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INTRODUCTION
INTRODUCTION

Since its inception in 2001, the NHLS has been on the forefront of academic achievement, actively promoting the teaching and research mandates of the organisation. The 2011/12 Annual Report celebrates these achievements by publishing a separate volume in the report dedicated to these mandates.

In collaboration with its academic partners, the NHLS has gone from strength to strength. This Academic Review acknowledges the much valued contribution by the joint staff (dual appointments by the NHLS and each university) of:

1. The University of Cape Town
2. The University of the Free State
3. The University of KwaZulu-Natal
4. The University of Limpopo (Medunsa)
5. The University of Pretoria
6. The University of Stellenbosch
7. The University of the Western Cape
8. The University of the Witwatersrand
9. Walter Sisulu University

The relationship between the NHLS and its academic partners is cemented in the Umbrella Agreement, signed in 2007. This agreement provides the framework for governance structures, the joint staff establishment, financial arrangements and uninterrupted and equitable access to both the academic and service platforms.

The NHLS academics are locally and internationally acknowledged for their teaching, research and community service activities and achievements. It is clear that NHLS is not only leading the way in South Africa and the SADC region, but its academic footprint is rapidly expanding into Africa and the rest of the world.

The training of health professionals is dependent on an integrated academic and service platform with the clear understanding and appreciation that in so far as health professional training is concerned, teaching, research and service delivery form a continuum and is inextricably linked. It is the statutory responsibility of the NHLS to train pathologists, medical scientists, technologists and technicians for the country as a whole. The close academic partnerships enable the NHLS and universities to produce skilled and dedicated health professionals in pathology.

Research lies at the heart of development and innovation. In collaboration, the NHLS and its academic partners are producing a wealth of ground-breaking, yet relevant, research to propel pathology into the second decade of the twenty first century. Combined, the NHLS – including the NICD and NIOH – and the universities boast a truly impressive research output, as is evident by the amount of grant funding received, peer-reviewed publications and presentations at local and international congresses.

The road ahead is filled with challenges and the 2012/13 year will witness the prioritisation of key objectives. These include, but are not limited to:

1. Improved registrar recruitment and retention drive;
2. Improving the equity profile of the students;
3. Translation of research into operational efficiencies; and
4. Translation of research to influence national policies.

In conclusion, I would like to express my sincere gratitude for the excellent contributions of our academic partners. I am looking forward to exciting developments and prosperous cooperation in the years ahead.
DIVISION OF ANATOMICAL PATHOLOGY

Head: Prof D Govender

DIAGNOSTIC SERVICES

The Division of Anatomical Pathology provides comprehensive diagnostic histopathological, cytopathological and autopsy services to Groote Schuur Hospital (GSH), Red Cross Children’s Hospital (RCCH), Somerset, GF Jooste and Victoria hospitals, which belong to the University of Cape Town’s academic hospital complex. The division also provides diagnostic services to 2 Military Hospital. There are separate laboratories at GSH and RCCH. In addition, diagnostic services are offered to the University of Cape Town (UCT) Private Academic Hospital and consultative and referral services to NHLS and private laboratories in East London, Port Elizabeth, Cape Town, Durban and Pietermaritzburg.

The RCCH histopathology laboratory has been South African National Accreditation System (SANAS)-accredited since 2005 and the GSH histopathology and cytopathology laboratories were accredited by SANAS in March 2011. These laboratories were re-accredited in March 2012.

During the reporting period, the GSH histopathology laboratory received 32,097 surgical pathology cases (including many cases with multiple specimens), the cytology laboratory processed 69,020 cases, of which 59,254 were cervical smears and 9,766 non-gynaecological cases. The cytology unit established a fine needle aspiration (FNA) clinic on site at GSH which began operating in July 2011. Since this service commenced, 119 FNA procedures were conducted. The electron microscopy unit at GSH processed 490 specimens, 223 of which were referrals from other laboratories. The immunohistochemical laboratory performed 18,826 tests. During the reporting period, 70 adult autopsies were performed. The foetal and perinatal service at GSH examined 134 foetuses and 904 placentas.

The RCCH histopathology service included paediatric patient referrals from Western Cape, Eastern Cape and KwaZulu-Natal. The laboratory received 2,783 paediatric surgical pathology cases. A consultative service for muscle biopsies is based at RCCH; during the reporting period, 133 muscle biopsies were processed, 113 of these were referrals. The electron microscopy unit at RCCH processed 270 specimens. A total of 39 paediatric autopsies were conducted.

Pathologists and registrars participated in 50 clinicopathological meetings per month held at both GSH and RCCH.

The division significantly increased its support in diagnostic services to the Eastern Cape, especially East London.

RESEARCH PROJECTS

The divisional staff is currently undertaking 20 research projects, of which the following two were initiated during the reporting period:

The immunoexpression of p53-cofactor JMY in prostatic adenocarcinoma and its effects on the cell cycle and cell adhesion molecules
Researchers: Dr M Otto, Prof D Govender (UCT)
Funding: NHLS Research Trust

Immunohistochemical evidence of proliferation, hypertrophy and differentiation of glomerular epithelial cells in HIV-associated nephropathy
Researchers: Dr F Botha, Dr M Duffield
Funding: NHLS Research Trust

HONOURS

The division was placed first among 67 international participating laboratories in the general diagnostic external quality assessment (EQA) module of the Royal College of Pathologists of Australasia (2011) and Dr K Pillay obtained 100% in the paediatric pathology diagnostic module.
Prof D Govender was elected president of the College of Pathologists of the Colleges of Medicine of South Africa. His three year term began in October 2011.

TEACHING AND TRAINING

Undergraduate
The consultant staff is responsible for delivering undergraduate teaching in anatomical pathology to MBChB students during semesters 3-5 in an integrated, problem-orientated (case-based) course, with computer-based tutorials and small-group teaching (i.e. museum and mortuary demonstrations). Lectures are also given to fifth year medical students during the gynaecology block rotations. A limited number of students gain access to a special study module in anatomical pathology in semester 4, currently the best and earliest opportunity of attracting future anatomical pathologists into the discipline. Anatomical pathology academic staff lectured in the newly introduced molecular medicine programme in the MBChB degree. Third year BSc (Occupational Therapy) and BSc (Physiotherapy) students are taught by anatomical pathology consultants as an integral part of the clinical sciences course for the allied health sciences. The division also hosted undergraduate medical students during their mandatory elective period in their fifth year.

Postgraduate
There were 11 registrars, three supernumerary registrars and two forensic registrars in training during the reporting period. Three registrars were successful in the FCPath (Anat) Part 1 examination and one registrar passed the FCPath (Anat) Part 2 examination.

Medical technologists
The division provides training for student histotechnologists and cytotechnologists. Two student histotechnologists were trained, one at each laboratory. They sat the Board examination in March 2012. One student cytotechnologist also sat the Board examination in March 2012. Two further student cytotechnologists started training in 2012.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated: 2 (MMed)
Postgraduate students enrolled: 23 (4 MSc, 16 MMed, 3 MPhil)
Student technologists: 3

RESEARCH OUTPUT

Publications


Lazarus J, Pillay K. Abdominal tuberculosis presenting as an inguinal hernia in a child. Urology. 2011; 1470-1471


**Conference presentations**

- **International**: 4
- **National**: 3
- **Local**: 3

"In July 2011, a fine needle aspiration clinic started operating at Groote Schuur Hospital”

**DIVISION OF CHEMICAL PATHOLOGY**

**Head**: Prof AD Marais

**DIAGNOSTIC SERVICES**

The integrated clinical pathology laboratory at GSH comprises chemical pathology, haematology, immunology, allergology, virology and microbiology and provides routine diagnostic services to the hospital, other Western Cape provincial healthcare facilities and the UCT Private Academic Hospital. It also offers a clinical trials service.

The laboratory, accredited by SANAS, offers a 24-hour service, and is highly automated. Specialised tests available include protein and lipoprotein electrophoresis and various manual assays and immunoassays. The radioimmunoassay (RIA) section of the laboratory offers a unique diagnostic service; four of the tests (active renin, aldosterone, 17OH-progesterone, acetylcholine receptor autoantibody) are not available elsewhere in the Western Cape and one other assay (11-deoxycortisol) is unique in the country. The laboratory also endeavours to perform any unusual RIA/immunoradiometric assays (IRMA) requested: leptin, adiponectin, plasma renin activity and atrial natriuretic peptide. The molecular laboratory offers a large repertoire of genetic testing, including certain pharmacogenetic tests. Newer tests added include the
androgen receptor and mitochondrial polymerase G gene sequencing. Streamlining of the existing tests is being undertaken for reasons of cost-effective practice.

The biochemical arm of the inherited metabolic disease (IMD) laboratory performs assays within the scope of its staff, equipment and budget according to the presentation of patients who need diagnosis of severe metabolic errors. Previously reported assays remain on offer while new assays will be undertaken as far as possible in collaboration with special clinics.

The RCCH laboratory received full SANAS accreditation on all routine, manual and specialist IMD diagnostic assays, including analysis of organic and amino acids by gas chromatography mass spectrometry (GC-MS). Test volumes within the IMD diagnostic service have continued to grow as samples are now analysed from the entire Southern African Development Community (SADC) region. A major achievement during the reporting period was the introduction of an interactive, searchable webpage listing of all the IMD tests available at the RCCH and GSH at www.madlab.uct.ac.za. All genetic and biochemical assays are listed. High throughput molecular newborn screening tests for glutaric aciduria type 1 (GA 1) and galactosaemia have screened close to 10,000 newborn children, confirming the high carrier frequencies of specific mutations for these disorders in the South African population and identified the first presymptomatic GA1 case South Africa. This child is currently on preventative treatment and will be closely monitored. The last year also saw the development and validation of a method for urine fractionated metanephrines by isotope dilution GC-MS.

Staff from the Department of Medicine assist the laboratory to provide tertiary patient care with tests that are not available in routine laboratories. This work is also supported by the MRC Cape Heart Group.

**RESEARCH PROJECTS**

**Investigation of high molecular weight adiponectin in HIV-infected patients on antiretroviral therapy**

**Researchers:** F Omar, JA King, TS Pillay  
**Funding:** NHLS Research Trust

The aim is to investigate the role of multimeric (high molecular weight) adiponectin in the development of metabolic disease resulting from antiretroviral therapy by quantifying the circulating levels of both total and high molecular weight (HMW) adiponectin and to establish whether a link exists between HMW adiponectin levels and susceptibility to HIV-induced lipodystrophy. Although total adiponectin levels have been shown to be significantly reduced in patients with HIV-induced lipodystrophy, there is no information on whether HMW adiponectin (the most biologically active form) is altered in highly active antiretroviral therapy (HAART)-induced lipodystrophy, and whether patients with low levels of the HMW form are more susceptible to lipodystrophy.

**Design and prototype of an interlaboratory quality assurance programme for urine bicarbonate**

**Researchers:** R Benjamin, P Berman, JA King  
**Funding:** NHLS K Grant

Renal tubular acidosis is an inexpensive condition to diagnose and treat and may initially present with failure to thrive. Current opinion is that every child with failure to thrive should be investigated. In South Africa about 500,000 such cases are identified per annum, of whom 250,000 are stunted or wasted. Thus, a need for the test exists. Laboratories should be and are reluctant to conduct the diagnostic tests (which include urinary bicarbonate) because the available methods – total CO2 and bicarbonate on a blood gas analyser – have not been validated for urine and EQA does not exist. By running the test, the laboratory risks accreditation and cannot allow the clinician to make confident diagnoses. The construction of an EQA programme for urine bicarbonate will allow laboratories to assist in the diagnosis of renal tubular acidosis. An EQA programme will facilitate screening of the at risk population, will allow the epidemiology to be explored, and the benefit of diagnosis to be determined. Furthermore, the test should be readily accessible through-out the country wherever blood gas analysers are used.

**Development and assessment of a DNA multiplex screening assay for detection of glutaric aciduria type 1 and galactosaemia in a large South African cohort**

**Researchers:** Dr GF van der Watt, Dr EP Owen, Prof HE Henderson  
**Funding:** NHLS Research Trust
This project was initiated because all known African patients diagnosed with galactosaemia or glutaric aciduria type 1 in South Africa have been homozygous for a single point mutation in each disease. A multiplexed molecular assay will be used to detect these mutations in low volume samples such as dried blood spots and the subsequent utilisation of this assay to screen for the mutations in a larger cohort of previously obtained dried blood spots. These data will be used to predict the expected disease burden for these disorders in the African population.

**Determination of sibutramine in contaminated herbal remedies using a rapid GC-MS method**

**Investigators:** Dr GF van der Watt, B Foster

This project was initiated based on queries from various investigators into the potential contamination of a number of herbal remedies with sibutramine, an amphetamine-related amine with anorexogenic properties. A GC-MS method using cation exchange extraction without derivatisation and a 10m Zebron AAA column (Phenomenex™) was developed to identify and quantify sibutramine within a total preparation and runtime of 20 minutes per sample. The method is being validated and performance characterised.

**Frequency of R563Q mutations in the epithelial sodium channel in South African populations**

**Researchers:** Dr E Jones, Dr EP Owen, Prof B Rayner

It was shown that the R563Q mutation, which possibly originated from the Kois, is found in multiple ethnic groups in South Africa, and is associated with hypertension. The results are important because hypertension resulting from the R563Q mutation is a common and treatable cause. It is recommended that hypertension units in South Africa screen for the mutation and alter treatment appropriately. A further recommendation is that a sodium channel inhibitor, such as amiloride, in an appropriate form, is registered in South Africa for the treatment of hypertension.

**Mutation screening in CTNS gene in a cohort of cystinosis patients**

**Researchers:** Dr EP Owen, F Leisegang, Dr J Nandhlal

A cohort of RCCH cystinosis patients is under investigation for pathogenic mutations within this transporter gene. Initial genomic DNA screening failed to clarify meaningful mutations but screening messenger RNA revealed a common mutation. This mutation will be studied in the general population.

**Plasma and urine sarcosine in patients with prostatic carcinoma**

**Researchers:** Prof L Boehm (Tygerberg Hospital); Dr G van der Watt

This project evaluates the utility of plasma and urine sarcosine levels in patients with prostatic carcinoma.

**Cystine urolithiasis in caracals**

**Researchers:** Dr A Tordiffe (Pretoria Zoological Gardens); Dr G van der Watt

This project investigates the high prevalence of cystine urolithiasis in African caracals and its health implications.

**Genetic causes of familial hypercholesterolaemia phenotype at the GSH Clinic**

**Researchers:** G Solomon, B Ratanjee, Dr D Blom, Dr K Wolmarans, Dr B Brice, J Barron, R Jooste, P Cowie, Dr D Blackhurst, Prof AD Marais

**Funding:** Medical Research Council (MRC)

Familial hypercholesterolaemia is a clinically distinct phenotype that is known to result from pathogenic mutations in the low density lipoprotein (LDL) receptor, apoB and proprotein convertase subtilin-kevin type 9 (PCSK9) gene. This project seeks to identify the genetic causes in a systematic manner. In a cohort of approximately 1,500 subjects, genotyping has identified about 1,100 subjects with 81 LDL receptor mutations, four apoB mutations and two PCSK9 mutations. Such genetic diagnoses permit cascade screening.

**Hyperalphalipoproteinæmia**

**Researchers:** G Solomon, B Ratanjee, Dr D Blom, Dr K Wolmarans, Dr B Brice, Dr R Benjamin, M Levey, Dr D Blackhurst, Prof AD Marais

**Collaborator:** Prof M Hayden (University of British Columbia)

**Funding:** MRC

This project continues with the initial findings that identified several subjects with endothelial lipase mutations. High concentrations of high density lipoprotein (HDL) cholesterol are not always protective against atherosclerosis and the genetic causes are not fully understood but may influence the risk of
Atherosclerosis. An electrophoretic analysis is being modified to describe the range of HDL species.

**Smith Lemli Opitz syndrome**

**Researchers:** G Solomon, A Mohamed, Prof AD Marais

The Smith Lemli Opitz syndrome is an uncommon, recessively inherited deficiency in 7-dehydrocholesterol reductase that results in hypocholesterolaemia and congenital malformations as well as developmental delay. As a result of limited equipment, a spectrophotometric assay has been set up and patients from Cape Town and Johannesburg have been diagnosed with the disorder. In the former case, the genotype is now identified while work-up continues in two other families with different mutations.

**Nutrition in hominins in the Cape Folded Belt region during middle to late stone age**

**Researchers:** K Kyriacou, Prof J Parkington, Prof AD Marais

This collaborative research between the archaeology and chemical pathology divisions seeks to link the findings at excavation sites and middens with nutritional supplies with special emphasis on energy, n-3 fatty acids, protein, and some micronutrients.

**Oxidative stress markers and anti-oxidant capacity in plasma of subjects with varying risk of cardiovascular disease**

**Researchers:** Dr D Ojji, Prof K Sliwa, Prof S Lecour, Dr D Blackhurst

This is a collaborative project in samples from a study in the Hatter Institute to determine whether graded cardiovascular risk and disease status correlates with markers of oxidative stress and anti-oxidant activity.

**TEACHING AND TRAINING**

**Postgraduate**

Registrars rotate between the different benches within the laboratories at GSH and RCCH to obtain skills across the discipline. They receive weekly tutorials on basic biochemistry, methodology, management, lipidology, molecular medicine and journal discussions. They also attend weekly journal clubs, endocrinology seminars and endocrinology ward rounds. There were five registrars in chemical pathology, including two supernumery (Malawi and Nigeria), and clinical pathology registrars rotated through chemical pathology as well.

**Medical technologists and technicians**

During the year under review, five medical technology interns and six third year medical technology students were trained in the laboratory; the latter group all passed the final exams and are now employed as student interns. Eight student phlebotomy technicians were employed for training, two of whom wrote and passed their exams in October 2011 while the others will write their final exams in October 2012.

**PROFESSIONAL DEVELOPMENT**

**Postgraduate candidates graduated:** 1 (PhD)

**Postgraduate candidates enrolled:**

18 (9 MMED, 6 PhD, 3 BSc(Hons), 7 MMED, 1 MSc)

**RESEARCH OUTPUT**

**Publications**


DIVISION OF IMMUNOLOGY

Head: Prof CM Gray

The Division of Immunology is involved with a range of activities from identifying the basic mechanisms of infectious disease immunity to translational clinical research on HIV and tuberculosis (TB) to diagnostic testing in tissue immunology, clinical immunology and allergy.

DIAGNOSTIC SERVICES

The main thrust of the diagnostic service of the division is provision of human leucocyte antigen (HLA) typing and cross-matching for bone marrow and solid organ matching, which takes place in the Laboratory for Tissue Immunology (LTI), and autoimmunity and allergy testing, which is performed in the clinical immunology and allergy laboratory. Both laboratories were inspected and SANAS-accredited.

Laboratory for Tissue Immunology

The LTI is the only tissue typing laboratory in South Africa and on the African continent to be accredited by the European Federation for Immunogenetics, which enables the division to provide the level of tissue typing acceptable to the World Marrow Donor organisation and other international institutions for HLA typing of patients and potential unrelated and related stem cell donors. Over the past year, 3,161 HLA types were performed by serological methods for solid organ donors (303), solid organ renal recipients (374), other organs (33, cardiac, liver, lung), stem cell transplant donors (1,307), stem cell transplant patients (355) and related stem cell donors (789). In addition to HLA typing, the LTI has also performed a total of 8,399 HLA class I cross-matches for either living related or cadaver donor solid organ transplantation. The LTI also provides HLA A, B and DR typings for all volunteer donors in the Western Cape, recruited by the Sunflower Fund for the South African Bone Marrow Registry (SABMR) and there was an agreed shift to start all DNA typing from January 2012 using DNA Luminex technology and to cease serological typing.

Clinical immunology and allergy

In total, 5,064 allergy-related tests were conducted during the past year, including identification of allergen-specific IgE, total IgE determination, analysis for the presence of autoantibodies against the high affinity IgE receptor, determination of Budgerigar, pigeon and parrot avian specific IgG antibodies as an aid in diagnosis of ‘bird-fanciers lung’ and assessment of aspergillus fumigatus-specific IgG antibodies which is important in allergic broncho-pulmonary aspergillosis. The laboratory now offers six new tests: anti-cardiolipin IgM, β2-glycoprotein 1 IgG, β2-glycoprotein 1 IgM, anti-tissue transglutaminase IgG, deamidated gliadin IgA and deamidated gliadin IgG.

South African Bone Marrow Registry

The SABMR is an independent non-profit agency, and has a close relationship with the NHLS and LTI, with two NHLS staff playing a crucial role in its functioning. The SABMR provides unrelated stem cell donors for all South African patients with blood diseases such as leukaemia who require matched unrelated donor (MUD) stem cell transplantation. This includes patients from other African countries who are referred to transplant centres in South Africa. The registry continues to be one of the main users of the LTI for patients who have been identified as possible candidates for unrelated bone marrow transplantation as well as their donors, both locally and internationally. All new potential unrelated bone marrow donors recruited by the Sunflower Fund in the Western Cape are HLA-typed in the LTI before being entered on the SABMR database. SABMR donors also continue to be of interest to international patients and this is demonstrated by the number of extended typing requests (±150) received from international registries, and blood samples of these are sent to the LTI for further typing. The cost of a local donor to a South African patient who is HLA-matched with an SABMR donor is
considerably less than if matched to an international donor. To date, March 2012, the SABMR has facilitated stem cell transplants for 255 patients in South Africa, in three transplant centres. Sixty-six patients (26%) received stem cells from local donors and 187 (74%) patients received international donors. While the total number of donors registered with the SABMR remains approximately 64,000, the number of DR-typed donors has continued to improve from 12.8% in March 2011 to 17.8% in March 2012. During the period under review, the SABMR provided peripheral blood stem cells from two local donors for international patients.

RESEARCH PROJECTS

As part of its research function, the division generates and characterises novel and unique transgenic mice, as animal models for human diseases and these have been used to make important advances in diseases, such as TB, bilharzia, African trypanosomiasis, candidiasis and cutaneous leishmaniasis, and contributed to a better understanding of immunological mechanisms on a cellular and molecular level. Basic research in the allergy section has focused on the T cell cytokine responses to allergens as well as the application of novel assays to identify sensitivity profiles. Research is also underway to understand adaptive and innate immune responses during acute HIV infection and in infants exposed to HIV in utero and upon receiving BCG vaccination. Projects in the division include:

HIV vaccine immunogen design
Researchers: Prof C Gray, L Zembe (postdoctoral fellow)
Collaborators: Prof C Williamson (Medical Virology, UCT); Prof U Ranga (Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India)

Innate, adaptive and mucosal immune responses in HIV-1-exposed uninfected infants: a human model to understand correlates of immune protection
Researchers: Prof C Gray
Collaborators: Dr H Jaspan (Division of Immunology, UCT); Prof K Rosenthal (McMasters University, Canada); Prof B Cameron (Ottawa Research Institute, Ottawa, Canada); Prof A Abimiku (Institute of Human Virology Nigeria)

A prospective observational study to analyse host and pathogen contributions in the emergence of extensively drug-resistant TB
Researchers: Prof C Gray, Dr C Riou
Collaborators: Dr D Fallows, Prof G Kaplan (Laboratory of Mycobacterial Immunity and Pathogenesis, New Jersey, USA)

Renal transplantation in South Africa: using HIV-positive deceased donors for HIV-positive recipients
Researchers: Prof C Gray
Collaborators: Dr E Muller (Department of Surgery, UCT)

Neurones and mycobacterium TB
Researchers: A/Prof M Jacobs; N Francesco, P Randall (PhD students); Dr N-J Hsu, Dr N Allie (postdoctoral fellows)
Collaborators: A/Prof D Lang, S Cooper, Prof L Kellaway (UCT); Prof V Quesniaux, Prof B Ryffel (CNRS-France)

Tumour necrosis factor and the central nervous system in TB
Researchers: A/Prof M Jacobs; B Sebesho (PhD student); Dr N Allie, Dr R Keeton, Dr N-J Hsu (postdoctoral fellows)
Collaborators: Prof L Kellaway (UCT); Prof V Quesniaux, Prof B Ryffel (CNRS-France)

Assessment of vaccine efficacy for malaria and TB in a co-infection setting relevant for disease-endemic sub-Saharan African regions
Researchers: A/Prof M Jacobs; Dr R Keeton (postdoctoral fellow)
Collaborators: Prof S Magez (Vrije Universiteit Brussels, Belgium)

Drug discovery for anti-TB agents from South African medicinal plants
Researchers: A/Prof M Jacobs; Dr N Allie (postdoctoral fellow)
Collaborators: Dr E Madikane, Prof P Smith (UCT); Prof P van Helden (University of Stellenbosch [US]); Prof P Folb (MRC); Dr C Parkinson (Council for Scientific and Industrial Research [CSIR]); Dr N Bagwandine

Investigating antimycobacterial activity of phenothionine derivatives
Researchers: A/Prof M Jacobs; S Salie (MSc student); Dr N-J Hsu (postdoctoral fellow)
Collaborator: Dr A Jardine
T cell responses to Mtb proteins in extensively drug-resistant TB patients  

Researchers: Dr B Nurse; M Davids (PhD student)

TEACHING AND TRAINING

The division teaches in the medical undergraduate syllabus and provides a more advanced course at the postgraduate level through the BSc Honours in infectious disease and immunology programme and advanced immunology.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated:  
3 (1 PhD, 2 BSc [Hons])

Postgraduate candidates enrolled: 17 (4 postdoctoral fellows, 6 PhD, 4 MSc, 3 BSc [Hons])

RESEARCH OUTPUT

Publications


Hong HA, Loubser AS, De Assis Rosa D, Naranbhai V, Carr W, Paximadis M, Lewis DA, Tiemessen CT, Gray CM. KIR genotyping and HLA-KIR-ligand identification by real-time PCR. Tissue Antigens 2011; 78: 185-194


“ The LTI is the only tissue typing laboratory in South Africa and on the African continent to be accredited”
DIVISION OF MEDICAL MICROBIOLOGY

Head: Prof M Nicol

DIAGNOSTIC SERVICES

The laboratory embarked on a major re-organisation of workflow, which has resulted in a central processing area, and improved efficiency. The laboratory’s accreditation with SANAS was renewed and awards were received for achieving EQA targets. The laboratory received requests from both Kenya and Pakistan to host visiting microbiologists for further training. The BacT-Alert (BioMerieux) blood culture system was validated; mycobacterial blood cultures are currently processed using this system, and normal aerobic and paediatric cultures will soon follow.

The diagnostic laboratory provides a service to colleagues engaged in clinical research. During the course of 2011/12, 11 new clinical studies commenced in the laboratory, and 20 trials were continued from the previous year. The majority of the studies are related to TB, and include diagnostic, treatment, vaccine and epidemiological studies.

The outreach programme to the Eastern Cape continued. Three visits were undertaken in the period April 2011-December 2011. Besides visiting the major hospitals in the region (Port Elizabeth and East London), the outreach included Graaf Reinett, Grahamstown, Cradock, Somerset East, Port Alfred, Uitenhage and Humansdorp. The division provides a 24-hour microbiologist on call service to provide advice for clinicians in the Coastal Region.

Members of the division are involved in infection control and antibiotic stewardship activities, serving on the infection control committees at RCCH and GSH, as well as on the provincial infection prevention and control committee. The antibiotic stewardship programme, which was based on an antibiotic restriction policy, has been altered to include a more active ward-round-based component as well as online tutorials for medical registrars on appropriate antibiotic use.

