-PCR for detection of new and traditional paediatric respiratory viruses in a routine diagnostic laboratory: assessment of the contribution and molecular epidemiology of respiratory syncytial virus, influenza virus A and B; adenovirus; parainflueanza virus 1, 2, 3, human boca virus, human coronaviruses and human metapneumovirus to acute lower respiratory tract infections in Gauteng.  
*Principal investigator:* Dr M Venter  
*Study leader:* MM van den Bergh  
*Funding:* NHLS Research Trust, Poliomyelitis Research Foundation
- Cytokine studies in children infected with respiratory syncytial virus and new respiratory viruses.  
*Principal investigator:* Dr M Venter  
*Funding:* University of Pretoria Development Grant
- Immunopathogenesis associated with lung cytokine production in infants with severe lower respiratory tract infection associated with respiratory syncytial virus, and the newly discovered human metapneumovirus, human boca virus and human coronavirus NL and HKU  
*Principal investigator:* Dr M Venter  
*Funding:* Poliomyelitis Research Foundation
- Association of HIV infection and genetics on severe respiratory syncytial virus infection in South African children.  
*Principal investigator:* Dr M Venter  
*Researcher:* Dr T Kresfelder  
*Funding:* Medical Research Council
- Molecular epidemiology of nosocomial outbreaks of respiratory disease at a secondary hospital in South Africa.  
*Principal investigator:* Dr M Venter  
*Study leader:* Ms Y Westerberg  
*Funding:* Poliomyelitis Research Foundation
- Association of genetic variation in respiratory syncytial virus proteins with disease severity and immune evasion.  
*Principal investigator:* Dr M Venter  
*Study leader:* Ms S Smit  
*Funding:* Poliomyelitis Research Foundation
- Mass-tag PCR for differential diagnosis of respiratory tract infections: identification of a new rhinovirus genotype associated with lower respiratory tract infections.  
*Principal investigator:* Dr M Venter  
*Collaborator:* Dr T Briese (Columbia University, New York)  
*Funding:* NHLS Research Trust
- Genetic determinants of West Nile virus pathogenesis: genome sequencing of highly neuroinvasive and mild South African strains for identification of molecular markers of pathogenesis.  
*Principal investigator:* Dr M Venter  
*Researchers:* Mr D Zaayman, Ms S Human  
*Funding:* NHLS Research Trust
- Association of Flavi and alpha viruses with neurological diseases cases in sentinel horses in South Africa.  
*Principal investigator:* Dr M Venter  
*Researcher:* Ms S Human  
*Funding:* National Research Foundation

- Virus discovery project: identification of undetermined causes of neurological diseases in sentinel animals in South Africa for detection and characterisation of potential zoonotic viruses.  
*Principal investigator:* Dr M Venter  
*Researcher:* Ms C van Eeden
- Development of molecular and immunological tools for diagnosis, prevention and control of West Nile virus in South Africa: impact and immune control of neurological cases in humans and horses.  
*Principal investigator:* Dr M Venter  
*Researcher:* Mr D Zaayman  
*Funding:* National Research Foundation
- Cytokine induction during the course of disease in a patient with a West Nile virus laboratory infection.  
*Principal investigator:* Dr M Venter
- Development of a recombinant West Nile virus vaccine using the baculovirus expression system.  
*Investigators:* Dr M Venter, Prof H Huisman (Department Genetics, University of Pretoria)  
*Researcher:* Ms J Mentoor

## Teaching and training

### Undergraduate

The department provides formal lectures and symposia in eight of the teaching blocks to undergraduate MBChB and BChD students in their 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, and 6<sup>th</sup> years. Teaching in the department also includes formal lectures to undergraduate students from the allied health professions (BCur, BDietetics, and BPhysT). Weekly tutorials are held with the intern medical technology students in the department.

### Postgraduate

The department offers formal lectures, tutorials and practical training to the basic science postgraduate students, the MMed (Path) Medical Virology/Medical Microbiology/Clinical Pathology registrars and to MMed students from other disciplines rotating through pathology.

MMed (Path) Medical Virology tutorials, attended by registrars and specialists from other academic departments and pathologists from the private sector, are held every week and departmental journal club and research meetings are held on a weekly basis. During the course of the year, the academic personnel and postgraduate students attended lectures and workshops presented by other departments and organisations.

### Honours

Ms JME Venter was the winner for a publication in the category 'Best publication by a young researcher (<35 years): non-clinical' in the Faculty of Health Sciences, University of Pretoria in 2008 for the publication: Venter JME, van Heerden J, Vivier JC, Grabow WOK, Taylor MB. Hepatitis A virus in surface water in South Africa: What are the risks? *Journal of Water and Health* 2007; 5: 229-240.

Dr K Richter received the award for outstanding MMed (Path) Medical Virology student from the Faculty of Health Sciences, University of Pretoria.

Dr LM Webber received the Laudation Medal from the Surgeon-General of the Medical Unit of the South African Defence Force for outstanding service as an advisor on HIV-related matters and the Phidisa Project.

Dr M Venter was a finalist in the Women in Science awards, category Best Emerging Woman Scientist, of the Department of Science and Technology. She was honoured as an 'Exceptional young researcher' at the University of Pretoria awards ceremony to recognise exceptional academic achievers.

Dr J Mans was awarded a post-doctoral fellowship by the Poliomyelitis Research Foundation for 2008/2009.

Dr T Kresfelder was awarded a travel grant from the African Centre of Gene Technologies to visit the laboratory of Dr R Jansen, Institute for Public Health and the Environment, Bilthoven, The Netherlands.

### Research output

Publications published: 17  
Conference presentations:  
    International: 6  
    National: 7  
    Local: 9

### Professional development

Postgraduate candidates enrolled: 11 (6 MSc, 3 PhD, 2 post-doctoral fellows)  
Postgraduate candidates graduated: 2 (MMed (Path) Medical Virology, *cum laude*)