The NICD satellite Molecular Epidemiology Unit, based in this division and in Medical Virology, provides molecular epidemiological services to investigate suspected outbreaks of hospital-acquired infection. In addition, the unit has developed molecular assays for the rapid identification of highly resistant carbapenemase-producing organisms and offers this service to other NHLS laboratories.

RESEARCH PROJECTS

The epidemiology of *Staphylococcus aureus* in patients with cystic fibrosis

Researchers: Dr A Whitelaw (UCT/NHLS); D Coertze, Dr L Edries, M Jansen van Rensburg (UCT)
Funding: NHLS Research Trust

The study aimed to describe the molecular characteristics of *Staphylococcus aureus* isolates from patients with cystic fibrosis. Twenty-three isolates, from 18 patients, were characterised by pulsed-field gel electrophoresis (PFGE), spa-typing and staphylococcal cassette chromosome (SCC)-mec typing. Eight isolates belonged to one PFGE cluster, suggesting a degree of cross transmission; however, the remainder of the isolates showed genetic diversity. The three methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were spa-1257 and contained SCC-mec-IV, suggesting they belonged to ST-612. This has recently been recognised as a common clone in Cape Town.

Screening for complement component C5 and C6 in patients with meningococcal disease

Researchers: EP Owen, F Leisegang, A Whitelaw (NHLS/UCT); J Simpson (NHLS); S Baker, P Potter (UCT); R Würzner (University of Innsbruck); A Orren (University of Cardiff)

People with defects in the complement pathway are known to be at higher risk of meningococcal disease and may develop recurrent meningococcal disease. Mutations in both the C5 and C6 components (C5D and C6Q0) have been described in indigenous populations in the Western Cape. This study aimed to determine the frequency of these mutations in patients presenting with meningococcal disease. Of the 109 patients screened, three had C5D, and 11 C6Q0. This suggests that screening for complement defects should be routinely carried out in patients with confirmed meningococcal disease.

A prospective study of *Clostridium difficile* infection in a tertiary referral hospital

Researchers: Dr N Rajabally (UCT/GSH); Dr A Whitelaw, Dr C Bamford (NHLS/UCT); Dr B Kullin, Prof S Reid, Prof V Abratt (UCT); A Klein (NHLS)
Funding: MRC, Department of Gastroenterology, UCT

This study aims to both describe the incidence and clinical characteristics of *Clostridium difficile* infection at GSH, and to ascertain the optimal diagnostic test for C.
*difficile* infection. Stool samples are being cultured onto three different selective anaerobic media for *C. difficile*, and toxin assays are being performed using commercial lateral flow assays, ELISAs and PCR. Clinical data are being collected prospectively on patients suspected of having *C. difficile* infection. Isolates will be ribotyped, and will be tested for susceptibility to metronidazole.

**The molecular epidemiology of *Streptococcus pyogenes* pharyngitis among children in the Vanguard Community (Bonteheuwel/Langa), Cape Town, and the development of a clinical prediction rule for streptococcal pharyngitis**

**Researchers:** Prof B Mayosi, Prof D Beatty, M Engel, B Muhamed (UCT); Dr J Dale (University of Tennessee); Dr A Whitelaw (NHLS/UCT)

**Funding:** National Institutes of Health (NIH)/DMID

The aim of the study is to describe the distribution of *emm*-types among isolates of *Streptococcus pyogenes* causing pharyngitis, in order to assess the possible coverage of a proposed 26-valent vaccine. Isolates have been collected from throat swabs from children presenting with sore throats to community health centres, as well as from asymptomatic children at schools in the region. Analysis of 143 isolates has been performed, and the results are being written up.

**Identification of ST612 methicillin-susceptible *Staphylococcus aureus* containing a partial excision of SCCmec**

**Researchers:** M Moleleki (MSc student); Dr C Bamford, A/Prof Gay Elisha (NHLS/UCT)

**Funding:** NHLS Research Trust

Building on previous work, in which a methicillin-susceptible *Staphylococcus aureus* (MSSA) isolate (ST612-MRSA-IV) with a genetic background similar to that of a locally prevalent MRSA clone was identified, competition assays were conducted to investigate the relative fitness of susceptible and resistant strains. Competition assays involving prolonged incubation over five days showed that this MSSA strain outcompeted three out of four ST612-MRSA-IV strains, suggesting that the carriage of SCCmecIV may impose a fitness cost on these isolates.

**Drug discovery for anti-TB agents from South African natural products**

**Researchers:** Dr VE Madikane (NHLS/UCT); Dr N Bhagwandin, Prof P Folb (MRC); Prof I Wid, Prof P van Helden (US); Dr L Weisner, Prof P Smith, (UCT); Dr C Parkinson (CSIR); Prof N Crouch (South African Biodiversity Institute)

**Funding:** Department of Science and Technology Innovation Fund

Originally, a specific antimycobacterial compound (F1082) with an minimum inhibitory concentration (MIC) of 11 µM against *Mycobacterium tuberculosis* was identified. Four additional compounds with suitable *in vitro* toxicity profiles have been identified through extensive and robust bioassay-guided chemical synthetic optimisations. Preliminary *in vivo* pharmacokinetic and toxicity studies are currently being conducted to ascertain the bioavailability and safety of these lead compounds in a mouse model. Gastric and microsome stability of these compounds is also being investigated in order to guide further formulation activities. An invention disclosure form has been completed and is being reviewed by the MRC’s Innovation Office.

**Integrated microanalytical extraction-amplification system for detection of TB in low resource settings**

**Researchers:** Prof M Nicol (NHLS/UCT); V Allen, Dr L Edries, Dr D Kelso (Northwestern University, USA)

**Funding:** Center for Point-of-Care Diagnostics for Global Health, PATH

The aim is to develop and test a low-cost, robust, point-of-care device for the diagnosis of TB in low resource settings. This involves the development of an integrated specimen collection/processing container and integration into a novel real-time PCR platform. The researchers have developed and determined the limit of detection of a novel real-time PCR assay as well as developed and field-tested a novel sputum collection device. The next stage will be to develop and test prototype assays using sputum samples from patients with suspected TB.

**Innate immunity to TB is lineage- and host-dependent**

**Researchers:** Prof M Nicol (NHLS/UCT); R Sarkar, K Wood (UCT); Prof R Wilkinson, Dr K Wilkinson (UCT, Imperial College, MRC UK)

**Funding:** National Research Foundation (NRF) Focus Area Award

Data from the first study demonstrated that there are indeed lineage-specific patterns of cytokine induction and that clinical strains may be better adapted to
in intracellular growth than laboratory-adapted strains. Experiments were conducted to compare the cytokine responses in donors of different genetic backgrounds, using multiplex (Luminex) cytokine analysis. The analysis of these complex results is now underway.

**The Drakenstein Child Lung Health Study**

**Researchers:** Prof M Nicol, Dr E Madikane (NHLS/UCT); Prof H Zar, Dr M Kaba, Dr E du Toit, F Dube, S Mohammed (UCT)

**Funding:** Bill and Melinda Gates Foundation

This large and ambitious study is a collaboration between the Department of Paediatrics and Child Health and Medical Microbiology at UCT. The study will enrol and follow up 500 mother-infant pairs from the Drakenstein region in the Western Cape over the first two years of life. The primary aim is to evaluate the aetiology and risk factors for the development of pneumonia in the first two years of life. A range of molecular and culture-based tools (including 33-plex real-time PCR) will be applied to identify the most important causes of respiratory illness in this cohort.

**The effect of early childhood exposure to environmental organisms on the development of wheezing and atopy in young children**

**Researchers:** Prof M Nicol (UCT/NHLS); Dr E du Toit (UCT)

**Funding:** Bill and Melinda Gates Foundation

The aim is to examine if high levels of exposure to environmental organisms (fungi, bacteria) influence the onset of childhood atopy and wheezing. Home dust samples will be collected antenatally, at six and 12 months using electrostatic dust collectors and the bacterial and fungal organisms present identified using denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphisms (T-RFLP) and metagenomic techniques. The level of endotoxins in the samples will be measured using the quantitative kinetic chromogenic Limulus amoebocyte lysate assay. The association between environmental exposure to bacterial and fungal organisms will be determined as well as endotoxin level, and the development of atopy and wheezing illness.

**The stool microbiota and its relationship to allergy and atopy**

**Researchers:** Prof M Nicol (UCT/NHLS); Dr E du Toit (UCT)

**Funding:** Bill and Melinda Gates Foundation

The aim is to identify the diversity and main components of the stool microbiota of infants and mothers over a two-year period at monthly intervals. This information will be related to the development of lung disease and allergy. Stool samples will be collected from the infants and mothers at birth and monthly thereafter. DNA will be extracted and the diversity will be measured using DGGE and T-RFLP. A clone library will be created and sequenced to identify the predominant members of the stool microbiota. Metagenomics will be used for deep sequencing purposes.

**Nsopharyngeal microbiome and pneumonia in young children from Drakenstein sub-district**

**Researchers:** Prof M Nicol, Dr E Madikane (NHLS/UCT); Dr M Kaba, F Dube, S Abdulgader, Prof H Zar (UCT)

**Funding:** Bill and Melinda Gates Foundation, Carnegie Foundation

The aim of this study is i) to longitudinally investigate the nasopharyngeal microbiome of a birth cohort of 500 infants (sampled two weekly over a two-year period), ii) to determine the nasopharyngeal pathogens associated with near-term progression to pneumonia in childhood during the first two years of life, and iii) to study the microbial diversity in infants sampled using metagenomic approaches.

**Characterisation and detection of Pseudomonas aeruginosa isolates from patients in GSH**

**Researchers:** N Minenza (BSc[Hons] student, UCT); R Jacobson (UCT/NHLS/NICD Molecular Epidemiology Unit); Dr Colleen Bamford (UCT/NHLS)

**Funding:** NRF

This project investigated local multidrug-resistant *Pseudomonas aeruginosa* strains. Molecular typing and antibiotic resistance gene screening confirmed an outbreak of VIM-2-producing *P. aeruginosa* of sequence type 233. One non-outbreak-related strain contained a blaGES-2 gene. Future studies are required to elucidate additional mechanisms of resistance, and the genetic structures and locations of the β-lactamase genes identified.
Association between a group of genetically related MRSA isolates and maternity and neonatal services in three hospitals and risk factors for the acquisition of MRSA among post-caesarean section patients in a maternity hospital in Cape Town

Researchers: M Mudau (UCT/NHLS/NICD Molecular Epidemiology/SAFELTP/University of Pretoria); Dr L Kuonza (SAFELTP/University of Pretoria); Prof M Nicol, Dr C Bamford (UCT/NHLS)

A retrospective cohort study confirmed the suspected association of a particular group of genetically related MRSA isolates with maternity and neonatal services in Cape Town. A case-control study was conducted to determine risk factors for MRSA acquisition among women who had caesarean section deliveries at a local maternity hospital over a two-year period. Results from 60 case-control pairs showed that blood transfusion, estimated intraoperative blood loss > 500ml and high birthweight were independent risk factors for MRSA acquisition. Maternal age between 20 to 35 years had an independent protective effect against MRSA acquisition.

Development of a novel, rapid diagnostic test for the detection of TB

Researchers: Prof M Nicol (NHLS/UCT); V Allen, Dr L Ah Tow (UCT)

Funding: NIH (PATH)

The aim is to develop a novel, rapid and easy-to-use diagnostic test that requires minimal energy so that it can be implemented in point-of-care facilities in resource-poor settings. The test is a nucleic acid amplification test (NAAT), involving the design and optimisation of a real-time PCR (RT-PCR) assay. A sensitive RT-PCR assay targeting the hypervariable V2 region of the 16S rRNA gene has been developed. The RT-PCR utilises TaqMan primers and probe (hydrolysis probe). The DNA limit of detection (LOD) of the assay is three Mycobacterium tuberculosis H37Rv (M. tb) genome equivalents. The RT-PCR assay will also be used as a means of assessing the efficiency of the specimen processing protocol (in this study the specimen being sputum). All aspects of sputum processing will be addressed. Making use of TB negative sputa that was spiked with known cell densities of M. tb H37Rv, the following processing steps could be addressed: sputum liquefaction, sonication (for cell lysis) of thinned sputum, DNA extraction/purification by magnetic bead technology and finally RT-PCR to detect the extracted DNA. This study showed that DTT/NaOH is the best sputum liquefaction agent, from a total of 14 tested. Sonication of the thinned sputa for two minutes in a cup-horn sonicator at amplitude 50%, yielded similar results as sonicating for 30 minutes with intermittent ‘rests’. DNA extracted and purified from the lysed cells, using a magnetic bead technology, is added as template for the RT-PCR assay. At present, certain steps along the sputum processing chain is still being optimised.

TEACHING AND TRAINING

Undergraduate

Although the majority of the teaching involves second and third year medical students, the division also teaches and examines students in years four to six. Teaching activities include facilitation of problem-based learning, lectures, tutorials and practicals. All practicals are now computer-based. The department hosted six second year students for four-week special study modules, and one fifth year student for an elective. In 2011 an intercalated molecular medicine course was presented for the first time. Nine students will take this course in 2012.

Postgraduate

The registrars are an integral part of the laboratory workforce and make a valuable contribution, particularly to liaison with clinicians. They gain knowledge and experience through daily laboratory bench work and participation in on-call rosters. They attend regular departmental meetings and journal clubs, as well as ward rounds and clinical meetings. There is a structured programme of tutorials, as well as opportunities to attend specialised NHLS training courses. The division’s postgraduate programme also includes training at Masters and Doctoral level.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated:
7 (1 MMed, 2 FCPath[SA] Micro, 1 FCPath[SA] Clin Pat, 2 MSc, 1 BSc Med [Hons])

Postgraduate students registered:
19 (4 Postdoctoral, 7 PhD, 3 MMed, 3 MSc, 2 BSc Med [Hons])
**Publications**


Conference presentations
International: 17
National: 11

MRC/NHLS/UCT MOLECULAR MYCOBACTERIOLOGY RESEARCH UNIT

Director: Prof V Mizrahi

The Molecular Mycobacteriology Research Unit (MMRU) was established in 2000 as a three-way partnership between the MRC, NHLS and the University of the Witwatersrand (Wits). From 2004-2010, the MMRU constituted the Wits/NHLS node of the Department of Science and Technology (DST)/NRF Centre of Excellence for Biomedical TB Research (CBTBR). In January 2011, the MMRU moved to UCT and, in partnership with Wits and US, now forms the UCT node of an expanded CBTBR. The move to UCT coincided with the appointment of Prof V Mizrahi as Director of the Institute of Infectious Disease and Molecular Medicine at UCT. The new academic home of the unit is the Division of Medical Microbiology, Department of Clinical Laboratory Sciences, Faculty of Health Sciences, UCT.

RESEARCH FUNDING

Local funding was received from UCT, Wits (Mellon Postgraduate Mentoring Award); MRC, NRF and the Technology Innovation Agency (TIA). International funding was awarded by the Howard Hughes Medical Institute, Swiss/South Africa Joint Research Programme, European Union FP7 Programme, and Foundation for the NIH.

RESEARCH PROJECTS

The mission of the MMRU is to carry out fundamental biomedical research on aspects of the physiology and metabolism of Mycobacterium tuberculosis of particular relevance to drug resistance and drug discovery. By adopting a research strategy that is based on investigating specific aspects of the physiology and metabolism of M. tuberculosis, the MMRU has positioned itself at the front-end of TB drug discovery research. Over the past year, research has focused on six main themes.

Mechanisms of DNA repair, replication and mutagenesis in mycobacteria

Researchers: Z Ditse, DE Ndandwe (Wits); Prof V Mizrahi, Dr DF Warner

Collaborators: Prof Č Venclovas (Institute of Biotechnology, Lithuania)
An integrated genetic, biochemical and physiological approach is being applied to investigate the molecular mechanisms underlying the maintenance of DNA integrity in *Mycobacterium tuberculosis*, as well as the role of mutagenic processes that contribute to strain evolution. The rationale underlying the work in this area is that these processes are intricately associated with some of the key defining features of *M. tuberculosis*, such as its extremely slow growth rate and its ability to acquire multidrug resistance by chromosomal mutagenesis.

**The biosynthesis, transport and function of vitamin B<sub>12</sub> in mycobacteria**

**Researchers:** A Moosa (Wits); Dr K Gopinath, Prof V Mizrahi, Dr DF Warner

**Collaborators:** Prof JD McKinney (École Polytechnique Fédérale de Lausanne, Switzerland)

This study forms part of Swiss/SA Joint Research Programme-funded study on the mechanisms of propionate catabolism in *Mycobacterium tuberculosis*. The role of the MMRU team is to investigate the biosynthesis and transport of vitamin B<sub>12</sub> in mycobacteria, and the role of vitamin B<sub>12</sub>-dependent enzymes in the metabolism of *M. tuberculosis*, with special reference to factors that might allow the use of alternate B<sub>12</sub>-dependent and B<sub>12</sub>-independent pathways for propionate metabolism under specific host conditions. A major highlight of this project was the identification and characterisation of a novel vitamin B<sub>12</sub> transporter in *M. tuberculosis*.

**The biosynthesis and function of molybdenum cofactor in mycobacteria**

**Researchers:** Dr MJ Williams, Prof V Mizrahi

**Collaborators:** Dr BD Kana (Wits); Prof G Kaplan (Public Health Research Institute, USA)

This study investigates the role of molybdenum cofactor in the pathogenesis of *Mycobacterium tuberculosis*. Dr Williams has been awarded a three-month CU-SA Fogarty AITRP fellowship which will enable her to carry out *in vivo* phenotyping of selected molybdenum cofactor-deficient strains in the laboratory of Prof G Kaplan in 2012.

This theme encompasses two major research projects within the unit that are being conducted as part of large, multinational TB drug discovery consortia led out of the USA and Switzerland. TB drug discovery research represents a major thrust of the work conducted in the MMRU, and builds on the key role in the Integrated Methods for Tuberculosis Drug Discovery project which was funded by the Bill & Melinda Gates Foundation from 2007-2010.

**Structure-activity relationships of novel anti-tubercular compounds**

**Researchers:** K Naran, Prof V Mizrahi, Dr DF Warner

**Collaborators:** Dr CE Barry III, Dr HI Boshoff (NIH); Prof K Chibale (UCT); Dr R Gordon (TIA) and others within the SATRII consortium

The third TB drug discovery project in the MMRU is funded by the TIA under the auspices of SATRII – a USA-SA TB drug discovery consortium that partners medicinal chemistry expertise with microbiological know-how. The role of the MMRU in this consortium – which was established in 2011 – is to provide SAR, target identification and validation services for novel anti-tubercular compounds developed by colleagues in the UCT medicinal chemistry group.

**Physiology of drug-resistant mycobacteria: implications for pathogenesis**

**Researchers:** A Koch, Prof V Mizrahi, Dr DF Warner

**Collaborators:** Profs R Warren, NC Gey van Pittius, TC Victor and others (US)

This is a new project that was initiated in 2011. The primary objective of this work is to evaluate the impact of specific drug resistance-associated mutations on the physiology of *M. tuberculosis*.
HONOURS

Prof V Mizrahi was invited to London for an interview under the auspices of the Exchanges at the Frontier programme of the UK-based Wellcome Collection on 21 October 2011. The interview was attended by approximately 200 people. An abridged version of the interview was broadcast by BBC World on 9 December 2011 to an estimated audience of 40-50 million listeners worldwide.

Dr DF Warner was the overall winner of the prestigious 2011 BioVision-Lilly Award in conjunction with the Academy of Sciences for the Developing World (TWAS). The award recognises young researchers from developing countries for outstanding scientific achievements in TB-related research. Dr Warner was honoured for his work on mycobacterial metabolism, which promises to have a major impact on understanding the development of drug resistance in Mycobacterium tuberculosis.

Dr MJ Williams and A Moosa were both awarded Keystone Symposia Global Health Travel Awards to attend the Keystone Symposium on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics, held in Vancouver in January 2011.

A Koch received the Benfara Scholarship from UCT and a DAAD-NRF Joint Scholarship from the NRF. K Naran was awarded a UCT Equity Scholarship and a DAAD-NRF Joint Scholarship from the NRF. Z Ditse was awarded the Marion Beatrice Waddel Scholarship and an Equity Scholarship from UCT and a DAAD/NRF Joint Scholarship from the NRF.

DE Ndwandwe and Dr DF Warner were awarded a K-RITH Collaborative Travel Award. Ndwandwe also received a Mellon Postgraduate Mentoring Award and a Postgraduate Merit award from Wits University.

A Moosa was awarded a Postgraduate Merit Award from Wits University.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 1 MSc
Postgraduate candidates enrolled: 9 (3 postdoctoral fellows, 5 PhD, 1 BSc (Hons))

RESEARCH OUTPUT

Publications


Conference presentations

International: 5
National: 10

TEACHING

Lectures were given in the medical microbiology component of intercalated MBChB programme, on TB in the ‘Defense and disease’ and ‘Two superbugs’ modules and in the postgraduate interactive writing workshop.
DIVISION OF MEDICAL VIROLOGY

Head: Prof C Williamson

DIAGNOSTIC SERVICES

The diagnostic laboratory has maintained its SANAS accreditation status. It offers a wide repertoire of tests in viral serology and molecular diagnostics with a special interest in molecular diagnosis with an extensive range of in-house and commercial molecular assays. It provides a comprehensive service to GSH and RCCH, and is the referral laboratory for the Western Cape province. It is one of two centres in the Western Cape where HIV viral load and PCR testing is done for the Comprehensive Care Management and Treatment (CCMT) programme.

From April 2011 to March 2012 the laboratory performed approximately 85,000 HIV viral loads and 10,000 HIV PCR tests. Turnaround time (TAT) has been a strong focus and a special effort has been made to shorten the TATs through restructuring and multi-task training. The average TAT for HIV viral loads improved from 63 hours in March 2011 to 32 hours in March 2012.

The use of multiplex PCR for diagnosis of respiratory virus infections continues to provide value to healthcare. Diagnosis of severe acute respiratory infections (SARIs) in young infants is useful for patient management and tracking local epidemiology of these infections. Seasonal prevalence of the different respiratory viruses, in particular that of human metapneumovirus, has been tracked, and this assay also facilitated the recognition of nosocomial outbreaks. Two outbreaks were investigated over the past year, one caused by influenza A virus in the RCCH intensive care unit (ICU) and a second due to respiratory syncytial virus (RSV).

The laboratory is involved in operational research, in collaboration with the UCT Department of Public Health, to assess the quality and impact of HIV early infant diagnosis in the Western Cape province. The goal of this research is to develop a rapid result notification system through the Corporate Data Warehouse (CDW) to clinics to facilitate follow up of HIV PCR-positive infants and thus improve service delivery at primary healthcare level. This work also strengthens the direct interaction between the NHLS pathology service and local health authorities.

Outreach

The diagnostic laboratory has an outreach programme to support the laboratories in the Eastern Cape. This support includes site visits, as well as providing remote support such as telephonic consultation on problems in the laboratory and EQA checking. The laboratory also acts as a referral centre for the less routine serology tests, and the non-HIV molecular diagnostic tests. A site visit was made to the Mthatha virology and serology laboratories as part of these laboratories’ preparation for SANAS accreditation, which they achieved later in the year.

RESEARCH PROJECTS

Molecular Epidemiology Unit (UCT/NICD)

This unit was established in the microbiology and virology diagnostic laboratories at GSH in 2010 to augment the capacity of the routine laboratory to investigate disease outbreaks and to train medical scientists. The following were projects performed in the unit over the past year:

Molecular epidemiology of human metapneumovirus

Investigators: Dr H Smuts, N-C Hu, Dr D Hardie

Human metapneumovirus (hMPV) is responsible for a spectrum of respiratory disease, especially in young children. Surveillance of infants with severe acute respiratory infections in the Western Cape hospitals was conducted over a 20-month period from 2009 to 2010. From the study, 4% (198/4,911) of respiratory samples were hMPV-positive. The virus was found predominantly in children less than 3 years of age, of which 55% were found in infants less than 6 months of age. Phylogenetic analysis of the fusion gene from the hMPV-positive samples showed genotype A2 was predominant in both 2009 and 2010, with co-circulation of genotypes B1 and B2.

Investigation of a nosocomial cluster of influenza A at RCCH

Investigators: M Mudau, Dr Z Valley-Omar, N-C Hu, Dr N-Y Hsiao

An investigation of a suspected nosocomial influenza outbreak between 25 May and 25 June 2011 in the cardiac ICUs of RCCH was carried out. Sequence comparison of the influenza haemagglutinin gene revealed four distinct lineages of influenza A that were circulating in paediatric hospitals around Cape Town, and one nosocomial cluster in the RCCH ICU was identified after combining epidemiological information with phylogenetic analysis.
Characterisation of measles virus from patients with sub-acute measles encephalitis

**Investigators:** Dr D Hardie, J Heckmann, C Albertyn, H Smuts  
**Funding:** Poliomyelitis Research Foundation (PRF)

A cluster of cases of sub-acute measles encephalitis (SME) was identified in patients infected during the 2009/10 measles epidemic in South Africa. Affected patients were all HIV-infected with very low CD4 cell counts. Analysis of brain virus from four patients revealed that the virus was very similar to the epidemic virus, but each patient had a distinct pattern of mutations in one or more of the major structural genes (matrix, fusion, haemagglutinin or nucleoprotein). Forty percent of mutations were A to G or U to C, a hallmark feature of measles virus from brains of patients with subacute sclerosing panencephalitis (SSPE). This suggests that a similar process to SSPE is occurring in SME. The lower number of mutations could reflect a shorter incubation time associated with SME.

**Evaluation of early infant HIV diagnosis services in the Western Cape, 2005-2011, using routine laboratory data**

**Investigators:** Dr N Hsiao, K Stinson, L Myer

This study determined the progress and barriers of the current early infant diagnosis programme by using archived CDW data of infant HIV diagnosis and linked, subsequent HIV viral load. The proportion of PCR-positive children declined steadily from 12% in 2005 to 3% in 2011. The delay between HIV diagnosis to initiation of antiretroviral therapy (ART) was reduced from 146 days in 2005 to 33 days in 2010. However, despite this improvement, a worrying 65% of HIV-infected children did not appear to have been enrolled to receive ART as no subsequent linked HIV viral load was found for these infants. This pilot study suggests that the CDW could be a powerful tool to facilitate result notification and help reduce the number of patients lost to follow up. In this way the NHLS could support the implementation of primary health programmes.

"The Corporate Data Warehouse could be a powerful tool to facilitate result notification"

Pan-polyomavirus screening of patients with meningitis and encephalitis: a pilot study

**Investigators:** Dr H Smuts, D Hardie

Polyomaviruses cause persistent infections in humans and may reactivate and cause disease in immunocompromised patients. The aim of this study was to use pan-polyomavirus primers to screen cerebrospinal fluid (CSF) samples from patients with meningitis/encephalitis by PCR to search for a novel virus or disease association. In total, 235 CSF samples were tested. One sample from a 3-year old girl with meningitis was positive. Sequencing of the PCR product identified a BK virus of genotype Ia. Meningitis associated with BK virus infection is rare and rearrangements of the non-coding control region (NCCR) may permit replication in the central nervous system (CNS). Amplification and sequencing of 10 clones of the NCCR region identified the archetypal virus. The significance of this is still under investigation.

**HIV VACCINE DEVELOPMENT GROUP AND HUMAN PAPILLOMAVIRUS RESEARCH GROUP**

Development of HIV vaccines

**Investigators:** Prof A-L Williamson, Prof C Williamson, Dr K Downing, Dr E Hurter, M Lambrick

**Funding:** South African AIDS Vaccine Initiative (SAAVI)/LifeLab, NRF, HIV Vaccine Trials Network (HVTN)
One long-term strategy for the control of the HIV/AIDS pandemic is the development of an HIV/AIDS vaccine. The first two vaccines, developed at UCT, tested in phase 1 clinical trial, are moderately immunogenic in humans. The next trial including these vaccines with a protein boost started recruiting at the end of 2011.

**Optimisation of rBCG as an HIV vaccine vector**  
**Investigators:** Prof A-L Williamson, Dr R Chapman, Dr GK Chege, M Lebeko, P Mbele, A/Prof EG Shephard  
**Funding:** NIH, NRF

BCG, the TB vaccine, is being developed as a potential HIV vaccine vector. The stability of rBCG and increased HIV protein expression were successful and the vaccines shown to be immunogenic in mice. The individual BCG vaccines expressing gag, RT and gp120 as well as a mix of these vaccines are immunogenic in that they prime the immune system for a SAAVI-MVA boost as observed by induction of cells recognising epitopes in all the vaccine HIV proteins that produce IFN-γ, TNF-α and IL-2. This was seen when the rBCG vaccines were tested individually or as a mix. The vaccines are presently being tested in non-human primates.

**Investigation into different strains of BCG to deliver vaccine transgenes**  
**Investigators:** Prof A-L Williamson, Dr R Chapman, Dr H Stutz, S Chetty, Dr G Chege, Dr N Douglass, Dr E Shephard  
**Collaborators:** W Jacobs Jr (Albert Einstein College of Medicine, USA); Aeras, B Ryffel (CNRS, France)  
**Funding:** NRF, NIH

Laboratory data suggest that the pan CD strain of BCG expressing HIV gag induces a better CD8 response to foreign proteins than the Pasteur strain of BCG. This is a novel strain of BCG (Pasteur) that has been disabled in the production of vitamin B5 (pan mutant). The mutant is subsequently unable to manufacture fatty acids or polyketides. Unlike BCG, the pan mutant (panBCG) does not kill SCID mice. After the boost, high frequencies of predominantly Gag-specific CD8+ T cells were detected when BCGpan-Gag was the prime in contrast to induction of predominantly Gag-specific CD4+ T cells when priming with BCG-Gag.

**Development of avipoxviruses as vaccine vectors**  
**Investigators:** Prof A-L Williamson, O Carulei, K Offerman, Dr N Douglass  
**Funding:** NRF

Interest in the avipoxviruses, notably fowlpoxvirus and canarypoxvirus, has increased due to their successful use as vaccines on commercial flocks and their extensive use and testing as vaccine vectors in humans and animals. The project was extended to identify other local avipoxviruses. Thirty-five local avian poxvirus isolates were acquired from around South Africa. These viruses were grown in embryonated hen’s eggs to produce virus stocks. Three genes from each of these viruses were amplified and sequenced for phylogenetic comparison to previously described viruses. This study showed that isolates from wild birds such as penguin, pigeon and lesser flamingo are genetically different to isolates from canaries and domestic chickens.

**Natural history of human papillomavirus infection in South African men and women recruited for a study on HIV discordant couples**  
**Investigators:** Prof A-L Williamson, ZZA Mbulawa, M Hoffman, D Coetzee, J Moodley, D Marais  
**Funding:** PRF, Swedish International Development Cooperation Agency, Cancer Association of South Africa (Cansa), NRF, MRC

During the past year, human papillomavirus (HPV) transmission between HIV-concordant negative, HIV-concordant positive and HIV-discordant (where one partner is HIV-positive) heterosexual couples was investigated. Female to male HPV transmission was more common compared to male to female HPV transmission. HIV-positive women were found to be at high risk of HPV transmission from their male partners compared to HIV-negative women. HIV-positive men with <350 mL CD4 counts had high risk of HPV transmission from female partners compared to HIV-positive men ≥350/mL CD4 counts.

**World Health Organization HPV LabNet for the African Region**

HPV is the cause of cervical cancer. HPV vaccination has not been introduced into the public sector in South Africa. Research is being done with a number of groups in South Africa to provide HPV typing and prevalence data in HIV-negative and HIV-positive women to provide some baseline data for vaccine introduction and also to inform policy on cervical screening.

**Sequence analysis of HPV**  
**Investigators:** Prof A-L Williamson, B Allan, D Marais, T Meiring, E Rybicki, J Moodley, A Salimo, XK Mndende  
**Funding:** PRF, NRF, MRC
Data on HPV incidence and genotype distribution are based on commercial HPV detection kits, but these kits may not detect all HPV types in HIV-positive women. Illumina sequencing identified a total of 16 HPV genotypes in the selected specimen, with four genotypes (HPV-30, 74, 86 and 90) not included in commercial kits. The significance of these types in relation to cervical disease remains to be investigated. Sequencing is also being done to determine which HPV variants are shared in South African couples.

**HIV DIVERSITY AND PATHOGENESIS GROUP**

**The role of CTL escape mutations in attenuating HIV-1 subtype C infection**

**Investigators:** Prof C Williamson, Prof CM Gray, RS Ntale, DR Chopera, Dr NK Ngandu  
**Co-investigators:** Dr K Mlisana; Prof S Abdool Karim  
(University of KwaZulu-Natal [UKZN])  
**Funding:** NIH/NAID

First generation CTL-based vaccines are likely to be only partially effective, and in individuals who become HIV-infected despite vaccination, immune responses to epitopes where escape is associated with a fitness cost to the virus might result in lower viral loads. Attenuating CTL escape mutations were identified in Gag associated with HLA-B*81:01 and B*39:10 alleles. These were also associated with lower viral load *in vivo*; however, the development of compensatory mutations coincided with spontaneous increase in viral loads. While these individuals still controlled virus to levels lower than the rest of the cohort, escape had a detrimental impact of B*81:01-mediated control of viraemia.

**Acute HIV-1 infection prospective cohort study**

**Principal investigator:** Dr B Haynes (Duke University, USA)  
**UCT investigators:** Prof C Williamson, M-R Abrahams, S Goodier, Prof CM Gray  
**Co-investigators:** Dr K Mlisana, Prof S Abdool Karim  
(UKZN)  
**Funding:** Centre for HIV/AIDS Vaccine Immunology (CHAVI), NIH/NAIAD

Understanding the characteristics of the transmitted virus, and its early diversification, is important for vaccines which may need to control early viral diversification. The CHAVI viral sequencing core generates HIV-1 sequences (env and full-length genome) and functional clones (env and full-length genome) from acutely and chronically infected individuals with the aim of elucidating properties of transmitted viruses and subsequently the genetic changes to the virus early in infection. In this study over 120 full-length sequences were generated from five individuals with acute subtype C HIV infection and the sequence of the virus responsible for clinical infection was derived. Viral evolution was then precisely monitored over six months. Evidence was found of CTL escape as early as two weeks post infection with most escape occurring in the viral structural genes within the first five weeks of infection and diminishing as viral load is controlled followed acute infection. These results illustrate the complexity and dynamics of early immune pressure across the genome which challenge subtype C vaccine design.

**Comprehensive Antibody Vaccine Immune Monitoring Consortium (CA-VIMC)**

**Principal investigator:** Dr D Montefiori (Duke University, USA)  
**UCT investigators:** Prof C Williamson, G Bandawe, R Thebus  
**Funding:** Bill and Melinda Gates Foundation

The goal of the CA-VIMC is to facilitate the discovery and timely licensure of a safe, effective and practical HIV-1 vaccine for the world. A further five years’ funding has been obtained for this project. To study the genetic and antigenic diversity of HIV-1 strains currently circulating in South Africa, and to ask how well the strains are represented by current vaccine immunogens and reference reagents, the investigators are currently establishing a subtype C panel to characterise neutralisation serotypes. To date, they have generated functional env clones from 143 participants.

**Sequencing of breakthrough infections from the Phambili Study/HVTN503 Phase IIb MRKAd5 HIV**

**Investigators:** Prof C Williamson, M Logan, C Rademeyer, Dr NK Ngandu, J Marais  
**Collaborators:** Prof G Gray (PHRU, Witwaterand University); Dr J Mullins, Dr JM McElrath (University of Washington, USA)  
**Funding:** HIV Vaccine Trials Network (NIH)

This vaccine trial did not prevent infection nor reduce viral load set-point. To determine whether evidence of vaccine effect can be seen, as in the sister trial in the USA, full-length genome sequences (5-10 participants)
were generated from 43 participants - 20 placebo and 23 vaccinees. The results demonstrate much weaker, if any, MRKAd5 sieve effects on the virus in the Phambili trial, which may be explained by few infection endpoints and thus limited statistical power; incomplete vaccination courses; and use of a clade B immunogen in a predominantly clade C region underscoring the potential importance of matching vaccine inserts to the predominant HIV-1 subtype of the region.

Sequencing of breakthrough Infections from the CAPRISA 004 tenofovir microbicide trial

Investigators: Prof C Williamson; Prof S Abdool Karim, S Goodier, Dr S Sibeko, Dr V Naranbhai, Dr T Ndugu (UKZN)
Funding: CONRAD (USA), Technology Innovation Agency (South Africa)

In the CAPRISA 004 trial, individuals in the placebo and tenofovir arm had differential disease progression based on viral load and CD4+ measurements at 12 months post infection. To determine if these differences can be attributed to mutations in gag, the investigators assessed the in vitro replication capacity of infectious molecular clones containing the gag-protease region from these participants. To date, they have obtained samples from 88 CAPRISA participants from the enrolment visit. The gag-protease region from 84 individuals was amplified and sequenced. Of these, 83 were infected with subtype C viruses and one was infected with a subtype A1 virus.

HIV MUCOSAL IMMUNOLOGY GROUP

Impact of genital inflammation, HIV co-infection and HPV-specific cellular immunity on HPV burden in the female genital tract

Principal investigator: Dr J-A Passmore
Co-investigators: Prof A-L Williamson, Dr F Little, Prof L Denny, Dr P Gumbi
Funders: CANSA

The study evaluated the level of genital tract inflammation in women in stable relationships with partners who are either concordant or discordant in HIV status; investigated the impact of inflammation on HPV viral load and/or persistence, and investigated HPV-16-specific T cell immunity in the female genital tract. Preliminary data indicate that several key biomarkers for genital tract inflammation are significantly elevated in genital secretions from HIV-negative women in discordant relationships with HIV-positive partners compared to HIV-negative women in concordant relationships with uninfected partners. In addition, HIV-negative women have levels of inflammation similar to HIV-positive women. HPV-16-specific immunity in the genital tract was more readily detectable in HIV-infected women than HIV-negative women although HIV-negative women had significantly higher CD4+ T cell responses to HPV-16 E7 antigens than HIV-infected women.

Impact of ART on HIV shedding, inflammation and the quality of HIV-specific immunity in the female genital tract of women chronically infected with HIV

Principal investigator: Dr J-A Passmore
Co-investigators: Dr W Hanekom, Dr G Walzl
Funding: MRC

Initiation of HAART in HIV-infected individuals is associated with rapid and highly effective control of systemic viraemia and rebound of blood CD4 cell counts over time. The study evaluated HIV shedding in the female genital tract over time in HIV-infected women on ART compared to those who are therapy-naïve, and explored the association between genital HIV shedding and genital inflammation, CD4 restoration and quality of mucosal immunity in the context of ART. It was shown that in HIV-infected women HAART is associated with significantly improved CD4 T cell counts both in blood and at the cervix. While HAART effectively suppressed both blood and cervical viraemia, HIV-specific CD8 T cell responses in blood were lost while those at the cervix were preserved.

Cervicovaginal specimen collection for evaluation of mucosal immune responses

Principal investigator: Prof JM McElrath (University of Washington, USA)
Co-investigators: Dr J-A Passmore, Dr R Kaul, Dr C Hendrix, Dr A Landay, Prof R Shattock
Funding: NIH

This international multicentre study seeks to standardise the procurement and processing of non-invasive specimens from the female genital tract for yields of viable leucocytes and their subtypes across six international sites in the USA, UK, Kenya and South Africa for roll-out to HIV vaccine trial sites. Cervical cytobrushes were obtained from 30 HIV-negative women attending the family planning clinic at GSH. Of these, 20 were using Depot medroxyprogesterone acetate (DMPA). The
number of CD45+, CD3+, HLA-DR+, CD11c+ lymphocytes in cytobrushes were quantified from each woman. Cape Town had significantly higher lymphocyte counts per cytobrush compared to the other international sites. DMPA did not significantly impact on the number of lymphocytes recoverable. Because the study focused only on women with no active sexually transmitted infections (STIs), inflammation as a result of STIs was not the cause of elevated numbers of cells recovered.

Role of genital tract immunity in prevention and control of HIV infection
Principal investigator: Dr J-A Passmore
Co-investigators: P Gumbi, Dr F Little, Dr D Coetzee, Dr H Jasp
Funding: South African HIV/AIDS Vaccine Platform (SHARP), LifeLab, DST

The study evaluates immunity in the female genital tract of women in discordant relationships that may be associated with protection from or susceptibility to HIV infection. It was found that the extent of CD4 depletion in the female genital tract was significantly associated with the level of CD4 T cell activation at the cervix. In HIV-infected women, the frequency of T cell activation in the female genital tract during HIV infection was broadly and significantly associated with genital tract viral loads, indicating that HIV shedding in genital secretions was related to the presence of high frequencies of activated T cells at this site. The study is ongoing and will lead to identification of biomarkers of reduced HIV susceptibility that would provide novel targets for therapeutics and candidate microbicide targets.

HONOURS

Denis Chopera was awarded a Canadian Institutes of Health Research Fellowship. Andile Nofemela was awarded the Fogarty-AIDS International Training and Research Program Fogarty Scholarship.

Clinical Infectious Diseases Research Initiative fellowships were awarded to Alfred Bere, Nonhlanhla Mkhize, Zizipho Mbulawa, Olivia Carulei, Cobus Olivier and Nobubelo Ngandu. Carnegie fellowships were awarded to Andreia Soares, Lindi Roberts, Marcel Tongo, Shameem Jaumdally, Gama Bandawe and Shivam Chetty.

Agano Kiravu won the Discovery Health Community Award for best presentation at 5th SA AIDS Conference, Durban in June 2011.


 Conference presentations

 International: 7
 National: 2

“A special effort has been made to shorten turnaround times through restructuring and multi-task training”
DEPARTMENT OF ANATOMICAL PATHOLOGY

Head: Prof CA Beukes

DIAGNOSTIC SERVICES

The five full time pathologists and one half day pathologist provided a diagnostic surgical pathology service to all the provincial hospitals in the Free State, and to some of the provincial hospitals in the North West Province. From time to time work was also sent from the Northern Cape. A consultation service was provided to numerous private pathology laboratories as well an immunohistochemical service for the Northern Cape. A cytopathology service was provided to the Free State, Northern Cape and North West provinces. During the year, 24,494 (compared to 22,529 the previous year) surgical pathology cases and 87,005 (previously 57,258) cytopathology cases were seen, which represents an overall increase of 31%. A post mortem service was also provided.

TEACHING AND TRAINING

Undergraduate
The department presents a module on general pathology to the second year medical students. Sessions on systematic pathology, which are integrated into system modules, are conducted with both second and third year students. The total contact time is approximately 140 hours per year. A short course in general pathology is provided to third year physiotherapy and occupational therapy students.

Postgraduate
Daily departmental and interdepartmental meetings are held. Registrars in forensic pathology rotate through the department for two years; registrars in oncotherapy spend three months in the department while orthopaedic, dermatology and ophthalmology registrars receive special training on a weekly basis for various lengths of time.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduating: 1
Postgraduate candidates registered: 6

RESEARCH OUTPUT

Conference presentations
International: 2

“Surgical pathology and cytopathology workload increased by 31% during the past year”

DEPARTMENT OF HAEMATOLOGY AND CELL BIOLOGY

Head: Prof M Coetzee

DIAGNOSTIC SERVICES

The department offers haematological diagnostic services to Universitas Hospital, and serves as a referral centre for laboratories in the Free State and Northern Cape as well as for some in the North West Province. The total number of tests performed was 103,307.

The Specialised Haemostasis Unit is offering tests for the activity of the ADAMTS13 enzyme using methods
that have significantly reduced costs. The Flow Cytometry Unit is offering quantitative enumeration of leukaemia cells in cerebrospinal fluid. A preliminary agreement was entered into with the University of the Free State under the Umbrella Agreement to offer molecular monitoring of chronic myeloid leukaemia testing.

In the Universitas Hospital haematology clinics, 3,213 patients were seen, while 188 were seen at the outreach clinics in Kimberley, Welkom, and Bethlehem.

**RESEARCH PROJECTS**

Ongoing projects include phenotypic and genotypic characterisation of patients with hereditary haemorrhagic telangiectasia in the Free State and Northern Cape, characterisation of platelet surface antigens of primates, thrombotic thrombocytopenic purpura models, and characterisation of bleeding disorders in the Northern Cape.

**Other projects are:**

**Thrombotic thrombocytopenic purpura in HIV patients**

*Researchers:* Prof M Meiring; Prof V Louw (Division of Clinical Haematology), and various postgraduate students

This project is investigating the pathogenesis of thrombotic thrombocytopenic purpura, focusing on the epidemiology, the role of microparticles in its pathogenesis, investigation of ADAMTS13, and improving diagnosis. The aim is to improve the understanding and improve the management of this condition and related disorders.

**Von Willebrand factor in health and disease**

*Researchers:* Prof M Meiring, and postgraduate students

*Funding:* NHLS Research Trust

Aspects included in this research project are the characterisation of a tissue factor inhibitory antibody fragment, the development of a cost-effective ADAMTS13 antigen assay, development of a cost-effective Von Willebrand factor-propeptide antigen assay, the effect of inflammation and thrombosis on ADAMTS13 and Von Willebrand factor expression in cultured endothelial cells, the selection of an antibody fragment to human factor VIII and the effect of different storage conditions on Von Willebrand factor activity.

**Preclinical haemostatic drug development**

*Investigators:* J Roodt, W van Rensburg

Various drugs are being tested on primate models, and new models are being developed.

**Investigation of other areas of the JAK2 gene in myeloproliferative neoplasms**

*Researchers:* Dr A de Kock, and students

*Funding:* NHLS Research Trust

Mutations in exons other than exon 14 (where the classic mutation lies) are being characterised. Novel techniques of using the patient’s own non-myeloid cells as controls are being investigated.

**Investigation of mutations of in the BCR-ABL fusion gene in chronic myeloid leukaemia**

*Researchers:* Prof C Viljoen, and students

*Funding:* GMO Testing Facility

The study investigates the use of high-resolution melting curve analysis and other techniques to identify mutations, and correlate these with disease progression and response to treatment.

**HONOURS**

Prof Muriel Meiring was awarded a C2 research rating by the NRF which recognises her as an established researcher with a sustained recent record of productivity.

At the Faculty Research Forum Dr L Pretorius was the Senior Winner, Clinical Poster, while W Allers received the Junior Winner, Laboratory Paper award.

The Dean’s Medal for the best Master’s student in the Faculty of Health Sciences at the Autumn Graduation Ceremony was awarded to Dr R Weyers and the best BMedSc(Hons) student in the School of Medicine was H du Plessis.

**TEACHING AND TRAINING**

**Undergraduate**

The undergraduate teaching involves the BSc Human Molecular Biology modules (Faculty of Natural and Agricultural Sciences), MBChB (a haematology and immunology module for second year students and various lectures to third, fourth and fifth year students) and to optometry students (haematology and immunology module).
The department also assists the medical laboratory technology students in their preparation for various examinations.

Postgraduate
Postgraduate courses offered by the department include BMEdSc(Hons) in haematology and human molecular biology, MMEd(Haemat), MMEd(Clin Path), a postgraduate diploma in clinical haematology, postgraduate diploma in transfusion medicine, and the School of Nursing’s short course in blood transfusion principles and practice.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 7 (2 MMEd(Haemat), 1 FCPath(SA)(Haem), 4 MMEdSc)
Postgraduate candidates enrolled: 21 (3 MMEd(Haemat), 1 MMEd(Clin Path), 11 MMEdSc, 4 BMEdSc(Hons), 2 PhD)

RESEARCH OUTPUT

Publications
Coetzee MJ. Inappropriate delegation. United Kingdom Casebook 2011; 19(2): 24


Conference presentations
International: 10
National: 14
Local: 11

DIVISION OF HUMAN GENETICS

Head: Prof M Theron

DIAGNOSTIC SERVICES

The division is SANAS-accredited and provides almost all human genetics services to the Northern Cape and parts of the Free State. The laboratories primarily render a comprehensive diagnostic service to the Universitas, 3 Military, Pelonomi, Kimberley, Upington, Bongani and Tshepong hospitals, surrounding clinics and various private pathology firms. Comprehensive genetic services encompass the three sub-disciplines: molecular genetics, molecular cytogenetics (or fluorescent in situ hybridisation) and cytogenetics.

Mutation screening in the molecular genetic laboratory is mostly PCR-based and population-directed. The division provides an extensive routine diagnostic screening for inherited breast cancer, and referrals throughout South Africa are diagnosed. The fluorescence in situ hybridisation (FISH) laboratory renders a pre-and post-natal screening programme based on microdeletions and common chromosomal aneuploidies as well as various haematological neoplasias. The cytogenetic laboratory provides a pre-and post-natal laboratory service for congenital and acquired chromosomal abnormalities. Cytogenetic analysis plays a major role in the diagnosis, prognosis and treatment of acquired genetic aberrations associated with haematological malignancies. Traditional cytogenetic analysis is performed on peripheral blood, bone marrow, amniotic fluid, products of conception and skin fibroblasts.

RESEARCH

Research is focused on familial breast cancer and includes the identification of pathogenic molecular lesions in the coloured and Sotho/Tswana populations of South Africa and the effect of other genes in the presence of the BRCA genes. Projects are all funded by the NHLS Research Trust.
HONOURS

Dr NC van der Merwe was awarded the Muller Potgieter Medal for best laboratory paper at the annual Faculty Forum.

TEACHING AND TRAINING

Intern medical scientists
The division is a HPCSA-accredited training facility for intern medical scientists. The vision of the division is the simultaneous incorporation of all intern medical scientists in full-time Master’s or Doctorate programmes.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled: 1 MMedSc

RESEARCH OUTPUT

Congress presentations
International: 6
Local: 3

DEPARTMENT OF MEDICAL MICROBIOLOGY AND VIROLOGY

Head: Dr E Elliott

DIAGNOSTIC SERVICES

The department provides a 24-hour microbiology and virology telephonic consultative service to Universitas, National and Pelonomi hospitals, and to the Free State as part of the training and outreach platform. It also provides information on request to medical practitioners and healthcare workers, and hospital patients are seen on a consultation basis.

The outreach programme to Kimberley and Bongani hospitals has been plagued by a shortage of staff and resources; only one visit was therefore possible during the year, but telephonic support of the management team with antibiotic issues and infection control matters has continued.

In the TB culture laboratory the initial implementation challenges remained due to the large volumes and plans have stalled to create sufficient space and facilities.

An increase in the work volume has been noted in line with the HIV burden; plans include capacity increase so that the service can accommodate the anticipated growth.

During the past year, the medical microbiology laboratory processed 301,334 bacteriology and 10,759 serology tests. The medical virology laboratory tested 12,914 serology, 119,436 viral load and 17,542 HIV PCR samples.

RESEARCH PROJECTS

Vector-borne and zoonotic viruses research group

Head of research group: Prof FJ Burt
Collaborators: Prof R Swanepoel, Prof J Paweska (NICD); Prof M Heise (Carolina Vaccine Institute, North Carolina, USA)

Research focuses on characterising humoral and cellular immune responses in patients with Crimean-Congo haemorrhagic fever (CCHF) virus and other arboviruses of medical significance, epitope exploration for vaccine development, development of molecular and serological assays for detection of arboviruses and other neglected diseases; and evaluation of vaccines against CCHF, yellow fever and Rift Valley fever viruses.

Human papillomavirus research group

Head of research group: Prof FJ Burt
Collaborator: Prof R Seedat (Otorhinolaryngology Department)

The research group focuses on the identification and characterisation of human papillomavirus associated with recurrent laryngeal papillomas.

HIV research group

Head of research group: Dr D Goedhals
Collaborators: Dr C Jansen van Vuuren, Dr D Steyn (Department of Internal Medicine, UFS); Dr C Seebregts (MRC); Dr T de Oliveira (Africa Centre, UKZN); Dr J Frater, Prof R Phillips (University of Oxford, UK)

The research group focuses on HIV drug resistance genotyping and surveillance in the public sector treatment programme and immunological studies including T cell function and viral adaptation in AIDS.

HONOURS

Prof FJ Burt was awarded a C1 NRF rating in January 2012 which recognises her as an established researcher.
with a sustained recent record of productivity enjoying considerable international recognition.

Prof Burt received the Roche Floating Trophy for best senior laboratory presentation at the Faculty of Health Sciences/AstraZeneca Forum, held in April 2011; L Matheng received runner up award for best junior laboratory presentation; S Smouse was the co-winner of the UFS 3 Minute dissertation competition for masters’ students; and Dr I Rossouw was the junior winner in the category: Clinical Poster Presentations.

S Smouse and H Hanekom, postgraduate MMedSc, students in virology, were awarded the prestigious Stars of Academe and Research scholarships from the Central University of Technology.

TEACHING AND TRAINING

Most undergraduate lectures are presented in English and Afrikaans. Lectures are presented to MBChB II and III, BSc MedMicro III, B Optometry III and to physiotherapy and occupational therapy students.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated:
5 (2 MMed, 1 MMed Sc, 2 BSc [Hons])

Postgraduate students enrolled:
13 (4 MMed, 2 PhD, 5 MMed Sc, 2 BSc [Hons])

RESEARCH OUTPUT

Publications


Burt FJ. Laboratory diagnosis of Crimean–Congo hemorrhagic fever virus infections. Future Virology 2011; 6(7): 831-841


Conference presentations
International: 4
National: 11
Local: 10
THE DEPARTMENT OF ANATOMICAL PATHOLOGY

Head: Prof PK Ramdial

DIAGNOSTIC SERVICES

The Department of Anatomical Pathology reported on 36,100 surgical pathology and 68 autopsy specimens. A total of 116,844 cytopathology specimens were processed; these included 103,648 gynaecological cases, 5,896 non-gynaecological specimens and 7,300 fine needle aspirates.

TEACHING AND TRAINING

Undergraduate

Teaching to undergraduate MBChB students included 60 lectures and 22 practicals to second year and six lectures to third year medical students at the Nelson R Mandela School of Medicine. The department was also involved in the end of theme and end of module assessments for the second and third years, and presented lectures to clinical science students.

Postgraduate

During the year, 33 clinicopathological meetings were undertaken with the clinical and surgical disciplines that allowed interaction with clinical colleagues and contributed to improved understanding of disease processes in general, and helped with management of individual patients in particular. The postgraduate training programme facilitated learning and intradepartmental interaction at 14 journal clubs, seven seminars, 15 short topics, eight post mortem seminars and 21 ‘box’ slide assessments.

Technologists/technicians

Two medical technicians passed the Professional Board Examination in histopathological techniques. Three student cytotechnologists wrote the Board exams; two distinctions were obtained and one student received the highest mark nationally for cytology. Three medical technicians completed their third year bridging course in biomedical technology.

RESEARCH OUTPUT

Publications


THE DEPARTMENT OF CHEMICAL PATHOLOGY

Acting head: Dr V Gounden

DIAGNOSTIC SERVICES

The Department of Chemical Pathology provides diagnostic and consultative services to the academic complex of King Edward VIII and Inkosi Albert Luthuli Central hospitals (IALCH). IALCH laboratory processes over 140,000 tests monthly and provides specialised services such as organic acid and very long chain fatty acid analysis as well as specialised endocrine assays and protein electrophoresis for the entire province. Outreach and consultative services are also provided to surrounding laboratories in Durban and the entire province.

Specialised endocrine tests that were introduced during 2011 include growth hormone, IGF1, adrenocorticotropicin, DHEAS and androstendione.
RESEARCH PROJECT

VISION study
Principal investigator: Dr B Biccard (Department of Anaesthetics, UKZN)
Co-investigators: Dr R Rodseth, Dr K de Vasconcellos (Department of Anaesthetics, UKZN); Dr P Naidoo

The vascular events in noncardiac surgery patients cohort evaluation (VISION) study is a multinational evaluation of major vascular events in patients undergoing noncardiac surgery. The study will also determine the extent of postoperative troponin leak, the proportion of postoperative myocardial infarctions that would have been missed without troponin analysis, and the long term prognostic implications of a postoperative troponin leak. The study includes an international collaboration of anaesthetists, cardiologists, surgical disciplines and chemical pathology. The IALCH Chemical Pathology laboratory provides the support for 24-hour troponin T analysis, which is not routinely analysed at this site. Diagnostic kits are sponsored by Roche Diagnostics.

TEACHING AND TRAINING

The department teaches undergraduate medical students, and trainee technicians and technologists. The department also conducts lectures for BTech Biomedical Technology students at the Durban University of Technology. As part of the department's outreach policy, the pathologists present lectures for continuing professional development at surrounding NHLS laboratories. Training of chemical pathology registrars is undertaken within the department.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 1 MTech
Postgraduate candidates enrolled: 2 MMed

RESEARCH OUTPUT

Publications


Conference presentations:
International: 1
National: 1
Local: 4

DEPARTMENT OF MEDICAL MICROBIOLOGY

Head: Prof K Mlisana

DIAGNOSTIC SERVICES

The microbiology laboratory at IALCH provides routine microbiological investigation as well as specialised reference tests to this and other hospitals based within the province. The mycology laboratory performs all mycology cultures and serves as a reference laboratory for KwaZulu-Natal.

The TB laboratory at IALCH was the only culture reference laboratory in KwaZulu-Natal until May 2010, when a TB culture laboratory was opened in King Edward VIII Hospital (KEH) along with a routine 24-hour bacteriology laboratory. The TB molecular reference facility (based at IALCH) offers genotype testing for the rapid diagnosis of multidrug-resistant (MDR)-TB from smear-positive sputum samples and smear-negative culture-positive samples. Other genotype PCR-based assays are also offered for the rapid identification of non-TB mycobacteria and members of the Mycobacterium tuberculosis complex. The IALCH TB laboratory processes an average of 18,000 sputum samples per month. The KEH laboratory processes 3,000 specimens for microscopy and culture of M. tuberculosis. The TB molecular laboratory at IALCH performs approximately 2,900 routine MTBDRplus tests per month.

The laboratory provides outreach support to Mahatma
Gandhi Memorial Hospital and King George V Hospital with one microbiologist based at each hospital.

RESEARCH

Closer collaborations have been established with the Centre for the AIDS Programme of Research in South Africa (CAPRISA), KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH) and Enhancing Care Initiative (ECI) research units at UKZN, as well as with Harvard University. To date, the research focus of the department has been on rapid diagnostic tests for TB and antimicrobial resistance. It is intended to extend the research capacity by increasing the numbers of honours and masters students in microbiology, while also building closer links with K-RITH.

RESEARCH PROJECTS

Causes of meningitis in the era of HIV
Principal investigator: P Ramjathan
Co investigator: Prof Y Coovadia

Meningitis is a life-threatening infection of the central nervous system. The causes of meningitis vary temporally and knowledge of the commonest aetiology is important to guide empiric treatment. There have been no studies in KwaZulu-Natal on the aetiology of meningitis in adult patients. In the 1980s the commonest causes of bacterial meningitis were Neisseria meningitidis, Streptococcus pneumoniae and Haemophilus influenzae. Other investigators have documented an increase in meningitis caused by Mycobacterium tuberculosis and Cryptococcus neoformans as a result of HIV infection. This study retrospectively investigates the spectrum of causative organisms in patients presenting with meningitis at KEH over a 30-month period. It also looks at cell counts, chemistry and demographic data of patients where available. This study aims to identify the spectrum of culture-positive meningitis at KEH and demonstrate the burden of opportunistic infections associated with HIV. It will also provide basic demographic data of these patients as well as reveal changing trends in the epidemiology of meningitis over the time period analysed.

Retrospective analysis of pathogens and anatomical sites of nosocomial infection and their antimicrobial susceptibility pattern in a level 1 trauma intensive care unit
Principle investigator: Dr Y Ramsamy
Supervisor: Prof DJJ Muckart; co-supervisor: Dr K Swe Swe Han

The study intends to determine the prevalence and distribution of pathogens and drug susceptibility of isolates originating from nosocomial infections in the trauma intensive care unit (TICU) as well as the rate of nosocomial infections. Antibiograms of pathogens isolated from patients with nosocomial infection will be obtained in order to guide antimicrobial therapy.

TEACHING AND TRAINING

Undergraduate
Departmental staff are involved with the development of the undergraduate medical (MBChB) curriculum from first to fourth year level. The consultants and registrars deliver lectures, conduct tutorials and, conduct computer-based sessions, set-up practical sessions, prepare assessments and evaluate feedback.

The BSc (Biomedical Science) course covers the pathogenesis, epidemiology and laboratory diagnosis of microorganisms that cause various clinical syndromes. The degree will be offered by the UKZN School of Laboratory Medicine and Medical Science in the future.

A series of lectures has been designed for undergraduate students in physiotherapy, occupational therapy, speech therapy and audiology.

Technologists/technicians
Five intern technologists were trained and wrote their Board exams in March 2012. Three intern technicians wrote and passed their Board exams in October 2011; another five are being trained and will write their exams in October 2012.

Postgraduate
Ten registrars and a supernumerary from the Democratic Republic of the Congo are in training; two registrars passed the Fellowship exams in 2011. Two virology registrars spent three months in the department and were trained on the role of the microbiology laboratory in the diagnosis of infectious diseases. The department also
offers a four-month training programme for registrars specialising in either adult or paediatric infectious diseases; they are taught the basics of microbiology, including specimen processing, identification and susceptibility testing. Time is spent in the TB, mycology and public health laboratories during this period. Two physicians and one paediatrician were trained in 2011.

The department presents an extensive academic developmental programme twice weekly. A combined multidisciplinary journal club and seminar presentation, and a combined multidisciplinary infectious disease case study/grand round are conducted. These activities are registered for continuing professional development points.

**PROFESSIONAL DEVELOPMENT**

**Postgraduate candidates enrolled:** 12 (8 MMed, 3 FCPath, 1 MSc)  
**Postgraduates candidates graduated:** 2 FCPath Micro

**RESEARCH OUTPUT**

**Publications**


**Conference presentations**  
**International:** 1  
**National:** 11

**DEPARTMENT OF VIROLOGY**

**Acting head:** Dr P Moodley

**DIAGNOSTIC SERVICES**

The number of HIV PCR tests increased considerably due to the expansion of HIV testing in infants. The number of HIV viral load tests decreased slightly following the recent start-up of HIV viral load testing in the Madadeni, Hlabisa and Addington hospital laboratories. The number of viral ELISA’s has increased due to the increase in requests for hepatitis B virus, cytomegalovirus and toxoplasma from the clinics in the CCMT programme.

The previous in-house molecular assays were replaced by new commercial assays for the molecular diagnosis of cytomegalovirus, varicella zoster-, herpes simplex-, parvo-, respiratory and enteroviruses. These passed all quality assurance and verifications before being offered as part of the diagnostic service.

The registrars spend one day a week in the CCMTP clinic at KEH and McCord Hospitals as part of an outreach programme. The department is involved in the provincial health department’s Antiretroviral Resistance Working Group. The input helps to facilitate clinical and scientific decision making in the CCMT programme.

**RESEARCH PROJECTS**

**Molecular epidemiology of HIV-2 infection in KwaZulu-Natal**

The study is undertaken in collaboration with the Africa Centre for Health and Population Studies and funded by the NHLS Research Trust.

**The frequency and characterisation of the JC virus in a cohort of HIV seropositive patients**

This collaboration is with the Department of Neurology, Nelson R. Mandela School of Medicine.
The clinical utility of real-time quantitative PCR in the diagnosis of cytomegalovirus pneumonia in HIV-positive children in a paediatric intensive care unit

The study is undertaken in collaboration with the Department of Paediatrics and funded by the Discovery Foundation Academic Fellowship Award and a NHLS Research Trust grant.

Identification of zidovudine and nevirapine resistance using ultra deep sequencing when dual therapy is used for prevention of vertical transmission of HIV

Funding: NHLS Research Trust

Inhibition of HIV-1 encapsidation by targeted poly-ribonucleotide decoys: a novel nanoparticle approach

This investigation is being done in collaboration with the School of Chemistry and is funded by the Discovery Foundation.

HONOURS

Dr R Parboosing received a 2011 Discovery Foundation Academic Fellowship Award.

TEACHING AND TRAINING

Undergraduate

In 2011, 24 BSc (Biomedical Science) students registered for the 16-credit module, Molecular Virology, at third year level in the Faculty of Science; 27 students registered for 2012.

The contribution to the medical student teaching includes lectures and assessments in the first three years of the curriculum. The curriculum is currently being upgraded to be in line with international trends.

Technologists

Five student medical technologists wrote the virology Board exams in March 2012. One student passed the exams in September 2011. Another three are currently in training.

Postgraduate

Eighty-two healthcare professionals registered for the module: ‘Introduction to the virology of HIV’ towards the postgraduate diploma in HIV/AIDS clinical management, presented via telemedicine in collaboration with the medical school’s Enhancing Care Initiative Unit.

RESEARCH OUTPUT

Conference presentations

International: 1
Local: 5

“The number of HIV PCR tests increased considerably due to the expansion of HIV testing in infants”

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled: 6 (4MMed, 2 PhD)
DEPARTMENT OF ANATOMICAL PATHOLOGY INCLUDING CYTOLOGY

Head: Prof NM Bida

DIAGNOSTIC SERVICES

A comprehensive surgical pathology, autopsy pathology and cytopathology service is provided to the Dr George Mukhari Hospital (DGM)/University of Limpopo (Medunsa campus) academic complex as well as to some regional health facilities in Gauteng, Limpopo, Mpumalanga and North West provinces. The laboratory was fully accredited by SANAS in November 2011.

The number of histology cases registered increased from 10,110 in the previous reporting period to 11,663, largely because of increased patient load in many of the referral centres, reflecting the general national trend of increasing hospital admissions as a result of the burden of HIV/AIDS. The number of cytology registrations increased from 57,614 to 71,875; this can be ascribed to the community outreach programmes with increased routine sampling especially in the neighbouring clinics.

During the reporting period, a total of 53 autopsies were performed, of which 14 were paediatric cases.

RESEARCH

Research is on-going in the following areas:

Breast cancer research
A project on the immunohistochemical profiling of variants of lobular carcinoma is being undertaken by Dr B Ratlabala.

Human papillomavirus in conjunctiva
Dr KC Skosana is investigating the prevalence of human papillomavirus (HPV) in conjunctival lesions of a HIV-positive cohort, and M Nkosi is undertaking molecular genetic characterisation of HPV in conjunctiva lesions in immunosuppressed patients for his PhD thesis.

TEACHING AND TRAINING

Undergraduate
The consultant staff is responsible for delivering undergraduate teaching in anatomical pathology to MBChB students and BDS students from the dental faculty during their third year. The departmental registrars provide lectures to the allied health sciences, which includes occupational therapy, dietetics, radiography and physiotherapy.

Postgraduate
There are currently four registrars in the department. From time to time, registrars from the Department of Forensic Pathology rotate through the department for one year as part of their training.

Technologists
One student histotechnologist and two cytotechnologists wrote the Board exams in March 2012.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates registered: 5 (4 Med, 1 PhD)

DEPARTMENT OF CHEMICAL PATHOLOGY

Head: Prof HF Joubert

DIAGNOSTIC SERVICES

The chemical pathology laboratory retained its accreditation status with SANAS during the re-assessment in February 2012. The laboratory is currently implementing electronic gate-keeping, which should ensure proper utilisation of laboratory tests.

The human genetics section processed 497 specimens, which is an increase of 32% compared to the previous year. About 60% of the specimens were referrals from other hospitals and clinics. Despite the implementation of cost-savings strategies at DGM and referral clinics, the chemical pathology laboratory processed 752,158 tests, an increase of 6, 2% compared to the previous financial
year. The department has regular meetings with the departments of internal medicine and paediatrics as well as some referral clinics and an endocrine clinic is run in conjunction with the Department of Internal Medicine.

RESEARCH PROJECTS

Comparative study of vitamin B₁₂ as a marker for vitamin B₁₂ deficiency at Dr George Mukhari Hospital

Researcher: Dr L Murray
Supervisor: Prof HF Joubert
Co-supervisor: Dr M de Jongh
Funding: NHLS Research Trust

The diagnostic sensitivity and specificity of total vitamin B₁₂ to active B₁₂ (holoTC) analyses in a population of patients attending at DGM were compared. Serum folate, full blood count (FBC), thyroid function test, homocysteine, serum total vitamin B₁₂, and active B₁₂ analyses were performed on 30 samples. It was found that the diagnostic sensitivity was the same and the total vitamin B₁₂ test’s specificity was better in comparison to the active B₁₂ analyses. Thus the active B₁₂ assay cannot be recommended for routine use, since it has no benefit.

Renal stone analysis: patient findings and Fourier transform infrared spectroscopy method validation

Researcher: Dr K Mentz
Supervisor: Prof HF Joubert
Co-supervisor: Dr M de Jongh
Funding: NHLS Research Trust

The study aimed to validate the Fourier transform infrared spectroscopy (FTIR) method used for renal stone analysis, establish prevalence of renal stones and obtain demographic information on patients presenting with renal stones. Renal stones are analysed by FTIR to determine the constituents of the stones, in order to assist with the appropriate treatment of the patient and prevent a recurrence of stone formation. Validation of methods is important to ensure accurate reported results. The majority of the renal stones analysed consisted of calcium in some form and the prevalence of stones was much higher in men (75%) than in women, which correlates to findings in other studies. The method is fit for the purpose of renal stone analysis using standard or mini pellets.

The role of brain natriuretic peptide (BNP), NT Pro-BNP and troponin I in the diagnosis, management and prognosis of congestive cardiac failure

Researcher: Dr J van Graan
Supervisor: Dr A Rab
Co-supervisor: Dr M de Jongh
Funding: NHLS Research Trust

Collaborative research with the haematology and obstetric and gynaecology departments are:

Retrospective review of laboratory findings in patients with multiple myeloma at Dr George Mukhari, 2004-2009

Researchers: R Rankapole, C Zelaya-Torres, B Sedumedi

Prevalence of Down syndrome in pregnant women attending Dr George Mukhari Hospital

Researchers: M Chabalala, S Monokoane, B Sedumedi

TEACHING AND TRAINING

Undergraduate

Departmental staff contribute to the integrated practice of medicine course of MBChB students in their second to fifth year. During the fourth year of study, a full subject-based course in chemical pathology is presented as well as a selective course. Case discussions are given to final year students.

Postgraduate

The department teaches and trains registrars in chemical pathology, haematology and internal medicine.

Technologists

Medical technology students are trained during their rotation in the department.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates registered: 4 MMed
DEPARTMENT OF
HAEMATOLOGICAL PATHOLOGY

Head: Prof V Moodley

DIAGNOSTIC SERVICES

A comprehensive 24-hour haematology diagnostic service is offered to the Dr George Mukhari Hospital. The laboratory serves as a tertiary referral centre to local hospitals and clinics and provides a service to the Medunsa Clinical Research Unit, supporting numerous clinical trials.

The number of tests for CD4 enumeration (which is part of the National Priority Programme) performed over the last financial year rose by 26.6%. Turnaround time has been a strong focal point over the last year and special effort has been made to shorten turnaround times.

The molecular diagnostic unit expanded its paternity service, DNA family studies and Y-chromosome testing for the law courts in the surrounding area.

The laboratory maintained its SANAS accreditation status in January 2012.

RESEARCH PROJECTS

Prevalence of paternity misidentification by the mother as compared to the DNA identification results
Researchers: Y Harris, AS Greef, I Ferreira, DJ Welgemoed
This is a retrospective study aimed at establishing the prevalence of incorrect paternity identifications by the mother.

Y-chromosome investigation of a male amelogenin dropout
Researchers: DJ Welgemoed, AS Greef, J Greef, Y Harris
The aim is to investigate the sequences of the Y-amelogenin profile.

A postulated point during sperm maturation where DNA mutations occur
Researchers: DJ Welgemoed, AS Greef, J Greef, Y Harris
An observational study is being carried out on the phase of sperm maturation where DNA mutations occur.

“CD4 tests rose by 26.6% over the past financial year”

TEACHING AND TRAINING

Undergraduate
The department teaches haematology as a fully integrated subject to MBChB students in the practice of medicine curriculum in the second and third year.

Postgraduate
An inclusive practical and theoretical training programme is in place for registrars in haematology over a four-year period. In addition, the department is involved in the training of registrars from the internal medicine and paediatrics as well as chemical pathology departments.

BSc (Hons) and MSc programmes are also offered.

Technologists
The department continuously trains medical technologists and technicians who are preparing for their final board examinations.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 1 MMed
Postgraduate candidates enrolled: 7 (4 MMed, 1 MSc and 2 BSc (Hons))

RESEARCH OUTPUT

Publications
Greef JM, Greeff FA, Greeff AS, Rinken L, Welgemoed DJ, Harris Y. Low non paternity rate in an old Afrikaner family. *Evolution and Human Behavior* 2012

**DEPARTMENT OF MICROBIOLOGICAL PATHOLOGY**

**Head:** Prof M Nchabeleng

**DIAGNOSTIC SERVICES**

Routine microbiology services are rendered to DGM Hospital and the surrounding clinics; the laboratory serves as a referral laboratory for some parts of Limpopo, North West and Mpumalanga provinces. TB volumes have been increasing and LPA (Hain) is running smoothly. An outreach programme to Limpopo has been strengthened. Three workshops in management of sexually transmitted infections, infection prevention and control and TB were conducted for nurses from the hospices, surrounding clinics and district hospitals.

**RESEARCH**

Research is focused on four areas, namely TB, sexually transmitted infections (STIs), antimicrobial resistance and contract research. For contract research, two clinical trial units have been established, namely Medunsa Clinical Research Unit (MeCRU) and Setshaba Research Centre (SRC), which are involved in HIV prevention clinical trials.

**RESEARCH PROJECTS**

Projects in the field of TB are:

**Comparison of agar proportion method, MGit susceptibility and GenoType MTBDrplus assays in the detection of drug resistance among Mycobacterium tuberculosis isolates from Dr George Mukhari laboratory**

**Researchers:** Dr J Molepo, Dr MBR Maloba, I Mabusa

**Funding:** NHLS Research Trust

Agar proportion, mycobacterium growth indicator tube (MGIT) drug susceptibility and Genotype MTBDRplus assays were compared in the detection of drug resistance among *Mycobacterium tuberculosis* isolates at DGM laboratory. The results confirmed that Genotype MTBDRplus was more sensitive in detecting resistance as compared to conventional drug susceptibility testing methods. The GenoType MTBDrplus assay had an overall sensitivity of 96% and the MGIT assay had a sensitivity of 88%.

**Molecular characterisation of mixed infections with different strains of Mycobacterium tuberculosis and/or nontuberculous Mycobacterium species**

**Researchers:** N Makhado, Prof M Nchabeleng

**Collaborators:** Prof B de Jong, Prof R Colebunders (UA)

The study is being undertaken in the area served by the DGM laboratory.

Projects in the field of STIs are:

**Prevalence and diversity of vaginal microbes in pregnant women and in women with adverse pregnancy outcomes**

**Researchers:** Dr MC le Roux, BE de Villiers, Dr M Ditsele

**Collaborators:** Prof TS Monokoane (Department of Obstetrics and Gynaecology)

**Funding:** NHLS Research Trust

Standard and PCR assays are being used to detect and identify vaginal microbes in pregnant women and compare with those in women with adverse pregnancy outcomes.

**Prevalence of STIs in women receiving termination of pregnancy at DGM**

**Researchers:** Dr MC le Roux, B de Villiers, Dr M Ditsele, L Machiane, K Matebane, N Mametja

**Collaborators:** Dr Mnisi, Prof TS Monokoane (Department of Obstetrics and Gynaecology)

**Funding:** NHLS Research Trust

In this ongoing project, the emphasis is on the mycoplasmas isolated, viz *M. genitalium* and *Ureaplasma* spp. These isolates will further be investigated for antimicrobial resistance using PCR and sequencing.

**Streptococcus agalactiae in pregnant women and their babies at DGM**

**Researchers:** Dr MBR Maloba, Prof M Nchabeleng

**Collaborators:** Prof S Moyo, Dr S Teffo (UNISA); Dr Tsepuane (Department of Obstetrics and Gynaecology)

**Funding:** NRF

The project determines *Streptococcus agalactiae* colonisation rate in pregnant women and their babies and characterises the isolates.
Antimicrobial resistance projects include:

**Antimicrobial susceptibility profile of non ESBL-producing, cefuroxime-resistant *Escherichia coli* isolates at DGM laboratory**

*Researchers:* Prof M Nchabeleng, B de Villiers, DL Nemutavhanani, IM Seabi, DP Mohale

*Funding:* NHLS Research Trust

The study aims to determine the antimicrobial susceptibility profile of non-extended-spectrum beta-lactamase (ESBL)-producing urinary *Escherichia coli* isolates and determine possible mechanisms of resistance to cefuroxime.

The prevalence and characterisation of beta-lactamases among Gram-negative uropathogens isolated from a selection of secondary and tertiary hospitals in South Africa

*Researchers:* Dr N Mbelle, L Fernandes, Dr M le Roux, B de Villiers, P Molefe, E Mogoloane

*Funding:* NHLS Research Trust

The prevalence and characterisation of *qnr* determinants in ESBL and non-ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates from the DGM laboratory

*Researchers:* Dr N Mbelle, L Fernandes, Dr M le Roux, B de Villiers, E Mogoloane

Characterisation and nasal carriage of MRSA isolates

*Researchers:* Prof M Nchabeleng, B de Villiers, TM Maleka

The study characterises MRSA isolates from wounds and nasal swabs from patients at DGM, and nasal swabs among selected patients at two local clinics and their household members in the GaRankuwa area.

Contract research is undertaken by MeCRU researchers Dr MP Mathebula, Dr N Carrim-Ganey and Prof M Nchabeleng and their team, and SRC researchers Dr K Ahmed, Dr R Mabo and the SRC team. Projects include:

- A phase III, multi-centre, randomised controlled trial to assess the safety and effectiveness of the vaginal microbicide 1% tenofovir gel in the prevention of HIV type 1 infection in young women, and to examine effects of the microbicide on the incidence of herpes simplex virus type 2 infection. Collaborators are the Follow on African Consortium for Tenofovir Studies, and the trial is funded by the DST, Bill & Melinda Gates Foundation and USAID;
- A phase II, randomised, double-blind, placebo-controlled trial to evaluate the immunogenicity and safety of a therapeutic, recombinant, biologically active HIV-1 tat protein vaccine in HIV-infected, anti-tat negative, ARV-treated adult volunteers. Collaborators are from the Walter Sisulu University and the trial is funded by ISS (Italian government).

HONOURS

The department was voted the best non-clinical department by final year medical students.

Dr MBR Maloba received a prize for best oral presentation for his paper titled ‘The role of intravascular catheter tip cultures in determining blood stream infections’ at the 7th Research Day of the University of Limpopo.

TEACHING AND TRAINING

Medical microbiology is taught to undergraduate students for several degrees, i.e. MBChB (I, II, III, IV), BDS, BCur and BSc Diet.

Six medical technologists and four medical technicians qualified.

PROFESSIONAL DEVELOPMENT

**Postgraduate students graduated:** 6 (1 MSc, 5 BSc [Med] Honours)

**Postgraduate students enrolled:** 15 (1 PhD, 5 MMed, 4 MSc, 5 BSc [Med] Honours)

RESEARCH OUTPUT

**Publications**


**Conference presentations**

**International**: 7
**National**: 12
**Local**: 8

**DEPARTMENT OF VIROLOGY INCORPORATING THE MRC/UL DIARRHOEAL PATHOGENS RESEARCH UNIT**

**Head**: Prof MJ Mphahlele

**DIAGNOSTIC SERVICES**

Routine pathology services are offered to DGM and surrounding private and public clinics. In 2011, centralisation of some diagnostic tests with high workload was implemented. DNA PCR testing for HIV-infected infants was moved to Tshwane Academic Division and DGM laboratory has taken over HIV quantitative testing as a central referral laboratory. A total of 14,140,814 samples were processed in 2011. The laboratory participated in the national antenatal HIV surveillance project of the national Department of Health for the 11th year in succession. The laboratory continues to serve as the referral site for a number of laboratories within and outside Pretoria, including peripheral laboratories in Gauteng, North West, Mpumalanga and Limpopo provinces.

**RESEARCH**

**MRC Diarrhoeal Pathogens Research Unit**

The MRC/UL Diarrhoeal Pathogens Research Unit (DPRU) was established in 1996 to address a national and continental under-researched disease need, particularly rotavirus. The DPRU established the African Rotavirus Surveillance Network, conducts groundbreaking research work in the field of rotaviruses and rotavirus vaccine trials, and provides training for postgraduate and African scientists. The DPRU has also been appointed as the WHO Rotavirus Regional Reference Laboratory (RRL) for Africa. In 2011, the RRL organised, in collaboration with WHO, the 11th African Region Rotavirus Surveillance Network Genotyping Workshop from 6 to 17 June 2011 on the Medunsa Campus. Seventeen participants from 13 African countries (Cote d’Ivoire, DRC, Ethiopia, Guinea Bissau, Kenya, Mauritius, Nigeria, Senegal, Togo, Tanzania, Uganda, Zomba, Zambia, Zanzibar and Zimbabwe) attended. The key objectives were to generate data on the circulating rotavirus strains in Africa and to provide training to participants.

**HIV and Hepatitis Research Unit**

The HIV and Hepatitis Research Unit (HHRU) studies the prevention and control of hepatitis B virus (HBV), co-
infection of chronic hepatitis viruses (HBV and hepatitis C virus [HBC]) and HIV, and conducts basic HIV research. During the past year, the HHRU conducted studies on virological dynamics of HBV and HIV co-infected adult patients on HAART; genetic characterisation of circulating HIV strains in Pretoria and surrounding areas; molecular characterisation of hepatitis B surface gene from selected HBV-infected babies in Pretoria; pre-vaccination exposure to HBV infection and protective efficacy of the HBV vaccine; molecular quantification and genotyping of HCV isolates in HIV patients on ARVs at DGM; explored the evolution of genetic diversity of HIV in cancer patients exposed to immunosuppressive therapy; and molecular characterisation of human papillomavirus types in South Africa.

South African Vaccination and Immunisation Centre

The Department of Virology hosts the South African Vaccination and Immunisation Centre (SAVIC) as a Centre of Excellence on vaccines and immunisation. SAVIC hosts a WHO Vaccine Safety Network (VSN) accredited website (www.savic.ac.za). The website was created to allow centralisation and sharing of data on vaccine preventable diseases and immunisation-related resources in South Africa, southern Africa and the rest of the world. This website serves as a prime source of information for promoting awareness on vaccine preventable diseases, supporting local and regional immunisation initiatives and promoting the use and benefits of vaccines in South Africa and southern Africa. The target audience includes health officials, healthcare workers, scientists, academics, the vaccine industry and scientific journalists. Since its launch in 2005, the SAVIC website has grown to be the main source of vaccination information in South Africa. The SAVIC website was first accredited by the WHO VSN in February 2007, and is now accredited for the second time since July 2010. SAVIC is proud to be the very first vaccine website in Africa to be accredited by the WHO’s VSN. The SAVIC website regularly receives queries on vaccine-related matters such as the MMR-autism issue.

HONOURS

Dr SG Selabe was the recipient of the university’s Best Overall Female Researcher award.

TEACHING AND TRAINING

The Department of Virology is involved in teaching medical microbiology (co-ordinated by the Department of Medical Microbiology) to undergraduate students in MBCHB I and III, BDS III, B Cur II, B Dent II and BSc Diet II, and various other courses to postgraduate students in MSc Med, MMed and PhD.

The virology diagnostic laboratory serves as a teaching platform for medical technologists and registrars in virological pathology and other pathology disciplines, and is part of external quality assurance programmes.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated: 1 MSc Med
Postgraduate students enrolled: 28 (7 PhD, 3 MMed, 6 BSc Med Hons, 12 MSc Med)

RESEARCH OUTPUT

Publications

Carlisle HJ, Luong TN, Medina-Marino A, Schenker L, Khorosheva E, Indersmitten T, Gunapala KM, Steele AD, O’Dell TJ, Patterson PH, Kennedy MB. Deletion of densin-180 results in abnormal behaviours associated with mental illness and reduces mGluR5 and DISC1 in the postsynaptic density fraction. J Neurosci 2011; 31(45): 16194-16207

“The Department of Microbiological Pathology was voted the best non-clinical department by final year medical students”


Forrest BD, Steele AD, Hiemstra L, Rappaport R, Ambrose CS, Gruber WC. A prospective, randomised, open-label trial comparing the safety and efficacy of trivalent live attenuated and inactivated influenza vaccines in adults 60 years of age and older. Vaccine 2011; 39(20): 3633-3639


Hayes VM, Venter PA, Mphahlele MJ. Helping hand for genomics in Africa. Nature 2011; 476 (7359):152


Michelmore A, Bryant PM, Steele AD, Vasilev K, Bradley JW, Short RD. Role of positive ions in determining the deposition rate and film chemistry of continuous wave hexamethly disiloxane plasmas. Langmuir 2011; 27(19): 11943-11950


Conference presentations
International: 8
National: 14

10 years of supporting academic excellence 47
DEPARTMENT OF ANATOMICAL PATHOLOGY

Acting head: Dr MW Kgoebane

DIAGNOSTIC SERVICES

Comprehensive diagnostic and prognostic services are supplied to Steve Biko Academic, Kalafong, Tshwane District and Mamelodi hospitals. The service also extends to all Tshwane Metro clinics as well as hospitals and clinics in Mpumalanga, parts of Limpopo and North West provinces. The department offers a consultation and support service to private pathology laboratories. Institutions under the Department of Health covered by this pathology service include Oral and Dental Department, 1 Military Hospital, Faculty of Veterinary Science at Onderstepoort, and Department of Forensic Pathology.

The department offers post mortem investigation services to Steve Biko Academic and Kalafong hospitals as well as to surrounding private hospitals. A cremation auditing/approval service is provided for the Tshwane Metro. A comprehensive cytology service is offered to the above mentioned health facilities.

RESEARCH PROJECTS

Projects completed during 2011 include:

Norpret study
Researchers: Prof G Dreyer and Dept of Anatomical Pathology

Birth asphyxia at Kalafong Hospital – an audit of placental pathology
Researchers: E Marais, S Delport

Undiagnosed fatal infections in children: a 10-year review of childhood post mortem findings
Researchers: C Crause, P Eyal, M Louw

TEACHING AND TRAINING

Undergraduate
The department trains medical, dental as well as allied health students. Lectures and practical training are delivered to the second, third, fourth and final year medical students.

Postgraduate
Currently, the department is training nine registrars. In addition, registrars from the oral and dental, and forensic pathology departments rotate through the department for 24 and 18 months, respectively.

Quarterly academic meetings are held with the internal medicine, general surgery, gynaecology and obstetrics, neurosurgical, and paediatric academic departments for discussion of unique and rare clinical cases.

In association with both the Steve Biko and Kalafong hospitals, the Department of Anatomical Pathology participates in training and assessment of Master’s and MMed students in the following disciplines/academic departments: internal medicine, paediatrics, general surgery, orthopaedic surgery, gynaecology and obstetrics, ophthalmology, neurosurgery, psychiatry, otorhinolaryngology and radiology. The department also trains and assesses postgraduate students in occupational therapy and radiography.

Medical technologists/technicians
One technologist and one technician passed their Board exams; three intern technologists are currently in training.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 1 MMed
Postgraduate candidates enrolled: 9 MMed

RESEARCH OUTPUT

Publications
Dinkel J. Lymph node biopsy: Some aspects revisited. CME 2012; 2(30): 72
DEPARTMENT OF CHEMICAL PATHOLOGY

Acting head: Dr N Oosthuizen

DIAGNOSTIC SERVICES

The core laboratory of the Department of Chemical Pathology provides diagnostic pathology services mainly to the Steve Biko Academic and Tshwane District hospitals, regional clinics and a number of referring NHLS laboratories. Although the total workload was only marginally higher than the previous year, referral tests increased by 23%. The process embarked on by the NHLS in February 2012 of consolidating smaller laboratories and rerouting tests from clinics to the larger centres, contributed to the increase in referrals. In order to manage the increased work volumes, additional staff members were appointed to expedite pre-analytical processing of specimens. As part of the total quality management system, several new tools for monitoring and addressing problems in the laboratory were implemented. The outcome of the annual SANAS inspection in February 2012 was retention of the laboratory’s status as an accredited facility for another year. Auto-reviewing of selected routine chemistry tests was implemented as an additional measure to shorten turnaround times.

RESEARCH PROJECTS

Investigation of the diagnostic utility of midregional pro-adrenomedullin (MR-proADM) and midregional proANP (MR-proANP) in COPD patients with cor pulmonale

Researchers: Dr SE Nagel, Dr NM Oosthuizen
Funding: BRAHMS (Thermo Scientific), University of Pretoria

This study investigated the diagnostic utility of MR-proADM and MR-proANP for the diagnosis of cor pulmonale in patients with chronic obstructive airways disease (COPD). Study participants included 31 patients recruited from the Pulmonology Clinic at Steve Biko Academic Hospital in Pretoria. Concentrations of biomarkers were compared to two-dimensional Doppler echocardiography and a six-minute walk test as indicators of impaired cardiac function.

Mutations in the androgen receptor gene and the fibrillin-3 gene in women with polycystic ovary syndrome

Researchers: Dr C van Niekerk, Dr NM Oosthuizen, M Nöthling
Funding: NHLS Research Trust, University of Pretoria

Polycystic ovary syndrome is the most prevalent endocrinological disorder in women worldwide, characterised by menstrual dysfunction, infertility, obesity and metabolic syndrome. This study investigates microsatellites and methylation differences in the androgen receptor and fibrillin-3 genes between cases and controls.

Identification of CYP21A2 mutations within South African patients suffering from congenital adrenal hyperplasia caused by 21-hydroxylase deficiency

Researchers: Dr C van Niekerk, Dr NM Oosthuizen, J Lombard

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder caused in 90% of cases by mutations in the CYP21A2 gene. The main aim of the study is to...
identify and compare mutations in South African CAH patients to those documented in European populations by utilising HRM-real-time PCR and sequencing.

HONOURS

The University of Pretoria, Faculty of Health Sciences prize for the Best Overall Publication – Clinical was awarded for the following article: Delport R, Bornman RMS, Macintyre UE, Oosthuizen NM, Becker PJ, Aneck-Hahn NH, De Jager C. Changes in retinol-binding protein concentrations and thyroid homeostasis with non-occupational exposure to DDT. Environmental Health Perspectives 2011; 119(5): 647-651.

TEACHING AND TRAINING

Undergraduate
The department contributes to lecturing and assessment of several systems-based blocks in the MBChB programme, including homeostasis, diseases of childhood, abdomen and breast, traumatology and genito-urinary disorders. The acting head of department is also chairperson of a two-week block for the final year MBChB students, focusing on diagnostic laboratory medicine.

Postgraduate
The department participates in the teaching and assessment of MMed students in paediatrics, neurology, medical oncology and nuclear medicine during their pathology rotations. Chemical pathology presents a series of tutorials to MMed anaesthesiology students in preparation for their primary examinations. MMed students enrolled in the clinical pathology training programme spend 18 months in the department.

Technologists
The department provides in-service teaching and training to student medical technologists registered for chemical pathology and clinical pathology. In the period under review, 26 CPD activities aimed at medical technologists and students were presented by registrars/pathologists in the core laboratory.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated: 1 BSc Hons
Postgraduate students enrolled: 12 (7 MMed, 4 MSc, 1 BSc Hons)

RESEARCH OUTPUT

Publication

Conference presentations:
International: 3
National: 3

“Several new tools for monitoring and addressing problems in the laboratory were implemented”
Laboratory and clinical services are rendered to the Steve Biko Academic, Tshwane District and Kalafong hospitals and surrounding clinics.

The number of specimens submitted to the flow cytometry facility for immunophenotyping continued to increase. New protocols were devised for the analysis of cerebrospinal fluid and for the monitoring of minimal residual disease. All data (figures and histograms) are now captured on a tablet computer for ease of storage and ready access.

The adult haematology clinic continues to grow and provides a consultation service both to the Steve Biko Academic Hospital and to the wider medical community of Pretoria and the surrounding areas. The clinic also serves as an important vehicle for the teaching of undergraduate medical students as well as haematology, and internal medicine registrars. During the period under review, 752 patients were seen at the clinic. Members of the department also provided valuable input into the paediatric haematology clinic.

A total of 303 visits were made to the haemophilia 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**

**A phase II/III, randomised, cross-over, open label trial to demonstrate superiority of prophylaxis over on-demand therapy in previously treated subjects with severe haemophilia A treated with plasma protein free recombinant FVIII formulated with sucrose**

*Researchers:* Prof R Pool, Dr JJC Potgieter, A Prinsloo

The primary objective of this trial is to demonstrate the superiority of prophylaxis over on-demand therapy by a clinically significant decrease in bleeding rate following 12 months of treatment with the study drug. Secondary objectives are to demonstrate the superiority of prophylaxis versus on-demand therapy, to evaluate the safety of the study drug and to assess its potential to induce the formation of inhibitory antibodies.

**Evaluation of the AMLprofiler® (acute myeloid leukaemia profiler) compared to the standard diagnostic procedures in patients with acute myeloid leukaemia**

*Researcher:* Dr E Beltchev

The aims of the study are to evaluate the benefits of the AMLprofiler® in the South African context. The project will compare the current diagnostic procedures performed routinely in patients with acute myeloid leukaemia to that of the AMLprofiler®. The variables to be compared will include cost, concordance between results obtained using current techniques and AMLprofiler® and time required to execute the tests.

**A method for establishing the presence of bcr-abl in patients with chronic myeloid leukaemia using flow cytometry**

*Researcher:* Dr G George

Flow cytometry allows for the identification of particles based on attributes such as size and fluorescence intensity. A cytometric bead array (BM™) system provides a way of coupling a soluble analyte with beads of known size or fluorescence, thus making it possible to detect analytes by flow cytometry. This technique will be employed to detect bcr-abl fusion proteins in human blood research samples.

**Epidemiological investigation of HIV-related lymphoma**

*Researcher:* Dr T Chetty

The project plans to obtain epidemiological data on HIV-related lymphoma. This will be a retrospective evaluation of both the incidence and prevalence of HIV-related lymphomas in the Gauteng province (initially) over the past three years (2009 to 2011). The rationale for this component of the study stems from involvement in a project that aims to develop an HIV-resistant immune system by means of generating CCR5 null haematopoietic stem cells for bone marrow transplantation with the objective of curing HIV/
AIDS. Along with collaborators in Geneva and Zurich, Switzerland, the project has progressed beyond proof of concept in vitro and is currently entering evaluation in a humanised mouse model.

Once the pre-clinical phase has been completed, clinical trials will be initiated in South Africa based on the high prevalence of patients with HIV-related lymphoma. These patients have been selected since many of them require bone marrow transplantation following chemotherapy. However, there are to date no reference epidemiological data on this population in South Africa. It is therefore critical that this population be accurately defined so as to ensure that the most appropriate sub-population be selected for the planned clinical trial.

**Method validation of an automated cell counter for CSF analysis**  
**Researcher:** M Mokgathi

The Advia 2120® cell counter has an FDA-approved automated cerebrospinal fluid (CSF) assay as part of its routine test repertoire. A series of CSF specimens will be analysed in terms of the red cell count, white cell count and white cell differential. Factors such as linearity, carry over and within run precision and accuracy will be assessed.

**Utility of the reticulocyte haemoglobin content in the diagnosis of iron deficiency in pregnant women**  
**Researcher:** D Matlebjane

Determination of the reticulocyte haemoglobin content (CHr) provides an early measure of functional iron deficiency because reticulocytes are the earliest red cell precursors released into blood and circulate for only 48 hours before maturing into erythrocytes. The clinical utility of CHr for the diagnosis of the anaemia of chronic disease has not been carefully studied. This project will look at the utility of the CHr, together with other traditional haematological and biochemical parameters in establishing the diagnosis of iron deficiency in pregnancy.

**Paroxysmal nocturnal haemoglobinuria diagnosis by flow cytometry using the FLAER method**  
**Researcher:** S Aboobaker

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired haematopoietic stem cell disorder leading to a partial or absolute deficiency of all glycosphingolipids (GPI)-linked proteins. FLAER is an inactive variant of aerolysin that retains specificity for GPI-linked structures but does not cause lysis of cells. The goal of this study is to develop a FLAER-based assay to diagnose and monitor patients with PNH and to improve detection of minor populations of PNH clones.

**Establishing a reference range of leucocytes in CSF of healthy individuals using the Advia 2120® automated cell counter**  
**Researcher:** A Kemp

Normal CSF may contain up to five white blood cells (WBCs) per mm$^3$ in adults and 20 WBCs per mm$^3$ in newborns. CSF will be obtained from patients undergoing spinal anaesthesia and the specimens will be analysed using the Advia 2120® haematology analyser to establish normal cell values for CSF at this institution.

**TEACHING AND TRAINING**

**Undergraduate**

The department participates in one teaching block and two special activities for undergraduate students.

The newly designed lecture series on homeostasis teaches MBChB II students to understand haematological changes in systemic disease, recognise the signs and symptoms of primary haematological conditions, to work-up, diagnose and treat the most common haematological conditions and to use the haematology laboratory in a rational and cost-effective manner.

Case studies for the haematological malignancy course for MBChB III students were revised and rotations were divided into laboratory, radiological and clinical modules. Assessment was also changed to include both a 600-word case report and a multiple choice, computer-based examination. 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 aspirations and biopsies, immunophenotyping, conventional cytogenetics, fluorescent in situ hybridisation and PCR.

Student interns (MBChB VI) are instructed on laboratory medicine. Topics covered include 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. The latter topic is particularly important in the light of the electronic gatekeeping programme which is soon to be introduced by the provincial authorities.

**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. Registrars in haematology are expected to attend and pass courses in research methodology and molecular biology within the first 18 months of starting in a registrar post. A formal course in management has been included in the MMed curriculum which includes modules on finance, inventory, laboratory design, human resources and quality assurance.

The BSc (Hons) Haematology degree is based on course work, tutorials, journal presentations and a dissertation. Compulsory modules include applied research methods, medical biostatistics and molecular pathology.

**PROFESSIONAL DEVELOPMENT**

**Postgraduate candidates enrolled:** 15 (6 haematology and 4 clinical pathology registrars 5 BSc [Hons])

**Student medical technologists enrolled:** 9

**RESEARCH OUTPUT**

**Publications**


**Conference presentations**

**Local:** 2

**DEPARTMENT OF IMMUNOLOGY INCORPORATING THE MRC UNIT FOR INFLAMMATION AND IMMUNITY**

**Head:** Prof R Anderson

**DIAGNOSTIC SERVICES**

Notwithstanding a wide range of services for the serodiagnosis of allergic, autoimmune and infectious diseases, the Department of Immunology also provides services for the laboratory diagnosis of leukaemias/lymphomas by flow cytometry (in partnership with the Department of Haematology), primary and acquired immunodeficiency disorders, and HLA typing (disease associations and clinical organ transplantation). A service for the serodiagnosis of paraneoplastic neurological diseases and other neurological disorders with apparent autoimmune aetiologies was introduced in 2011/2012.

**RESEARCH**

Although primarily laboratory-based, research in the Department of Immunology is undertaken mainly in collaboration with clinical colleagues and has clear translational objectives. It is focused on the immunopathogenesis and immunopharmacotherapy of acute and chronic inflammatory disorders of both infective (HIV/AIDS, TB, severe pneumococcal disease) and non-infective (bronchial asthma, rheumatoid arthritis, toxicity of heavy metals in both the environmental and occupational settings) origin, pharmacogenetics, as well as stem cell harvesting and expansion with a view to future therapy of HIV infection and neuromuscular disorders. Research highlights from several of these programmes are described below.

**Infectious diseases**

**Researchers:** Prof R Cockeran, Dr MC Cholo, Prof A Theron, Prof R Anderson, M Potjo, ND Mutepe, Prof C Feldman, Prof TJ Mitchell, Dr A Von Gottberg, Prof K Klugman

Highlights of this research programme are as follows: Exposure to cigarette smoke condensate activates the production of biofilm by *Streptococcus pneumoniae* and also causes functional inactivation of the pro-inflammatory, pneumococcal toxin, pneumolysin. Up-regulation of biofilm formation is accompanied by selective activation of genes encoding a two-


“component system linked to production of biofilm. These mechanisms are likely to favour pneumococcal colonisation of the airways, and may underpin the susceptibility of smokers to severe pneumococcal disease.

The major K+ transporters of Mycobacterium tuberculosis appear to be relatively redundant in environments of neutral pH and high K+ concentrations. However, when the extracellular pH becomes mildly acidic, there is a marked increase in the expression of these genes, compatible with a role for the K+ transporters in promoting intra-vacuolar and intra-granuloma survival, possibly by removal of protons.

Inflammation

Researchers: Prof A Theron, Dr HC Steel, Dr MC Cholo, Dr C Gravett, M Mokgobu, Prof GR Tintinger, Prof R Anderson, Dr P Meyer, Dr B Hodkinson, Prof M Ally, E Musenge, Prof A Wadee, Prof M Tikly

The major highlights of this research programme are:

Tetracycline antibiotics appear to interact pro-oxidatively with human neutrophils by acting as calcium inonophores, thereby potentiating the harmful, pro-inflammatory activities of these cells.

The heavy metal, manganese, at concentrations which are representative of occupational exposure to the metal, potentiate the production of the toxic reactive oxygen species, hydrogen peroxide and hypohalous acids, by activated human neutrophils and monocyte-derived macrophages. This is a consequence of the superoxide dismutase mimetic activity of the metal and may underpin its neurological and respiratory toxicity.

Predisposition to rheumatoid arthritis and seropositivity for citrullinated peptide antibodies are associated with a high frequency of the HLA-DRB1 genotype. These, together with high levels of certain circulating cytokines, especially vascular endothelial growth factor and interferon-gamma, appear to identify a subset of patients at risk for development of severe disease.

These infectious diseases/inflammation research programmes are supported by research funding awarded by the MRC, NRF, and European Union.

TEACHING AND TRAINING

Undergraduate

Teaching/training in basic/applied immunology is offered to student medical technicians/technologists, 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 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 equipment and supervision to researchers from other departments and academic institutions.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 3 (2 PhD, 1 MSc)
Postgraduate candidates enrolled: 23 (11 PhD, 12 MSc)
RESEARCH OUTPUT

Publications


Dodgen TM, Cromarty AD, Pepper MS. Quantitative plasma analysis using automated on-line solid-phase extraction with column switching LC-MS/MS for characterising cytochrome P450 2D6 and 2C19 metabolism. Journal of Separation Science 2011; 34: 1102-1110


**Congress presentations**

International: 7
National: 14

**DEPARTMENT OF MEDICAL MICROBIOLOGY**

**Head:** Prof AA Hoosen

**DIAGNOSTIC SERVICES**

Comprehensive diagnostic laboratory services are provided to the Steve Biko Academic and Tshwane District hospitals and a referral microbiology service for Kalafong and Mamelodi hospitals as well as for a number of primary healthcare clinics in the Tshwane District. The TB laboratory at the Tshwane Academic Complex serves as the referral laboratory for TB for the NHLS’ Northern Region.

The Department of Medical Microbiology is involved in the infection prevention and control programmes for the hospitals mentioned above and partners the Gauteng Department of Health in co-ordinating the infection prevention and control programme for the Tshwane region.

The medical microbiology laboratory serves as an enhanced surveillance site for the NICD and participates in the GERMS-SA surveillance programmes for enteric pathogens, mycology, parasitology and respiratory and meningal pathogens as well as the recently introduced pneumococcal vaccine surveillance programme. The latter programme is also co-ordinated for Kalafong Hospital.

**RESEARCH**

There are four research programmes in the Department of Medical Microbiology. The TB research programme includes projects for the development of rapid diagnostic tests, detection of antibiotic resistance profiles, characterisation and genotyping of *Mycobacterium* species. The second programme focuses on the detection and prevalence of antibiotic resistance genes of emerging and re-emerging pathogens of clinical importance using molecular-based detection and typing methodologies. The third programme focuses on the surveillance and monitoring of clinically important pathogens circulating in the hospital and which are associated with outbreaks. The fourth is the STI research programme, which investigates the rapid identification and characterisation of sexually transmitted organisms.

“The TB laboratory at the Tshwane Academic Complex serves as the referral laboratory for TB for the NHLS’ Northern Region”
RESEARCH PROJECTS

TB research programme
Fifteen projects are ongoing or nearing completion and a study to evaluate the risk of contracting TB in the healthcare environment has been initiated.

Studies completed during the year, include:

Researchers: H Said, S Omar, A Osman (PhD students); A Bulane, L Khathi, Mohlabeng, J Bezuindenhoudt (MSc students); Dr AW Dreyer, Dr E Hoosien (MMed students)
Supervisors: Prof MM Ehlers, Dr MM Kock, Prof B Fourie, Dr K Baba, Dr Lekalakala, Dr NA Ismail
Collaborators: Dr M van der Walt (MRC); Dr AM Dyrr-Riise, Prof N Langeland (University of Bergen, Norway); Dr A Friedland, Dr G Coetzee, Dr L Erasmus (National TB Reference Laboratory); Prof N Beyers (DTTC); Dr C Boehme (FIND); Dr J Fischer (LHVD)
Funding: Incentive Funding for Rated researchers, NRF; NHLS Research Trust, Faculty of Health Sciences, UP Research Committee, DTTC/USAID/TREAT TB

Susceptibility testing and resistance mechanisms to second-line agents against Mycobacterium tuberculosis in Pretoria
In this study, 384 consecutive MDR-MTB isolates were collected and analysed for first-line anti-TB drugs using the standard agar proportion method. Susceptibility testing of the MDR-MTB isolates was also conducted for second-line anti-TB drugs using the standard agar proportion method followed by analysis using the GenoType® MTBDRsl (Hain Lifescience, Germany) assay. Isolates that showed discrepant results were sequenced. The lower performance of the GenoType® MTBDRsl assay in this study could be due to differences in the frequency of mutations in drug-resistant M. tuberculosis isolates which can affect the test performance. The genotyping results showed a high diversity of drug-resistant genotypes, indicating that transmission of drug-resistant strains in this setting is not related to clonal spread of a specific M. tuberculosis strain.

Detection and characterisation of ESBL-positive Klebsiella pneumoniae clinical isolates
Researchers: GI Manenzhe, I Barakzai, MB de Jesus, JE Louw (MSc students); N Schoonraad, W Strasheim, R dos Santos (BSc Hons students)
Supervisors: Dr MM Kock, Prof MM Ehlers
Funding: RESCOM; BMAZD IRT, UP

In this study, 150 Klebsiella pneumoniae clinical isolates were collected from the diagnostic laboratory within a four-month period. The isolates were analysed and characterised using phenotypic and genotypic methods.

The aim of the study was to investigate the prevalence of ESBL-producing K. pneumoniae isolates and, secondly, to compare the sensitivity and specificity of the Vitek2
advanced expert system (AES) and multiplex PCR assay against the combination disc method (gold standard) in detecting ESBL-production. Lastly, the study investigated the clonal relatedness of the collected isolates using ERIC, REP and BOX PCR fingerprinting assays. The prevalence of ESBL-positive K. pneumoniae isolates according to the combination disc method was 57.4%. The sensitivity and specificity of the Vitek2 AES in detecting ESBL production was 99% and 98%, respectively, when compared to the combination disc method. The sensitivity and specificity of the multiplex PCR assay were 96% and 98%, respectively. Genotyping revealed a high level of similarity among ESBL and non-ESBL-producing K. pneumoniae isolates.

Evaluation, assessment and characterisation of the activities of carbapenem antibiotics against Pseudomonas aeruginosa and Acinetobacter baumannii complex from hospitalised patients

Researchers: NHN Ahmed (PhD student)
Supervisors: Dr K Baba, Prof SY Essack

The presence of multidrug-resistant Acinetobacter baumannii raises a big therapeutic challenge in the hospital. The study evaluated the in vitro activity of tigecycline against carbapenem-resistant A. baumannii complex. Consecutive clinical isolates of carbapenem-resistant A. baumannii complex were collected between February and July 2010. Species identification and susceptibility testing was performed by Vitek-2 colorimetric compact system with AES. All carbapenem-resistant isolates were found to be fully susceptible to colistin; amikacin and tigecycline susceptibility was 78% and 76%, respectively. Treatment options for infections due to carbapenem and multidrug-resistant A. baumannii organisms are limited and hence tigecycline and amikacin may be considered.

Carbapenem resistance in A. baumannii and Pseudomonas aeruginosa infections are major health problem in the hospital. The in vitro activity of fosfomycin, an old forgotten antimicrobial agent, against carbapenem-resistant A. baumannii complex and P. aeruginosa was evaluated. A total of 190 carbapenem-resistant clinical isolates were collected; 95 A. baumannii complex and 95 P. aeruginosa. All the isolates were susceptible to colistin and fosfomycin. All the P. aeruginosa isolates were susceptible to fosfomycin. As treatment options for infections due to carbapenem- and multidrug-resistant A. baumannii complex and P. aeruginosa organisms are very limited, fosfomycin may be considered as a therapeutic option for managing infections caused by these bacteria.

Surveillance research programme

This programme focuses on the continuous monitoring and surveillance of clinical pathogens such as MRSA strains associated with outbreaks in ICU wards and the risk to public health. A study to characterise and genotype MRSA isolates from a healthcare centre in Ogun state Nigeria was initiated.

Ongoing studies include:

Antimicrobial profile and molecular characterisation of MRSA isolates from patients attending the Tshwane Academic Hospital Complex

Researcher: Dr GS Mahlangu (MMed student)
Supervisor: Dr K Baba

MRSA causes serious infections in hospitalised patients and presents a serious infection control problem. To determine the antimicrobial susceptibility pattern to selected antimicrobials, the Etest was used. None of the isolates were resistant to vancomycin; daptomycin and fosfomycin. With regard to mupirocin, no high level resistance was detected. The MRSA isolates showed good susceptibility to vancomycin, teicoplanin, daptomycin, fosfomycin and linezolid. Tigecycline also showed good in vitro activity when tested against the MRSA isolates. No heteroresistant glycopeptide intermediate Staphylococcus aureus (hGISA) was detected. The susceptibility of the MRSA isolates to an older drug like fosfomycin, which has no current indication for MRSA infections, offers hope for the future should resistance to newer drugs occur.

Comparative evaluation of BD Phoenix™ automated microbiology system with the Vitek 2 system and manual methods used for the identification and susceptibility testing of Gram-positive bacteria from clinical isolates

Researcher: Dr B Magazi (MMed student)
Supervisor: Dr K Baba

Eighty-seven enterococci were identified with 95% concordance rate between the methods; 134 staphylococci were tested with a concordance rate of 95%. The BD Phoenix™ automated system compares favourably with the laboratory protocol and is therefore
a reliable diagnostic tool for the identification and antimicrobial susceptibility testing of enterococci and staphylococci species, and *S. agalactiae*.

**STI research programme**

Research in this area focuses on the detection, identification and characterisation of sexually transmitted pathogens with emphasis on *Trichomonas vaginalis*, *Neisseria gonorrhoeae* and *Ureaplasma* species, using molecular and phenotypic tools. Ongoing studies include:

- The association between genital mycoplasmas and bacterial vaginosis in pregnant women with or without genital symptoms; and
- The prevalence of the aetiological pathogens of vaginal discharge and male urethritis syndromes in the Tshwane region.

New projects include:

- The prevalence of *Neisseria gonorrhoeae* and *Trichomonas vaginalis* in pregnant women with or without genital symptoms; and
- Mycoplasmas and ureaplasmas associated with HIV-positive men and women.

**MISCELLANEOUS RESEARCH PROJECTS**

**Detection of atypical pathogens of pneumonia**

**Researchers:** Dr E Hoosein, Dr BT Magazi (MMed students)

**Supervisor:** Dr MR Lekalakala

The aim of the project is to diagnose the aetiologic agents of pneumonia in patients admitted with a diagnosis of community-acquired pneumonia in medical ICU using both conventional methods of respiratory tract diagnosis such as microscopy, culture and sensitivity and newer molecular techniques. The results will provide information on how good the molecular techniques are, especially for organisms that are known to be difficult to culture or taking long to culture in a diagnostic laboratory. The study will also provide information about the prevalence of atypical organisms in these patients.

**Prevalence and characterisation of *Streptococcus agalactiae* colonisation in pregnant women**

**Researchers:** Dr E Hoosein, Dr BT Magazi (MMed students)

**Supervisor:** Dr MR Lekalakala

The study will determine the rate of group B streptococcal (GBS) carriage among pregnant women and the GBS antimicrobial susceptibility profiles at Tshwane District Hospital; this will inform policy on screening for GBS and development of appropriate chemoprophylaxis and drug treatment regimens. Serotyping is of importance in understanding the epidemiology of virulent strains and the development of relevant vaccines. This study will inform on the hospital’s GBS serotype and subtype distribution, thereby enabling choices on appropriate GBS vaccine candidates.

The risk-based strategy for screening mothers for GBS carriage is logistically easy and has few cost implications but it misses 45-50% of infected term infants. The updated CDC guidelines discourage the use of the risk-based screening method and advocate for culture-based screening. Penicillin is used as the drug of choice in the management of women colonised with GBS and for the treatment of invasive GBS disease. While it is universally accepted that GBS is susceptible to penicillin strains, tolerance to penicillin has emerged. Drug resistance to second line drugs, clindamycin and erythromycin, is known to occur.

**Detection and characterisation of *Bordetella pertussis* from children at the Kalafong Hospital**

**Researcher:** Dr M Hnaya

**Supervisor:** Dr MM Kock

**Collaborator:** Prof T Avenant (Department of Paediatrics)

**HONOURS**

Prof MM Ehlers received for the second time a NRF C2 rating as a scientist for the next five years (2012 to 2016).

**TEACHING AND TRAINING**

**Undergraduate**

Teaching and training is provided to medical technologists, medical and dental students, as well as to allied healthcare students in the disciplines of nursing, physiotherapy and dietetics. Interaction with medical
students is over the six-year study period while intensive teaching is done during the second semester of the second year for medical and dental undergraduates.

Postgraduate
The Department of Medical Microbiology has been accredited by the HPCSA as a training facility for intern medical scientists specialising in microbiology; as such, programmes are offered to BSc Hons, MSc, MMed and PhD postgraduates.

The CPD-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. An infectious diseases ward round is hosted in collaboration with the Infectious Diseases Division of the Department of Internal Medicine.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled: 37 (8 BSc Hons, 10 MSc, 8 PhD, 11 MMed)
Postgraduate candidates graduated: 11 (3 BSc Hons, 2 MSc, 3 MMed, 2 FCPath, 1 MSc Public Health)

RESEARCH OUTPUT

Publications


Lekalakala R. Infection prevention and control for viral infections. CME 2011; 29: 214-216

Lekalakala MR. The laboratory diagnosis of Clostridium difficile. In: Biotechnology, Medicinal Applications and Implications. 2012

Mahomed H, Fourie PB. Clinical trials of TB vaccines: Harmonization and cooperation. Tuberculosis 2012; 9251: 521-524


Visser A, Hoosen A. Human papillomavirus (HPV) vaccines. CME 2011; 29: 214


Conference presentations
International: 9
National: 5
Local: 12
DEPARTMENT OF MEDICAL VIROLOGY

Head: Prof LM Webber

DIAGNOSTIC SERVICES

The department offers a service covering viral serology and viral detection techniques with an emphasis on HIV-1 DNA PCR. Serological assays for human T-lymphotropic virus type I (HTLV-I) were introduced and real-time PCR/RT-PCR assays are offered for the detection of herpes simplex virus, enterovirus, and influenza. Papillomavirus genotyping and cytomegalovirus viral load assays are also available.

The department’s outreach activities include: i) one of the pathologists assisting weekly at the HIV Clinic at the Tshwane District Hospital; ii) pathologists and registrars attending clinical ward rounds at the Steve Biko Academic Hospital; and iii) pathologists, registrars and medical scientists presenting lectures, workshops and providing other academic support to healthcare professionals in the Pretoria and other geographical regions.

RESEARCH

The Department of Medical Virology has four main research focus areas: i) the bloodborne virus research programme which addresses clinical and epidemiological aspects of HIV-1, hepatitis B virus (HBV) and hepatitis C virus (HCV); ii) the human papillomavirus and cervical cancer research programme which addresses cervical cancer screening in South Africa; iii) the enteric virus and environmental research programme which includes projects on the molecular epidemiology of gastroenteritis viruses, the development of sensitive methods for the recovery, detection, characterisation and molecular epidemiology of food- and waterborne viruses, and iv) viral zoonoses. The Viral Zoonoses Unit was established as part of the University of Pretoria Institutional Research theme ‘Biotechnology of animal and zoonotic diseases’, and is housed in the University of Pretoria-based BSL3 laboratory.

BLOODBORNE VIRUS RESEARCH PROJECTS

New projects initiated are:

HBV genotype distribution among different HBV serological patterns in AIDS patients and HIV-negative people in a Gauteng population
Principal investigator: Dr S Mayaphi
Co-investigators: Prof DJ Martin, Dr TM Rossouw, Dr SAS Olorunju
Funding: PRF, NHLS Research Trust

Optimising and developing more sensitive assays for surveillance, diagnosis and characterisation of unique variation in African isolates of HBV, HCV and HIV in mono- and HIV co-infected patients
Project leader: Dr SM Bowyer
Co-investigators: Dr S Mayaphi, Prof LM Webber
Researchers: LS le Clercq, Dr O Laguda-Akingba

Using the power of position-specific scoring matrices to analyse viral variation and evolution in Africa
Project leader: Dr SM Bowyer
Researcher: LS le Clercq

HONOURS

MM Lassaunière was a joint winner in the category ‘Best publication by a young researcher (<35years): non-clinical’ in the Faculty in 2010 for the publication ‘A novel multiplex real-time RT-PCR assay with FRET hybridisation probes for the detection and quantitation of 13 respiratory viruses.’

At the Faculty of Health Sciences Faculty Day 30-31 August 2011, the following awards were made:

- Dr S Mayaphi was awarded first place in the category Researcher: clinical [poster] for his poster entitled ‘HBV/HIV co-infection: correlation of HBV serology and viral loads with CD4+ cell counts in patients with AIDS’;
- M Magwalivha was the second runner-up in the category ‘Best publication by a young researcher (<35years): non-clinical’ for the publication ‘High prevalence of species D human adenoviruses in faecal specimens from urban Kenyan children with diarrhoea’;
• Prof LM Webber was the second runner-up in the category ‘Best overall publication: qualitative/education/health systems research’ for the publication ‘From a practising medical doctor to a confident researcher: starting an academic paper’;
• Dr J Mans was the second runner-up for a publication in the category ‘Best publication from a team effort’ for the publication ‘Emerging norovirus GI.4 2008 variant detected in hospitalised paediatric patients in South Africa’; and
• Dr M Brauer was awarded third place in the category ‘Researcher: basic sciences [oral]’ for her presentation entitled ‘Evaluation of the p24 antigen component of a rapid fourth-generation HIV assay’.

T Yasvoin was awarded the prize for the ‘Best platform presentation’ at the 2nd Regional Conference of the Southern African Young Water Professionals held in July 2011.

TEACHING AND TRAINING

Undergraduate
Lectures, symposia and tutorials are presented to MBChB and BChD students in the second to sixth years. Teaching also includes lectures to allied health professions (BCur, BDietetics, and BPhysT) students.

Technologists and technicians
The department provides weekly lectures/tutorials to the intern medical technology and technician students. One technologist and one technician qualified during the year.

Postgraduate
The department is a HPCSA-accredited training facility and offers lectures, tutorials and practical training to basic science students, MMed virology and medical microbiology registrars and MMED students from other disciplines rotating through pathology. The weekly MMED virology tutorials are attended by clinicians and pathologists from the private sector. Journal club meetings are held on a weekly basis. Numerous presentations were given to other academic departments, societies and NGOs.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled:
23 (5 BSc [Hons], 9 MSc, 4 PhD, 4 MMED, 1 Post-doctoral fellow)

Postgraduate candidates graduated:
6 (1 MMED, 1 PhD, 4 MSc)

RESEARCH OUTPUT

Publications
Bowyer SM, Sim JGM, Webber L. Current laboratory diagnosis of hepatitis B virus infection including 8 years of retrospective laboratory data. CME 2011; 29: 210-213

Erasmus M. Viral infections of the central nervous system. CME 2011; 29: 190-193


Van Niekerk S, Venter M. Replacement of previously circulating respiratory syncytial virus subtype B strains with the BA genotype in South Africa. *J Virol* 2011; **85**: 8789-8797


Webber L. Guest Editorial. *CME* 2011; **29**: 189

**Conference presentations**

**International**: 11

**National**: 13

**Local**: 15

“Cervical cancer research is one of the four main focus areas of the Medical Virology Department”
DIVISION OF ANATOMICAL PATHOLOGY

Head: Prof JW Schneider

DIAGNOSTIC SERVICES

A comprehensive diagnostic service is provided to Tygerberg Hospital and about half of the Western Cape public health sector and consultation services are offered to NHLS laboratories in the Eastern Cape and the private sector. The division also offers special services and expertise including electron microscopic support for service and research, a fine needle aspiration (FNA) clinic, rapid on site cytology diagnostic services, and consultation services especially in the fields of dermatopathology, electron microscopy, neuropathology, renal pathology and perinatal pathology. Specialised services include the application of flow cytometry in the diagnosis of lymphomas using material obtained from FNA of lymph nodes. The prion laboratory - the only one of its kind in Africa - offers specialised skills and infrastructure as a referral laboratory for specimens with suspected prion disease. The electron microscopy unit provides a service to the public- and private sectors and to researchers from other Faculties at Stellenbosch University and other higher educational institutions, including the University of the Western Cape and the MRC.

During the reporting period, the surgical pathology laboratory processed 24,729 cases, the electron microscopy laboratory processed 431 cases, and the immunohistochemistry laboratory performed 14,805 immunohistochemical stains and 1,276 direct immunofluorescence stains. Staff conducted 101 adult and 108 paediatric autopsies. The cytopathology unit processed 61,981 gynaecological cases, 4,663 non-gynaecological cases and 9,657 (FNAs), including the performance of on site FNA on 3,922 patients in the FNA clinic and on 540 patients in theatre. The growing demand by clinicians and patients for this service during the past year is reflected by an increase of 19% and 20% in the number of cases for the FNA clinic and on site FNA, respectively. The cytopathology laboratory obtained SANAS accreditation.

The Pathology Research Facility facilitates the development and introduction of new diagnostic molecular pathology tests through collaboration with national and international collaborators. In addition to supporting clinical geneticists, various molecular tests are offered for the diagnosis, prognostication and therapeutic interventions of various haematological malignancies, colorectal carcinoma and carcinoma of the breast.

During 2011, the division expanded the paediatric pathology service to meet the growing demands from neonatologists, paediatricians, paediatric surgeons and obstetricians. A major contribution is made to improve the understanding and management of poor foetal outcome and to assist clinicians with the management of patients in both the public and private sectors.

The division sustained its on-going support of diagnostic services to pathologists in the Eastern Cape through assistance with excessive routine workloads and diagnostic consultations, including a telepathology service to pathologists in Mthata and East London.

The SAFRI fellowship programme continued in 2011. It aims to improve the quality of healthcare of southern African communities by improving health professions education. To date, there are fellows from South Africa, Malawi, Mozambique, Sudan, Uganda, Botswana, Zimbabwe, Ethiopia, Nigeria, Rwanda, Madagascar and Zambia participating in the programme.

RESEARCH PROJECTS

The quality of specimens obtained by FNA: does training make a difference?

Researchers: J Goedhals, CA Beukes (Department of Anatomical Pathology, University of the Free State [UFS]); G Joubert (Department of Biostatistics, UFS); CA Wright

This study was conducted to determine the outcome of a one-hour training session on the correct technique of fine needle aspiration biopsy (FNAB) on adequacy of FNA specimens received from clinicians at an academic hospital. It included a questionnaire to determine the subjective assessment of the clinicians’ perceived value of the training on their aspiration technique. Six
Clinicians were recruited and their aspirates six months prior and post the training session were evaluated and graded for advocacy. The adequacy of the aspirates for all clinicians did not improve, but decreased although this was not statistically significant. They subjectively perceived quality of the aspirates to have improved and recommended the training session to their colleagues.

**Transbronchial FNAB in patients with superior vena cava syndrome**

**Researchers:** Dr K Brundyn, Prof CA Wright; Dr C Koegelemen (Division of Pulmonology, US); Prof A Diacon (Division of Medical Physiology, US)

Transbronchial FNAB has been evaluated in patients with superior vena cava syndrome (SVCS) in respect to yield, safety and spectrum of pathology. The study showed that flexible bronchoscopy with transbronchial needle aspirations has a high diagnostic yield and is safe in the setting of SVCS. With the addition of rapid on-site evaluation, tissue biopsy is required in the minority of cases, minimising the risk of complications.

**Paediatric rhabdomyosarcomas in a resource-constrained public sector hospital**

**Researchers:** Dr L de Villiers, Prof C Wright, Prof J Bezuidenhout; Dr C Stefan (Department of Paediatrics, US)

**Funding:** NHLS Research Trust

This is a descriptive review that assessed whether immunohistochemistry, PCR and FISH significantly improve the diagnosis of rhabdomyosarcomas in this laboratory, justifying the expense of including these in routine diagnostic workup of these tumours. The study confirmed that in a resource-constrained environment the diagnosis of rhabdomyosarcomas can be made by routine hematoxylin and eosin stains and myogenin immunohistochemistry as the contribution of FISH does not justify the additional cost.

**Morphological changes in the placenta of HIV-positive mothers and association with the degree of immune suppression**

**Researchers:** Prof CA Wright, Dr PT Schubert, Dr A Vermaak

**Funding:** NHLS Research Trust

Placentas of HIV sero-positive mothers who deliver live-born babies or stillbirths are evaluated to establish whether any specific histopathological features are associated with the degree of immune suppression and to identify factors that may have been associated with adverse pregnancy/neonatal outcome.

**Clinicopathological features of disseminated cutaneous histoplasmosis and other fungal infections and the detection of fungal organisms in skin lesions by PCR**

**Researchers:** Dr M Smit, Prof JW Schneider; Prof S Engelbrecht (Division of Medical Virology, US); Prof HF Jordaan (Division of Dermatology, US); Prof E Wasserman (Division of Medical Microbiology, US)

**Funding:** NHLS Research Trust

This study confirmed that, using commercially available oligonucleotide probes for *Histoplasmosis capsulatum* and other dimorphic fungi, RT-PCR appears to be a rapid, sensitive and specific method to identify *H. capsulatum* DNA obtained from human skin biopsies in a clinical setting. This study furthermore strongly suggests that fungal organisms other than *H. capsulatum* may account for many disseminated fungal infections in immunocompromised patients.

**Factors that impact upon postgraduate workplace-based learning**

**Researchers:** Prof J Bezuidenhout; Dr R Hoffmann (Division of Medical Microbiology, US); Prof V Burch (UCT); J Grant (Open University, United Kingdom); D Manning (Wits); M van Rooyen (University of Pretoria); J van Wyk (UKZN); M van Heusden (US)

The aim of this collaborative study is to determine the factors that impact on teaching and learning of postgraduate anatomical pathology trainees in South Africa.

**Non-alcoholic fatty liver disease: genotype and phenotype expression**

**Researchers:** Prof MJ Kotze, LR Fisher, Dr K Brundyn, Dr G Swart; Prof M Kidd (Centre for Statistical Consultation, US); C Daniels, Dr FC Kruger (Gastroenterology Unit, Durbanville Medi-Clinic)

**Funding:** MRC

Non-alcoholic fatty liver disease patients recruited for this study are subdivided into four clinical subgroups relating to disease severity (fatty liver disease, non-alcoholic steatohepatitis, no/mild fibrosis, severe fibrosis) to identify biomarkers that may distinguish between patients at increased risk of cardiovascular disease vs. liver disease.
Development and application of a pathology-supported genetic assay to assess the impact of hereditary factors on health outcomes in individuals subjected to a wellness screen

Researchers: Prof MJ Kotze, Dr DP van Velden, D Geiger; Prof J Mannewick (Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Cape Town [CPUT]); Prof L van der Merwe (Biomedical Sciences, Faculty of Health and Wellness, CPUT); Prof SJ van Rensburg, Prof R Erasmus (Division of Chemical Pathology, US)

Funding: Winetech and THRIP

Chronic, multi-factorial conditions caused by a complex interaction between genetic and environmental risk factors frequently share common disease mechanisms, as evidenced by an overlap between genetic risk factors for cardiovascular disease (CVD) and Alzheimer’s disease. A method has been developed and implemented for assessment and treatment of CVD risk factors in midlife as a preventable cause of cognitive decline, morbidity and mortality in old age.

The effect of regular alcohol consumption interacting with genetic risk factors for CVD on the lipoprotein profile, oxidative stress and inflammation

Researchers: Dr DP van Velden, Prof MJ Kotze; Prof J Mannewick (Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, CPUT); Prof M Kidd (Biomedical Sciences, Faculty of Health and Wellness, CPUT); Dr D Blackhurst (Cape Heart Centre, UCT)

Funding: Winetech

The study compares the effect of moderate red wine consumption to brandy on the atherogenic lipoprotein profile, oxidative stress and inflammatory status in individuals with and without features of the metabolic syndrome.

The contribution of the placenta to the diagnosis of congenital TB

Researchers: U Rabie, Prof CA Wright, Dr PT Schubert; Dr KHoeck (Division of Medical Microbiology, US); Dr A Bekker (Division of Neonatology, Department of Paediatrics and Child Health, US); Dr S Gebarhdt (Department of Obstetrics and Gynaecology, US); Prof R Warren (Division of Biochemistry, Department of Biomedical Sciences and Human Genetics)

Funding: NHLS Research Trust

The study aims to determine if examination of the placenta can expedite the diagnosis of congenital TB in neonates born to mothers with untreated TB or with TB diagnosed within a three-month period prior to delivery.

Histopathology of the placenta in HIV-positive women

Researchers: Dr MS Zungu, Prof D Hall (Department of Obstetrics and Gynaecology, US); Dr PT Schubert, Prof C Wright

Placental changes related to HIV infection are being investigated, and these are correlated with simple maternal characteristics and neonatal outcomes.

Automated sputum screening using the BD Focal Point™ Slide Profiler: correlation with tranbronchial and transthoracic needle aspirates

Researchers: Dr PT Schubert, Prof C Wright, GS Neethling

Funding: NHLS Research Trust

The possibility of automated sputum screening on pre-bronchoscopy sputum from patients attending the TTNA/TBNA clinic with clinically and radiologically suspected intrathoracic neoplasms is being investigated.

The clinicopathological spectrum of cutaneous granulomatous inflammation

Researchers: Dr S Fakir, Dr W Visser (Division of Dermatology, US); Prof JW Schneider

This retrospective, descriptive study focuses on the classification of granulomatous dermatitis as defined by the aetiology and histomorphology of granulomas in skin biopsies and the importance of clinical correlation and ancillary investigations to arrive at a specific diagnosis.

The clinicopathological spectrum of panniculitis

Researchers: Dr F Ahmed, Prof HF Jordaan (Division of Dermatology, US); Prof JW Schneider

This retrospective, descriptive study focuses on the classification of panniculitis as defined by the aetiology and histomorphology of panniculitis in skin biopsies and the importance of clinical correlation and ancillary investigations to arrive at a specific diagnosis.
The clinicopathological spectrum of trichoepitheliomas

Researchers: Dr J du Toit, Dr W Visser (Division of Dermatology, US); Prof JW Schneider

This retrospective, descriptive study aims to document the clinicopathological spectrum of trichoepithelioma and especially its familial variant in a South African population group.

HONOURS

Dr A van Wyk received the University of Stellenbosch medal for the best 2011 Masters Student, Faculty of Health Sciences.

MSc student N van der Merwe was awarded with a certificate of excellence for a poster presentation entitled ‘CYP2D6 genotyping in South African patients with breast cancer: clinical implications with use of antidepressants’ at the Biological Psychiatry Congress, Cape Town in September 2011.

TEACHING AND TRAINING

Undergraduate

In addition to the module on Essentials of Disease Processes, consultants also lecture in clinical modules in the third, fourth and fifth years of the MBChB programme. The division is involved with a practical module in the fourth and fifth years where students visit the laboratories, receive training in the performance of FNAs and other basic procedures, and perform case studies that involve all the pathology disciplines. Other undergraduate teaching activities include supervision of MBChB students’ research assignments, teaching of basic pathology to students in physiotherapy and occupational therapy, participation in the Faculty Committee for Undergraduate Teaching, MBChB Programme Committee, and close involvement with the development and introduction of the revised MBChB programme.

During the past year, pathologists from the division served as external examiners for undergraduate medical students at the College of Health Sciences, Department of Pathology, Makerere University, Kampala, Uganda and Walter Sisulu University in Umtata.

Postgraduate

There are 10 anatomical pathology registrars in the division. A further two registrars in oral pathology (University of the Western Cape) and two registrars in forensic medicine share the same platform and anatomical pathology consultants are directly involved with their training.

Other postgraduate teaching activities include lectures to BScHons (Reproductive Biology) students, involvement with development of and teaching in the MPhil (Health Science Education) research methodology module, teaching of MMed students in family medicine on how to perform FNAs, participation in the Faculty Committee for Postgraduate Teaching, and teaching of anatomical pathology and normal histology to registrars from clinical disciplines to assist them with preparation for part 1 MMed or College of Medicine of SA examinations.

The HPCSA-registered MSc (Cytopathology) programme runs over a minimum of two years and has a modular design in keeping with modern international trends. The programme is offered by distance education and it includes student contact via satellite broadcasting and WebCT. On-going national and international moderation of the programme confirmed the high quality and standard of this programme, which is the only cytopathology degree programme in Africa. Four students completed the programme and graduated in 2011.

Medical technologists and technicians

The division provides training for student cyto- and histotechnologists. Successful candidates who passed the Professional Board examinations included one histotechnologist and two cytotechnologists.

Outreach

The division is involved with the training of medical staff and healthcare workers at regional hospitals and clinics to develop the skills and confidence to perform FNAs for cytology. Academic support is offered to pathologists in the Eastern Cape and there is close collaboration with pathologists at Walter Sisulu University utilising telepathology to exchange teaching material and to offer comments on diagnostically challenging cases. The division also provides specialised pathological tests and consultations on various tissue samples from the NHLS anatomical pathology laboratories at Nelson Mandela Academic Hospital in Mthatha and in Port Elizabeth.

Staff continued to teach FNAB technique to clinicians as part of the maintenance of competence initiative by the Western Cape health department, and organised and participated in outreach projects in Worcester, Port Elizabeth and East London to train clinicians and nursing sisters in the optimal technique in FNAB to ensure that better quality specimens reach the cytology laboratories.
PROFESSIONAL DEVELOPMENT

Postgraduate students graduated:
9 (3 MMed, 1 M (Pathology), 4 MSc, 1 PhD)

Postgraduate students enrolled:
25 (10 MMed, 10 MSc, 4 M (Pathology), 1 PhD)

RESEARCH OUTPUT

Publications


Wright CA. Fine-needle aspiration biopsy of lymph nodes - fine-needle aspiration biopsy (FNAB), when performed by trained operators, and for the correct indications, is a safe and minimally invasive procedure, with an excellent diagnostic yield. CME 2012; 30(2)

Conference presentations
International: 7
National: 5
Local: 4
DIVISION OF CHEMICAL PATHOLOGY

Head: Prof RT Erasmus

DIAGNOSTIC SERVICES

Comprehensive diagnostic chemical pathology services are provided to Tygerberg Hospital and consultation services to the Green Point laboratories. The laboratory maintained its accreditation status and expanded the test repertoire on serum protein electrophoresis, urine chemistry and vanillylmandelic acid. Continuous improvement was carried out in the laboratory support area to streamline workflow and improve turnaround times, e.g. re-arrange workstations and workflow, implement a peg system to expedite centrifugation and registration. The method for free light chains was evaluated and validated and reference values established. Methods for C-reactive protein and vitamin D were also evaluated and the former introduced as one of the tests in the division.

There is an established interest in laboratory management and quality assurance within the division. Several audits were carried out to improve the quality of service. The division initiated one of the first studies in Africa to use the Six Sigma principle to evaluate laboratory quality. The division has also established a working group on point-of-care (POC) which will liaise with the divisional audit committee. It has also recently established guidelines for selecting POC instruments and has evaluated a POC instrument for measuring ketones in an acute care setting. Currently the division is liaising with the national QA Division to evaluate various POC instruments.

RESEARCH PROJECTS

Relationship between microalbuminuria, obesity and components of the metabolic syndrome
Researchers: Y Essack, RT Erasmus; S Hassan, T Matsha (CPUT)
Funding: NHLS Research Trust

The relationship between micro-albuminuria with other components of the metabolic syndrome in 8-18-year-old learners in Western Cape communities was studied.

Role of viral factors and membrane fatty acids in multiple sclerosis
Researchers: D Hon, T Matsha (CPUT); SJ van Rensburg, RT Erasmus
Funding: CPUT Research Fund

The role of specific viral factors which might contribute to the loss of membrane integrity in multiple sclerosis patients (through changes in fatty acids) is being investigated.

Molecular investigation of genetic factors contributing to obesity
Researchers: Y Yako, SJ van Rensburg, RT Erasmus; T Matsha (CPUT)
Funding: NHLS Research Trust, US

This completed project studied the various genes involved in the leptin-melanocortin pathway as a possible contributor to the development of overweight and obesity in children and adults living in semi-urban/rural areas in the Western Cape.

Association of HbA1C with cardiovascular risk factors (or disease) and metabolic syndrome
Researchers: RT Erasmus; S Hassan, T Mouton, T Matsha (CPUT)
Funding: CPUT

This ongoing project is examining the association of HbA1C levels with specific cardiovascular risk factors such as serum total cholesterol and LDL cholesterol, and metabolic syndrome in a Cape Town community with normal and impaired glucose tolerance.

Prediction of impaired glucose tolerance and diabetes mellitus
Researchers: T Mouton, T Matsha, S Hassan (CPUT); RT Erasmus
Funding: CPUT

This ongoing project examines the use of glycosylated haemoglobin in screening for diabetes mellitus and impaired glucose tolerance.

Improving the quality of life in patients diagnosed with multiple sclerosis
Researchers: M Kotze (Pathology Research Facility, US); SJ van Rensburg, RT Erasmus
Funding: US
The inter-relationship of environmental and genetic factors that could impact on the quality of life in patients with multiple sclerosis is being investigated. The aim is to develop intervention programmes that, through modification of the external environment, may influence gene-environment interaction, thereby having an impact on the course of their disease.

**Serum concentration of iron carrier proteins and involvement of genetic factors in influencing serum iron concentration**

*Researchers:* SJ van Rensburg, M Rensburg, RT Erasmus  
*Funding:* NHLS Research Trust

This is a study of the interaction between iron carrier proteins and certain iron regulating genes and how they influence serum iron levels.

**Determination of reference values in healthy South African and Kenyan adults**

*Researchers:* R Erasmus, M Hoffman, Y Essack, T Matsha, P Ojwang, J George, J Wassung, K Ichihara  
*Funding:* Beckman, NHLS Research Trust, Japan Fund

**TEACHING AND TRAINING**

**Undergraduate**

Lectures and practical sessions are presented for MBChB students in the Essentials of Disease Processes module; consultants also lecture in clinical modules in the third, fourth and fifth years. The division is involved with a practical module in the fourth year where students visit the laboratories and perform case studies that involve all the pathology disciplines. Tutorials are given to third and sixth year students.

**Postgraduate**

The division revised the module on laboratory management. The training laboratory which was established in collaboration with the Division of Haematology to assist registrars and other postgraduate students to be proficient in laboratory techniques has continued to flourish. The division co-ordinated the postgraduate modules in molecular pathology and laboratory management. The division continued to be involved in the postgraduate exams conducted by the Aga Khan Medical University, Nairobi, Kenya and contributed to further improving their academic programme.

**OUTREACH**

Locally, the division collaborates with the Multiple Sclerosis (MS) Society of the Western Cape, and makes an important contribution to inform patients about recent developments on the biochemical basis of MS. Staff offered updates on the utilisation of laboratory services to clinicians at regional hospitals in the Western and Eastern Cape. Continuing medical education was given at Green Point and RCCH laboratories and GSH. The division is involved with the development of an academic programme in chemical pathology for Walter Sisulu University. This university and its teaching hospital were visited by a consultant member of staff who presented several lectures.

On the African continent, the divisional head is involved in the development of collaborative links with Nigeria and Ghana. Links were also established with the department of Pathology, Aga Khan University, Nairobi, Kenya and the Department of Pathology at Muhimbili Research Centre in Tanzania. The MODY (maturity onset diabetes of the young) project is an international collaborative project between South Africa and Kenya, with one student from the Aga Khan using the project for her MMed degree The division is also involved in co-ordinating the Africa Reference Ranges project which involves South Africa, Nigeria and Kenya. The project is now integrated into the Global Reference Range Study which is being led by Prof K Ichihara from Japan.

A laboratory management workshop was given in Nairobi, Kenya from 27 to 29 September, 2011 at the invitation of the International Federation of Clinical Chemistry.

**PROFESSIONAL DEVELOPMENT**

**Postgraduate candidates graduated:**  
8 (2 FCPath, 2 MSC/MTech 4 HonsBSScMedSc)

**Postgraduate students enrolled:**  
19 (5 MMed, 5 Doctoral, 3 MSc, 6 HonsBSScMedSc)

**RESEARCH OUTPUT**

**Publications**


Matsha T, Hassan S, Erasmus RT. The 30-year Cardiovascular Risk Profile of South Africans with Diagnosed Diabetes, Undiagnosed Diabetes, Pre-Diabetes or Normoglycaemia: The Belville-South Africa Study. *Cardiovascular J Africa* 2012; 23: 5-11 DOI: 105830/CVJA-2010-087

Rensburg MA, Matsha T, Hoffmann M, Hassan MS, Erasmus RT. Distribution and association of hs-CRP with cardiovascular risk variables of metabolic syndrome in adolescent learners. *AJLM* 2012; 1(1)


**Conference presentations**

**International:** 14

**National:** 10

**Local:** 3

“**The Division initiated one of the first studies in Africa to use the Six Sigma principle to evaluate laboratory quality**”
DIVISION OF HAEMATOLOGICAL PATHOLOGY

Head: Prof A Abayomi

DIAGNOSTIC SERVICES

A comprehensive 24-hour service is provided to Tygerberg Hospital and some of the clinics and secondary hospitals in the Western Cape region. This service was expanded from April 2011 to include Eerste River Hospital and more peripheral clinics. The division is one of the referral centres for samples from the Eastern Cape, particularly from Nelson Mandela Hospital, Livingstone Hospital and East London Hospital.

An antenatal blood group typing service is provided to the hospital and large regions in the Western Cape in association with Groote Schuur Hospital.

Some of the specialised haematology tests performed at Tygerberg include hereditary thrombophilia screening assays, platelet function analysis, flow cytometry used in the diagnosis of haematological malignancies, cytogenetic analysis of haematological malignancies and genetic tests.

The following new molecular-based tests have been introduced by the division in conjunction with the Pathology Research Facility: FLT-3 is an important poor prognostic indicator in patients with acute myeloid leukaemia; and a RQ-PCR method to assess response to treatment by measuring BCR-ABL transcript levels in patients receiving treatment for chronic myeloid leukaemia was validated.

RESEARCH

The TB Cytokine Group was developed by the Division of Haematopathology research committee during the past year. Together with the Tygerberg Lymphoma Study Group and the HIV Activation and Inflammation Group, which were developed in 2010, a number of projects for Honours, Masters and PhD students have been created.

The division is involved in a collaborative study on paediatric HIV-exposed and -uninfected infants with the Immunology Unit.

RESEARCH PROJECTS

The Tygerberg Lymphoma Study Group: Malignant lymphoma incidence and HIV-related lymphoma subtypes

Researchers: EA Abayomi, R Grewal, A Sommers, G Sissolak, F Bassa, D Maartens, P Jacobs, C Stefan; LW Ayers (Department of Pathology, The Ohio State University, USA, and Sub-Saharan Africa Lymphoma Consortium)

Funding: The NIH through the Sub-Sahara Africa Lymphoma Study and the AIDS Cancer Specimen Repository

This is a continental and international multidisciplinary approach to improve the understanding of the how HIV is transforming the incidence, pattern, prognosis of malignant lymphoma, and to define a cost-effective policy model and strategic approach to management in the Western Cape and the sub continent.

HIV Activation and Inflammation Group: Development of a cost-effective panel of biomarkers of immune activation and inflammation in asymptomatic HIV-infection

Researchers: H Ipp, S Mburu, B Nkambule; R Glashoff, T Reed, S Loots (Division of Virology, US); L-G Bekker (Desmond Tutu HIV Centre, UCT); A Zemlin (Division of Chemical Pathology, US)

Funding: SHARP/PRF, NHLS K-funding

This project aims to delineate cost-effective and easily measurable markers of the activation and inflammatory status of the immune system in asymptomatic HIV infection, in order to identify patients who may be at increased risk of progressive disease or adverse events. This would lead to the design of a panel of affordable tests for application in resource-limited settings that have direct impact on the management of patients in the chronic stage of HIV-infection. The implementation of this approach would facilitate earlier access to treatment and thereby ultimately assist in delaying the onset of AIDS. A secondary objective is the identification of points of potential therapeutic interventions in the inflammatory signalling cascade and antioxidant metabolic pathways.
Pilot study of innate immune abnormalities in HIV-exposed uninfected infants

Researchers: M Cotton (Department of Paediatrics, US); MM Esser (Division of Microbiology and Immunology, US); T Kollmann, D Speert (University of British Columbia, Vancouver, Canada)

Collaborators: H Ipp; J Steenkamp; C de Beer; S Naidoo (Division Microbiology and Immunology, US)

Funding: Burroughs Wellcome

The primary aim is to investigate the innate immune response in 25 HIV-exposed uninfected (HEU) and 25 HIV-unexposed infants at 2 and 6 weeks of life. HIV PCR will be repeated on all babies at 12 weeks of life. The secondary aim is to process the study results for recommendations of HEU infant follow up. The objectives will be to determine: i) the difference between HEU and UE infants in innate immune function, and ii) if documented, the significance of this difference.

Pilot study of innate immune abnormalities in HEU infants

Researchers: J Steenkamp, H Ipp, A Abayomi; M Esser (Division of Immunology, US)

Funding: Burroughs Wellcome

A long term follow up of the infants in the above study will continue at ages 6 and 12 months.

TEACHING AND TRAINING

Undergraduate

The haematology module is presented to MBChB III students and encompasses the important basics of haematological disease. Lectures and tutorials as part of the essentials of disease processes module is presented to MBChB I students. MBChB IV and V students visit the laboratory as part of their middle clinical rotation. This rotation involves a more intimate teaching environment and exposes the students to the laboratory as well as haematology case studies. The registrars in the division participate in this programme which runs throughout the academic year. The Division of Haematopathology together with Clinical Haematology run a two-week collaborative programme for final year medical students and senior resident students from Nebraska (USA).

Postgraduate

In the past year, two haematopathology registrars were recruited, one being a supernumery from Libya. The division also contributes towards training a clinical pathology registrar every year, as well as internal medicine registrars rotating in clinical haematology every three months. During 2011, two BSc honours students and one Masters Science student were trained.

Outreach and community interaction

An outreach trip to Nelson Mandela Hospital in Mthatha was undertaken in September 2011 where seven haematology-related lectures were presented. Staff also lectured as part of the outreach programme at the NHLS Green Point complex in March 2012.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated:
(2 MMed, 1 MSc, 2 BSc Hons)

Postgraduate candidates enrolled:
13 (8 MMed, 4 FCHaem, 1 PhD)

RESEARCH OUTPUT

Publication

Conference presentations
International: 7
National: 7
Local: 9
DIVISION OF MEDICAL MICROBIOLOGY

Head: Prof E Wasserman

DIAGNOSTIC SERVICES

The Division of Medical Microbiology has been SANAS-accredited since February 2009 and offers diagnostic services in microbiology, immunology and serology.

The molecular facility to aid in the early diagnosis of TB, is a fully operational diagnostic service, and has significantly reduced the turnaround time of TB specimens and the screening of resistance to first-line agents.

New tests implemented in the past year are the 16S RNA PCR plus sequencing for identification of bacteria, and pan-fungal PCR plus sequencing for identification of fungi.

Consultants conduct regular ward-rounds and clinical discussions and offer an academic programme for clinicians and postgraduate students. The division’s service platform has been extended to incorporate Karl Bremer Hospital and the surrounding clinics.

Regular expert committee meetings with UCT and Green Point laboratories are attended to discuss issues such as standardised testing, postgraduate programmes and a syllabus for staff training.

This division again collaborated with colleagues at UCT to publish an updated booklet on antimicrobial guidelines for academic hospitals; this booklet is distributed to doctors at Tygerberg and Groote Schuur hospitals, as well as those in peripheral hospitals.

RESEARCH

The division has three well-defined research focus areas:
- The laboratory diagnosis of TB;
- The epidemiology and pathogenesis of staphylococcal infections; and
- Immunopathology in HIV-exposed children.

The following projects are currently being undertaken:
- Full participation in the national surveillance programmes directed by the NICD;
- A collaboration concerning the pathogenesis of staphylococcal infection with researchers at Würzburg University, Germany; W Oosthuysen, a PhD candidate, spent six months (July 2011 – January 2012) at the Julius-Maximilian-Universität in Würzburg, Germany to complete his research project on the virulence factors in Staphylococcus aureus in complicated infection. This research is funded by the NRF and an equivalent German partner agency. Prof Wasserman is also co-supervising another PhD student working at Würzburg;
- In collaboration with the University of British Colombia, a 24-month pilot birth cohort study on HIV-exposed uninfected infants was completed. A funding application with the Peter Wall Institute of Advanced Studies from Vancouver was successful for a larger birth cohort study to focus on the immune and environmental risk parameters of HIV-exposed infants which will commence in 2012. Dr Esser and Prof M Cotton from the Children’s Infectious Disease Clinical Research Unit (KIDCRU) Unit in Tygerberg Hospital are the local investigators for the study;
- The Primary Immunodeficiency Registry of South Africa, principal investigator Dr M Esser, continues to promote the networking for laboratory diagnosis and treatment of patients with primary immunodeficiencies. Almost 200 patients are recorded nationally;
- Close collaboration with the African Society of Immunodeficiencies (ASID) was again established with a teaching school for primary immunodeficiencies (PID) hosted at the Allergy Congress in Sun City in September 2011 by Dr Esser. A national working group for PID was constituted under the auspices of the Allergy Society of South Africa, with Dr Esser chairing the group.

RESEARCH PROJECTS

Evaluating the capability of a recently qualified medical microbiologist to practice in the rapidly changing South African healthcare environment

Principal researcher: Dr R Hoffmann
Population structure, host cell interaction and pathogenesis of *Staphylococcus aureus* strains isolated at Tygerberg Hospital  
**Principal researcher:** Prof E Wasserman

A phenotypic and genotypic characterisation of strain types, virulence factors and agr groups of colonising *S. aureus* strains associated with bloodstream infection  
**Principal researcher:** Prof E Wasserman

Primary Immunodeficiency South African Registry/database  
**Principal researcher:** Dr M Esser

Pilot study of innate immune abnormalities in HIV-exposed uninfected infants  
**Principal researcher:** Dr M Esser

Investigation of biofilm formation in *S. aureus* causing bacteremia in patients admitted to Tygerberg Hospital  
**Principal researcher:** Prof E Wasserman

The relevance of anti-cyclic citrullinated peptide measurement in the HIV-infected patient  
**Principal researcher:** Dr M Esser

An evaluation of the diagnostic utility of peripheral blood culture versus bone marrow culture in children with suspected disseminated TB  
**Principal researcher:** Prof E Wasserman

Immunological parameters of HIV-exposed uninfected infants with severe infectious morbidity  
**Principal researcher:** Dr M Esser

Real time PCR as a diagnostic tool for detecting BTK mutations in agammaglobulinaemia  
**Principal researcher:** Dr M Esser

Development and validation of flow cytometry-based assays as alternatives to the isotope incorporation assay for the diagnosis of immune dysfunctions  
**Principal researcher:** Dr M Esser

Characterisation of *S. aureus* causing bacteremia in patients admitted to Tygerberg Hospital  
**Principal researcher:** Prof E Wasserman

Improving laboratory diagnostic techniques to detect *Mycobacterium tuberculosis* complex and *C. neoformans* as the causative agents of chronic meningitis in the cerebrospinal fluid of adult patients  
**Principal researcher:** Dr K Hoek

Molecular diagnosis of *M. tuberculosis* from urine and stool specimens of patients with suspected TB disease  
**Principal researcher:** Dr K Hoek

HONOURS

Two lecturers from the Division of Medical Microbiology received awards for the most influential lecturer contributing to the academic success of first year medical students four years in a row: Prof Arderne Forder received the award for three consecutive years 2009-2011 and Prof Elizabeth Wasserman received it in 2012.

TEACHING AND TRAINING

Undergraduate

The division is extensively involved in training MBChB students in their different modules. Various modules are presented in the BSc Dietetics programme.

Trainee medical technologists from the Cape Peninsula University of Technology have done their practical training in the division.

During 2011 the division received a two-year accreditation from the HPCSA for the training of an intern scientist. While the immunology section received a five-year accreditation for the training of three intern scientists. Members of the HPCSA were specifically impressed with the extensive exposure to clinical work that interns would receive.

Postgraduate

Registrars rotate on a regular basis to Green Point, Worcester and Paarl laboratories, and rotate for six months through the virology division and three months in the infection control unit at Tygerberg Hospital.
PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated:
3 (1 MSc MedSc, 2 Hons BSc)

Postgraduate candidates enrolled:
9 (4 MMed (Micro), 2 doctoral, 2 MSc MedSc, 1 Hons BSc)

RESEARCH OUTPUT

Publications


Conference presentations
International: 3
National: 2
Local: 3
DIVISION OF MEDICAL VIROLOGY

Head: Prof W Preiser

DIAGNOSTIC SERVICES

The diagnostic section’s workload increased by 26,000 tests to a total of almost 170,000, while requests for HIV viral load tests increased by 21% and those for HIV PCR by 9% and requests for genotypic HIV drug resistance testing doubled, underlining its rapid emergence as an important issue.

Although the 2011 influenza season was relatively mild, more respiratory samples were tested for the national Viral Watch programme than the year before, and once again, around 10,000 serum specimens from the Western Cape were tested as part of the HIV and syphilis annual antenatal survey.

The Seeplex RV15 panel was complemented by the addition of cytomegalovirus (CMV) testing, and the Argene CMV viral load test was introduced and accredited. In-house RT-PCR tests for mumps and enteroviruses as well as sequencing of the HIV integrase gene were added to the routine test repertoire but have yet to be accredited.

This laboratory is the only NHLS facility in the Coastal Region to routinely perform cell culture and offer virus isolation. It thus provides training also for registrars and student medical technologists from Groote Schuur Hospital laboratory. Tygerberg is also the only laboratory in the whole country still performing Coxsackie virus neutralisation assays.

Optimisation of work hours and internal workflow patterns resulted in significantly improved turnaround times for the majority of tests offered. Diagnostic staff undertook outreach visits to NHLS laboratories in Vredendal, Vredenburg, George, Knysna, Oudtshoorn and Mossel Bay, and to Calvinia Provincial Hospital to give CPD talks to laboratory staff, district nursing staff and local clinicians. The laboratories in Paarl and Worcester were also visited to suggest some improved methodologies and help with referral problems and injury-on-duty protocols.

RESEARCH PROJECTS

The division’s research focuses on HIV-1 as one of the major health problems in South Africa and the region, encompassing projects on genetic, immunological and clinical/translational aspects; this is complemented by research on selected other relevant topics.

Molecular characterisation and diversity of HIV

Researchers: Prof S Engelbrecht, Dr R Glashoff, Dr E Vardas, Dr GB Jacobs (post-doctoral fellow), E Wilkinson (PhD student), S Isaacs (MSc student), PW Msimanga (MSc student), MM Seleka (MSc student), C Tamandjou (BSc Hons student)

Collaborators: Prof S Seedat (Department of Psychiatry, US); Dr T de Oliveira (Africa Centre for Health and Population Studies, UKZN); Prof J Joska (Department of Psychiatry, UCT); Prof R Paul (Department of Psychology, University of Missouri, St. Louis, USA)

Funding: PRF, University of Stellenbosch, NHLS Research Trust, NIH, Wits Health Consortium

One of the features of HIV-1 is its extreme genetic diversity, which impacts on diagnostic assays, ARV treatment, prevention and vaccine development. It is well known that HIV-1 subtype C strains predominate in South Africa. However, during the last year, an unusually large number of subtypes and unique HIV-1 recombinants (URFs) have been identified. Pure subtypes detected include subtypes A, B, C, D, and G. URFs detected include BC, AC, AK, CK, CD and complex recombinants (ACD and AFG). This may be an indication that the homogeneous subtype C epidemic in the country is changing. It is essential to study HIV-1 non-subtype C viruses on an ongoing basis in order to gain a better understanding of their characteristics and the viruses in circulation in South Africa. Ongoing projects include investigating the history and the evolution of the HIV-1 epidemic in the southern African region and the effect of different tat mutations on toxicity and apoptosis in cell culture. Another project aims to characterise monocyte-associated HIV strains and viral copy numbers in patients with HIV-associated dementia (HAD). HAD is not well studied in areas of the world with predominantly HIV-1 subtype C. The aim of the study is to determine whether HIV-1 subtype C targets monocytes and also to assess the relative contribution of activated macrophage-like monocytes (CD14+CD16+) to the risk of HAD development and the viral copy numbers associated with monocyte subsets.
Studying patients in rural Mpumalanga, a further project aims to investigate viral diversity and the prevalence of viral co-infections in the area and the level of general immune activation by cytometric bead array profiling of plasma cytokines. The findings will give a clearer picture of the HIV-1 epidemic in this remote part of South Africa close to the Mozambican border.

**HIV immunology relevant to the development of vaccines and immunotherapeutic approaches**

**Researchers:** Dr R Glashoff, Dr H Ipp; D de Swardt (PhD student), K Poovan (MSc student), T Reid (MSc student), S Loots (MSc student), K Reddy (MSc student)

**Collaborators:** A Zemlin (Division of Chemical Pathology, US); Prof LG Becker (Desmond Tutu HIV Foundation/UCT)

**Funding:** DST SHARP, PRF, NHLS Research Trust

Several projects are currently studying immune activation and inflammation in chronic HIV infection, its impact on immune cell numbers, phenotypes and function and the potential for immunotherapeutic intervention to minimise activation-associated pathology. Two major aims are to define novel cost-effective surrogate markers of immune activation for use in the South African setting and to investigate the neuropeptide vasoactive intestinal peptide (VIP) as a potential therapeutic agent. Included in these studies are HIV-TB co-infected individuals and individuals on short-term ART to assess the impact of both on immune cell activity and marker expression.

Sub-projects are investigating the following aspects: modulation of apoptosis of CD4+ T cells from HIV-infected individuals with the neurotransmitter VIP and other biomediators; blood dendritic cells in HIV-infected individuals and the impact of TB co-infection and immune modulation; B-lymphocyte activation and exhaustion in chronic HIV infection: novel surrogate markers of generalised immune activation and selective modulation of aberrant B cell responses using VIP; erythrocyte apoptosis (erythroptosis) and anaemia in chronic HIV infection: relationship with immune activation and viraemia; monocyte/macrophage function in chronic HIV infection; impact of immune activation and modulation with VIP; and monocytes in chronic HIV infection: expression of gut-associated chemokine homing receptors, bcl-2 and PPAR-γ and their relationship to immune activation.

**Antiretroviral drug resistance: epidemiological, clinical and diagnostic aspects**

**Researchers:** Dr GU van Zyl (virological pathologist enrolled for part-time PhD), Prof W Preiser, Prof S Engelbrecht, R Fisher (PhD student), M Claassen (medical technologist enrolled as part-time MSc student), D Hart (MSc student)

**Collaborators:** Prof M Cotton (Paediatric Infectious Diseases Unit, US); Dr M Zeier, Dr J Taljaard (Adult Infectious Diseases Unit, US); Dr C Scheller, Dr J Bodem, Prof A Rethwilm (University of Würzburg, Germany); Dr E Goemaere, Dr G van Cutsem, Dr C Malavazzi (Médecins Sans Frontières, South Africa); D Smith, R Haubrich (University of California, San Diego); S Travers, N Wood (University of the Western Cape); Dr T de Oliveira (UKZN); Prof R Shafer (Stanford University)

**Funding:** PRF, NHLS Research Trust, Department of Health CCMT Grant, Centre for AIDS Research Sub-Award, University of California San Diego

An ongoing major project to assess the relevance of ARV drug resistance in the context of the South African roll-out programme has yielded some important results: it confirmed that prevention of mother-to-child transmission (PMTCT) using two drugs halves the mother’s likelihood of being left with drug resistance HIV; dual PMTCT has since been rolled out countrywide.

It also showed the sequential acquisition of drug resistance mutations in patients failing first-line ART, highlighting the need for regular monitoring by viral load testing, as opposed to the paucity of drug resistance in patients failing second-line ART where poor adherence is far more important. Dr van Zyl and Prof Preiser are part of the working group formulating the new antiretroviral resistance treatment guidelines to be issued by the Southern African HIV Clinicians’ Society.

The use of nevirapine for PMTCT poses a risk of inducing resistance in the infant, should he/she become infected. However, viral variants harbouring these resistance mutations may wane over time and are therefore often undetectable by standard resistance testing, using bulk sequencing, when patients require therapy. Furthermore, deep sequencing methods used to detect minor resistant variants may not reliably quantify these variants due to PCR or sequencing error. A different platform to quantify these minor variants with a reduced error rate is being investigated.
Stavudine was replaced by tenofovir (TDF) in adults and by abacavir (ABC) in children as a component of first-line ART in the national antiretroviral roll-out since April 2010. To study the effect of these changes on resistance patterns information from request forms and resistance mutations detected in the laboratory were recorded. The guideline change was associated with a rapid increase in TDF use from <2% between 2006-2008 to 41% patients in 2011 associated with an increase in the frequency of the K65R mutation. Similarly, in children ABC use increased with an associated increase in the L74V mutation.

Deep sequencing sheds light on low levels of protease inhibitor (PI) resistance in South African patients who failed a second-line lopinavir/ritonavir-based regimen. While standard sequencing revealed no PI resistance, deep sequencing revealed low abundance PI-resistant HIV variants in five of seven patients. These variants probably do not emerge to become predominant due to inadequate drug pressure in patients with inadequate adherence. This may help to explain the higher probability of PI resistance in patients with intermediate to high levels of adherence.

While transmitted and – in infants – initial drug resistance are still rare in our setting, an international collaboration has found a substantial rate of resistance mutations in patients prior to starting ART. The WHO is currently considering a revision of surveillance recommendations for transmitted HIV drug resistance, to take into account these findings.

Opportunistic viral infections and related conditions

Researchers: Dr MI Andersson, Dr C de Beer, Prof WP Reiser, Dr R Glashoff, Prof S Engelbrecht, Dr W Liebrich, T Maponga (MSc student), N Chotun (MSc student), J van Staden (BSc Hons student), I Smit (BSc Hons student)

Collaborators: Dr S Ijaz, Prof R Tedder (Health Protection Agency, London, UK); Dr M Esser (Immunology Unit); Dr J Dempers (Department of Forensic Pathology and Western Cape Forensic Services); Dr M Zeier ( Infectious Diseases Clinic, Tygerberg); Dr H Botha (Gynaecological Oncology Unit, Tygerberg); Prof M Cotton (Paediatric Infectious Diseases Unit, Tygerberg); Prof G Theron (Dept Obstetrics & Gynaecology, Tygerberg); Dr ENel (Department of Paediatrics, Tygerberg); Prof SMoore (Paediatric Surgery, Tygerberg); N du Toit (Dept Oncology, Tygerberg); Drs M Joffe, P Ruff, A Sparaco (Wits); DrsaB Robertson, W Spearman, Prof M Kew, (UCT); Drs P Veersamy, V Fredlund (UKZN); Dr RSantella, Prof J Jacobson (Columbia University, New York); Prof H von Briesen (Fraunhofer-Institut für Biomedizinische Technik, Germany); Prof DC Montefiori (Duke University, USA); Prof T Kollmann, D Speert (University of British Columbia, Vancouver, Canada); Dr A Giuliano (Moffitt Cancer Research Institute, Tampa, USA)

Funding: Wellcome Trust, PRF, NHLS Research Trust, NIH, Bill & Melinda Gates Foundation

Ongoing studies analysing the prevalence and character of chronic HBV infection in HIV-infected and HIV-uninfected women and in infants born to HIV-positive mothers have yielded first results. In addition, a new multi-site study aims to assess whether there is any increased risk of developing hepatocellular carcinoma (HCC) in those who are HIV-positive and will describe the demographics, survival and risk factors associated with HCC.

While the success of PMTCT programmes is astounding, there is increasing evidence suggesting immune abnormalities in uninfected children born to HIV-infected mothers (HIV exposed uninfected [HEU]). HEU infants far outnumber HIV-infected infants. They are a frequently overlooked risk group for infectious and non-infectious morbidity which is higher than in unexposed infants. Known causative factors include poor placental transfer of maternal antibodies, perinatal exposure to ARV drugs and increased exposure to pathogens from immunodeficient individuals in the household. Studies investigating innate immune abnormalities and immune activation, apoptotic and memory B cell markers in HEU infants and unexposed controls are being published.

Maternal HIV infection has been identified as an independent risk factor associated with increased mortality, for necrotising enterocolitis (NEC), the pathogenesis of which remains elusive. This may relate to enhanced endothelial activation and inflammation in HIV-infection. This study aims to evaluate how HIV exposure impacts on NEC pathogenesis, prevalence and severity. Clinical markers of disease severity and mortality as well as serum levels of inflammatory cytokines are evaluated in infants with severe NEC referred for surgical evaluation.

An internationally collaborative evaluation of HPV vaccination in young women as a preventative measure against HIV-1 acquisition is being conducted in preparation for a larger phase III being trial planned for 2014/2015. The research will focus on HPV genotyping to assess subtype distribution and the impact of sexually
transmitted co-infections. To investigate CMV epidemiology in infants, urine and blood samples are being collected from HIV-exposed and HIV-unexposed babies soon after delivery and at follow-up visits up to two years of age. Urine samples are tested for CMV by RT-PCR and blood samples for CMV antibodies. Demographic and clinical data will be correlated with molecular and serological results and comparisons drawn between HIV-exposed and HIV-unexposed babies.

The majority of sudden unexpected death in infancy (SUDI) cases occur in infants between one and six months of age. Inflammatory changes in the upper and lower respiratory tract in SUDI cases are a frequent finding and respiratory tract infection in the days preceding death had also been documented repeatedly. Furthermore, viral respiratory infections have commonly been found in these autopsy samples. Although viral infections have often been implicated in SUDI and routine virological investigation often forms part of the laboratory investigation, there is very little guidance as to which viruses should be included in this protocol. There is also no nationally accepted infant death investigation protocol available in South Africa and this study will investigate lung samples from SUDI cases from the Tygerberg medico-legal mortuary for the presence of 16 different respiratory viruses.

South Africa is the primary site for the Global HIV Vaccine Research Consortium (GHRC), which is one of the grantees of the Bill & Melinda Gates Foundation within the Collaboration for Aids Vaccine Discovery (CAVD). This consortium is developing and standardising cryotechnology and cryoprocedures through establishment of a fully functional cryolaboratory in Medical Virology, US. The university is also the only primary site in the CAVD and is responsible for sample collection of recently infected HIV strains. Peripheral blood mononuclear cell (PBMC) isolation is done according to specifically developed protocols in the ChameleonLab system. Plasma and serum are stored at -80°C, and PBMCs are stored in the cryolaboratory in liquid nitrogen, using an Askion workbench with computer-controlled cooling rates and an access tower with computerised access and a temperature controlled environment. This access tower facilitates the placement of samples in liquid nitrogen tanks and captures all the information from the microchips in an electronic database. These samples are being used by global collaborators involved in vaccine and neutralisation assay development and optimisation.

Development and improvement of diagnostic laboratory technologies

Researchers: Prof W Preiser, Dr GU van Zyl, Prof S Engelbrecht, Dr J Maritz (MMed student), N Sampson (BSc Hons student), D Njenda (BSc Hons student)
Collaborators: Prof C Wright (Division of Anatomical Pathology)
Funding: PRF, NHLS Research Trust, NHLS K-funding

Immunohistochemistry (IHC) and immunocytochemistry (ICC) are indispensable techniques for the diagnosis of viral disease in organs and tissues, as they combine the visualisation of characteristic morphological changes indicative of pathogenic processes with the detection of viral antigen proving the presence of a specific viral infection. In situ hybridisation (ISH) allows the detection of specific viral genome sequences in tissues and cells by means of specific oligonucleotide probes followed by detection of probe binding. It is increasingly used and has the same advantages as antigen detection in combining the detection of a specific virus with the assessment of its effect on the infected cell or tissue. The use of appropriate positive and negative controls is critical for diagnostically sound IHC, ICC and ISH testing.

The control material should be fixed and processed in a similar manner to the sample, with the positive control consisting of tissue or cells known to contain the viral target antigen. Obtaining such control material for viral IHC, ICC and ISH poses a challenge as it currently relies on using patient specimens. These are scarce and often problematic in that they may be inadequately processed, contaminated, lacking in quantity or give poor IHC results. The use of patient-derived material left over after routine testing also raises substantial ethical concerns. Readily available control materials were developed for viral IHC and ICC as well as ISH, processed either as ‘mock tissue’ blocks or liquid-based cytology preparations, by using virus-infected and uninfected cells propagated in vitro. A South African provisional patent application has been filed for this invention and it is already being used routinely in the Tygerberg histopathology laboratory.

The division is working to develop alternative assays for important markers. This includes allele-specific RT-PCR, pooled resistance testing of tagged samples by next-generation sequencing, pooled qualitative PCR to replace viral load testing followed by sequencing to methods for HIV-1 infant diagnosis and ARV mutation detection that have the potential to be performed as point-of-care assays as no thermocycling is required. However, their design is complicated by the high degree of HIV-1 diversity.
Potentially zoonotic and emerging viral diseases

Researchers: Prof W Preiser, NL Ithete (PhD student), N Sampson (MSc student), A Adams (BSc Hons student)  
Collaborators: Prof S Matthee (Department Conservation Ecology & Entomology, US); Prof D Krüger (Institute for Virology, Charité, Humboldt University, Berlin, Germany)  
Funding: Deutsche Forschungsgemeinschaft, PRF, NHLS Research Trust

This project aims to identify and characterise novel viruses occurring in small mammals in southern Africa that could result in zoonotic transmission and human disease. Animal tissues are screened for the presence of viral genome. In addition, patients matching a clinical case definition of possible disease are tested using molecular and serological assays and serosurveys are conducted to assess the prevalence of virus-specific antibodies in different populations as a marker of previous exposure.

HONOURS

S van Zyl and Prof W Preiser received Stellenbosch University's Rector's awards for general performance. Dr M Andersson was given the opportunity to attend the EPIC summer school study at the University of Columbia as part of the D43 NIH grant. She was also appointed as a junior member of the Hepatitis Scientific Committee of the International Maternal Pediatric Adolescent AIDS Clinical Trials Group.

TEACHING AND TRAINING

Undergraduate

Lectures and practical sessions were presented to MBChB students, most of them as part of the modules on essentials of disease processes and infections and immunology; and for a variety of postgraduate courses within the faculty and elsewhere, both nationally and internationally.

Postgraduate

Postgraduate teaching activities comprise the South-to-South Partnership for Comprehensive Paediatric HIV Care and Treatment, the postgraduate diploma in infection control, the immunology interactive forum, which includes basic aspects of immunology and advanced aspects of immunology, and the diploma in tropical medicine and hygiene course at the London School of Hygiene and Tropical Medicine. Another notable postgraduate activity is the international research training group ‘HIV/Aids and associated infectious diseases in southern Africa,’ in cooperation with the University of Würzburg in Germany and UCT, funded by the NRF and the Deutsche Forschungsgemeinschaft. This is still the only such international structured PhD training programme for the whole of Africa. The second phase of the programme has been applied for.

Medical technologists

Six intern medical technologists were in training last year; two have passed their Board exam. After qualification, one was placed at the NHLS laboratory in Mthatha and the other at NHLS Dora Nginza.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 10 (1 PhD, 2 MSc, 6 BSc [Hons], 1 MMed)  
Postgraduate candidates enrolled: 19 (4 PhD, 8 MSc, 3 BSc [Hons], 4 MMed)

RESEARCH OUTPUT

Publications


Van Zyl GU, Van der Merwe L, Claassen M, Zeier M, Preiser W. Antiretroviral resistance patterns and factors associated with resistance in adult patients failing NNRTI-based regimens in the Western Cape, South Africa. *J Med Virol* 2011; 83(10): 1764-1769

Van Zyl GU, Rabie H, Nuttall JJ, Cotton MF. It is time to consider third-line options in antiretroviral-experienced paediatric patients? *J Int AIDS Soc* 2011; 14: 55


Conference presentations

**International**: 31

**National**: 18

“Immunohistochemistry and immunocytochemistry are indispensable techniques for the diagnosis of viral disease in organs and tissues”
DEPARTMENT OF ORAL AND MAXILLOFACIAL PATHOLOGY

Head: Prof J Hille

DIAGNOSTIC SERVICES

Diagnostic histo- and cytopathology services of the oral and head and neck regions are rendered through the anatomical pathology laboratories at Tygerberg Hospital. NHLS services have also been expanded and consolidated to the regional and academic hospitals in the greater Western Cape region and to the NHLS’ anatomical pathology laboratories in the eastern part of the Coastal Region (Port Elizabeth, East London and Walter Sisulu University) by means of referral. The department delivers direct consultation services to the maxillofacial and oral surgery departments of Groote Schuur Hospital and Red Cross Children’s Hospital through the anatomical pathology laboratories of these hospitals.

Furthermore, the department provides clinical oral diagnostic services to the oral medicine clinics of the University of the Western Cape’s Oral Health Centre and the ear nose and throat clinics at Tygerberg Hospital and consultations are given to the Dental Genetics Unit at Red Cross Children’s Hospital. The department has a representative on the weekly combined head and neck oncology clinics of Tygerberg Hospital for clinico-pathological correlation and to enhance the treatment of head and neck cancer patients.

RESEARCH PROJECTS

Diagnostic accuracy of liquid-based brush cytology of laryngeal mucosal dysplasia and malignancy

Researchers: Dr A Afrogheh, Prof J Hille; Prof CA Wright, Dr A Pelser, P Schubert (US)

This study intends to establish an endoscopic brush biopsy procedure and evaluate the adequacy of cytomorphic criteria that differentiate truly ‘risky’ from reactive/’non-risky’ cell samples derived from either suspicious laryngeal mucosal lesions or those lesions of a low level of clinical concern using a standardised liquid-based collection and smear preparation method that satisfies the criteria of economy, low turnaround times, consistent superior quality and highly reproducible. The project is based on the findings of a previous study indicating that the PapSpin liquid-based cytology (LBC) method in association with the transepithelial brush biopsy technique of oral lesions is a highly sensitive, specific, and economical screening test. It uses a novel cytological scoring scheme showing promise as a reliable and reproducible system. The diagnostic accuracy of LBC will be compared with the results of the histopathology examination of the biopsies of the remainder of the laryngeal lesions.

The development of a clinical trial for the Oral Cytology Global Network

Researchers: Prof J Hille, Dr A Afrogheh; Prof R Mehrotra (Moti Lal Nehru Medical College, India); Prof MW Lingen (University of Chicago, USA); Prof J Epstein (City of Hope NCI –designed Comprehensive Cancer Centre, USA); Prof F Koch (University Medical Centre Mainz, Germany); Dr A Chaturvedi (National Cancer Institute, NIH, USA)

Funding: NHLS Research Trust, NIH

This international trial focuses on the usefulness of cytological diagnosis and use of biomarkers in the early diagnosis of oral potentially malignant and malignant lesions. The standardising of oral cytology and its interpretation as a reliable diagnostic tool – akin to the time-tested Papanicolaou smears extensively used in the detection of cervical malignancies – is envisaged. The specific aims are to: a) establish and test the cytomorphic criteria that differentiate truly ‘risky’ from reactive/’non-risky’ cell samples derived from suspicious oral lesions using a standardised liquid-based collection and smear preparation method that satisfies the criteria of widely accessible, economical, low turnaround times and consistent high quality; and b) establish a panel of biomarkers to identify the potentially malignant/dysplastic lesions which have a high propensity to develop into malignancy. Thus far, the researchers have concentrated on the development of tools for data management and oversight of the research; the development of a trial design and other essential elements of the study, such as the protocol, recruitment strategies, and procedure manuals; and to collect feasibility data.
Epidemiology of oral cancer in South Africa

Researchers: Prof JJ Hille, Dr MK Ndui

Oral cancer is characterised by marked geographic differences in frequency and site preference. Following previous studies, it was investigated whether the incidence of oral squamous cancer had changed and whether there is a trend that younger individuals are being affected by the disease. The NHLS’ National Cancer Registry records of cases of oral cancer were analysed over a seven-year period from 1996 to 2002 (n=9,702). The male:female ratio was 1:3. Age specific incidence rates (ASIRs) were the lowest (0.64/100 000 per year) among African females and the highest was 13.4/100 000 per year among the coloured male population. Cumulative rates were 1.83 and 1.319 for males and females, respectively. Unexpectedly, the ASIR of oral cancer remained largely unchanged in South Africa. The HIV pandemic appears to have had little or no effect on the incidence rates of oral cancer.

Diagnostic accuracy of autofluorescence microscopy of Pap smears for oral Candida hyphae

Researchers: F Titinchi, J Du Toit, Prof JJ Hille, G Neethling
Funding: NHLS Research Trust

Autofluorescence microscopy allows for the rapid detection of pathogens like mycobacteria and fungi in Pap-stained smears at a relatively lower power magnification because of better pathogen discrimination against a dark background. This study investigated the sensitivity and specificity of this technique to detect the presence of Candida spp in oral smears from 80 randomly selected patients. Two parallel exfoliative smears were prepared from each brushing sample and respectively stained with the PAS and Pap techniques. The randomised Pap slides were then viewed under autofluorescence microscopy by two independent observers. Results of the autofluorescence viewing were then correlated against the control group (PAS). The sensitivity was found to be 30-35%, specificity 83-95%, and diagnostic accuracy 64-68%. The orientation of the Candida hyphae and autofluorescing squamous cells affected screening results. Hyphae otherwise undetected under light microscopy may be identified under autofluorescence microscopy, and vice versa. However, autofluorescence microscopy alone was inadequate in the detection of oral Candida.

TEACHING AND TRAINING

Undergraduate
The department offers courses to BChD (dental) students in the second, third and fourth years and a basic pathology course to oral hygiene students.

Postgraduate
Training is provided to MChD, MSc Dent, MChD Pros and MSc Forensic Dentistry, as well as to Postgraduate Diploma in Forensic Dentistry (PDD) students.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled:
5 (2 MChD, 2 MSc, 1 PDD)
Postgraduate students graduated:
4 (1 MChD, 2 MSc, 1 PDD)

RESEARCH OUTPUT

Publications


Conference presentations
International: 1
National: 1
Local: 1
DEPARTMENT OF ANATOMICAL PATHOLOGY

Head: Prof MJ Hale

DIAGNOSTIC SERVICES

The Department of Anatomical Pathology provides all the pathology requirements inclusive of histology, cytology and autopsy pathology for patients admitted to the academic hospitals in the Johannesburg region, namely Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath, Helen Joseph and Coronation hospitals. In addition, the department offers similar care to all provincial hospitals in Gauteng and North West Province.

During the reporting period, the department based at CMJAH saw a total of 44,571 cases. A total of 143 neuropathology cases were processed. Diagnostic opinions were provided for 364 renal biopsies and immunofluoresence was done on 78 skin biopsies. Oral pathology diagnosed 1,805 cases. A total of 220 specimens required electron microscopy and 10,764 immunohistochemistry investigations were performed each month. A total of 984 PCR tests were performed including 152 B-cell rearrangement studies and 130 T-cell rearrangement studies; 582 mycobacterial DNA studies; 39 Bartonella studies; 59 human herpesvirus 8 (HHV 8) studies; one HPV study; and 21 RT-PVR studies (synovial sarcomas). Epstein Barr virus studies and HPV testing have been replaced with in situ hybridisation.

Sixty-three post mortems were performed from the Charlotte Maxeke and Helen Joseph hospitals. A total of 1,874 bone marrow trephines were processed.

A total of 116 consultations and reviews received from Namibia, Kenya, Polokwane, Cape Town, East London, Port Elizabeth and Bloemfontein were seen by consultants in the department; these also included consultations from private practice laboratories in Johannesburg, Pretoria, Durban, Port Elizabeth and Cape Town.

The histopathology unit sited at Chris Hani Baragwanath Hospital was responsible for diagnostic histopathological opinion on a further 13,254 patients and 39 post mortems. Increasing use of immunohistochemistry, introduced in the previous year, was evident with 3,000 of these investigations being performed.

The cytology unit reported 111,932 Pap smear cases, 14,880 non-gyneacologic exfoliative cases, 12,767 FNA cases including palpable masses, radiologically guided FNAs and intraoperative FNAs.

The department is accredited with SANAS and SMILE (Patient Safety Monitoring in International Laboratories), which is part of the AIDS Malignancy Consortium and AIDS Clinical Trial Group.

RESEARCH

Sub-Saharan Africa Lymphoma Consortium

Principle investigators: Dr Y Perner; Prof W Stevens (Molecular Medicine and Haematology, Wits)
Collaborator: Prof LW Ayers (Department of Pathology, Ohio State University, USA)
Funding: US National Cancer Institute

The subtypes and distribution of non-Hodgkin’s lymphoma in southern Africa, especially in patients with AIDS, is largely unknown. The Mid-Region AIDS and Cancer Specimen Resource, (ACSR), has launched the Sub-Saharan Africa Lymphoma Consortium to address this deficiency. Approximately 200 cases of lymphoproliferative disorders were retrieved from the anatomical pathology archives. These were reviewed with a panel of expert haematopathologists at Ohio State University. In addition, a core biopsy from each paraffin-embedded tissue block was subjected to tissue microarray analysis (TMA). On completion of the review and TMA analysis, it is anticipated that areas of diagnostic discrepancy will be highlighted and clarified.

The histopathological analysis of cellular elements, accessory molecules and cytokines in mycobacterial granulomas from HIV-positive and negative individuals

Investigators: Dr R Wadee, Prof MJ Hale; Prof AA Wadee (Department of Immunology, Wits)
Funding: NHLS Research Trust
The immune response to infection with *Mycobacterium tuberculosis* involves interactions between macrophages, cytokines, accessory molecules and T-helper cells. Mycobacteria evade the host’s immune response while granulomata are important in the host’s defences. These responses may be associated with immune-pathology especially in patients with HIV. This study investigates the cell-mediated immune mechanisms in granulomata and assesses the presence of macrophages and other cellular elements including CD4+ and CD8+ lymphocytes and accessory molecules in granulomata. The accessory molecules include HLA class I and II and intercellular cell adhesion molecule-1. The study aims to identify the presence of several pro-inflammatory and anti-inflammatory cytokines in granulomas, and compare the various molecules, cellular elements and cytokines in granulomas from HIV-positive and HIV-negative individuals. Control specimens are in the form of foreign body granulomas.

**Molecular profiling of colorectal cancer**

**Principle investigator:** M McCabe  
**Supervisor:** Dr Y Perner  
**Collaborator:** Dr L Cronje

This project aims to define the molecular pathogenesis of colorectal cancer (CRC) according to currently known parameters in a random cohort of South African individuals who have had biopsy samples or colorectal resections reported by the CMJAH. These data will provide insight into the CRC molecular subtypes prevalent in South Africa, a heretofore largely unassessed aspect of the disease, with a view to developing a cost-effective method for assessing the molecular profile of these tumours for prediction of response to individualised treatment and prognosis in patients with this disease.

**HPV in Africa**

**Investigator:** Dr P Michelow  
**Collaborators:** This is a combined study between the London School of Medicine and Tropical Hygiene, University of Montpellier France, University of Ouagadougou, Burkina Faso and the Wits Health Consortium in Johannesburg.  
**Funding:** European Union (EU)

The NHLS will be performing the laboratory part of the study for the Johannesburg site, reporting on the Pap smears, performing quality assurance on the smears from Ouagadougou and writing up the cytological aspects. Enrolling of participants began in January 2012. The aim of the study is to determine the best way of screening HIV-positive women to detect cervical cancer and its precursor lesions. Six hundred eligible women (1,200 in total) in both Johannesburg and Ouagadougou will undergo cervical cytology, HPV testing, visual inspection of the cervix and a screen for sexually transmitted diseases followed by biopsy. These procedures will be repeated in 18 months. The results of the study will help determine what modality is best to screen HIV-positive women in low-resource communities, the progression of HPV-related cervical disease in HIV-positive women and the most cost-effective interval between screenings.

**ACTG A5282 trial**

**Investigator:** Dr P Michelow  
**Funding:** Department of Health and Human Services, NIH (through the National Institute of Allergy and Infectious Disease [NIAID]), USA

The AIDS clinical trials group (ACTG) is the largest HIV clinical trial organisation in the world, playing a major role in setting standards of care for HIV-positive patients. Cervical cytology and HPV testing will be compared in HIV-positive women in low-resource communities. The trial has 11 study sites (3 in South Africa, 1 in Zimbabwe, 1 in Botswana, 1 in Zambia, 1 in Malawi, 1 in Haiti, 1 in Peru, 2 in India). A random selection of five Pap smears from all 11 sites will be sent to the Johannesburg cytology laboratory for review at six-monthly intervals for the duration of the study.

**Screening for anal dysplasia by using anal cytology and HPV testing in HIV-infected women**

**Collaborators:** Dr P Michelow; Dr E Jong, Dr C Firnhaber (Clinical HIV Research Unit, Helen Joseph Hospital, Johannesburg)

An increased risk of anal carcinoma has been found among HIV-infected individuals. ART has been shown to have little effect on anal dysplasia. As more people have access to ART, it is hypothesised that the incidence of anal cancer in sub-Saharan Africa will increase. Anal smears for cytological evaluation and HPV testing will be performed to provide epidemiological data in this regard and compare the findings to cervical cytology.

**MYC rearrangements in extra-oral plasmablastic lymphoma**

**Researcher:** Dr Y Perner  
**Supervisors:** Prof S Meer, Dr P Willem  
**Funding:** NHLS Research Trust
Thirty plasmablastic lymphoma cases from the anatomical pathology archives are being reviewed histologically; these are representative of extra-oral tumours from individuals of both known and unknown retroviral status. A tissue microarray (TMA) has been created using a manual tissue arraying instrument. An immunophenotypic profile has been established against which all lymphoma diagnoses are being assessed. MYC rearrangements are assessed on TMA by interphase fluorescence in situ hybridisation. Translocations commonly associated with diffuse large cell lymphoma are explored by FISH, specifically t (8; 14), t (11; 14), BCL2 and BCL6 rearrangements. The relationship to Epstein Barr virus and clonality studies are examined by chromogenic in situ hybridisation. Results are pending.

**Her2 over-expression in gastric carcinoma**
*Researcher:* Dr T Pitjadi  
*Supervisor:* Dr P Swart  
*Funding:* NHLS Research Trust

The prevalence of Her2 over-expression in gastric carcinoma in the South African population and the concordance between FISH and BDISH is being investigated.

**Classification of hydatidiform moles**
*Researcher:* Dr M Kaake  
*Supervisor:* Prof M Hale  
*Funding:* NHLS Research Trust

The accurate classification of hydatidiform moles using p57 immunohistochemistry and molecular genotyping is being determined in 50 recent cases at Chris Hani Baragwanath Hospital.

**HIV and HPV**
*Researcher:* Dr D Fassom  
*Supervisor:* Prof M Hale  
*Funding:* NHLS Research Trust

The question posed in this retrospective study is whether HIV infection affects the integration of the HPV; p16 is used as a marker.

**TEACHING AND TRAINING**

**Undergraduate**
Consultants and registrars within the department contribute with lecturing and offering practical theme sessions to graduate entry medical programme (GEMP) 1 and 2 and all registrars participate as facilitators in the GEMP small-group problem-based learning. Students undergoing their surgical rotations in GEMP 3 and 4 receive weekly clinicopathological teaching at both CMJAH and Chris Hani Baragwanath Hospital.

Dental and pharmacy students as well as students of physiotherapy and occupational therapy attend half-courses in anatomical pathology during their third year of study.

**Postgraduate**
Anatomical pathology lectures are given over four blocks during the academic year to MSc students in physiotherapy and occupational therapy, and departmental consultants teach pathology within their areas of specialisation for the Diploma in Tropical Medicine and Hygiene.

All registrars are registered for the MMed (Anatomical Pathology). In addition to their surgical pathology training, registrars follow a formal academic programme.

**Technologists and technicians**
Medical technologists and technicians are given theoretical and on-the-bench training. Laboratory supervisors give a one hour lecture per week, and also give the students assignments/tasks to complete. From Tuesdays to Thursdays of every week, the students are given one and a half hour each day for studying, completion of assignments or other matters related to their training.

**PROFESSIONAL DEVELOPMENT**

**Postgraduate candidates qualified:** 3 FCPath (Anat)  
**Postgraduate candidates enrolled:** 25 MMed

**RESEARCH OUTPUT**

**Publications**
Geel AG, Bennett KG, Rigby JM, Poole JE. Gorlin Syndrome.  
*SA J Child Health* 2011; 5(1): 21-23
Grayson W. Recognition of dual or multiple pathology in skin biopsies from patients with HIV/AIDS. *Pathology Research International* 2011: 1-7


Pantanowitz L, Michelow P. Review of Human Immunodeficiency Virus (HIV) and squamous lesions of the uterine cervix. *Diagnostic Cytopathology* 2011; 29(1): 65-72


**Conference presentations**

*International*: 1

*National*: 15

“ All histology, cytology and autopsy pathology requirements are provided to academic hospitals in the Johannesburg region”
DEPARTMENT OF CHEMICAL PATHOLOGY

Acting head: Dr N Lutchman

DIAGNOSTIC SERVICES

As a very extensive repertoire of tests is performed in the academic complex, consisting of laboratories at both CMJAH and Chris Hani Baragwanath (CHB) Hospital, this unit serves as a reference laboratory to other NHLS laboratories. The automated laboratories at both sites are high volume 24-hour seven days a week facilities. The laboratories continue to experience a high turnover of technologists; with the average time to train a technologist to be competent in all areas of the auto laboratories being 12-18 months, this gives rise to many challenges. Both laboratories maintained SANAS accreditation.

The CMJAH biochemistry routine laboratory runs on average 187,000 tests per month while the laboratory at CHB performs on average 170,000 tests per month. The CMJAH laboratory successfully established a point-of-care testing (POCT) service in the diabetic clinic in order to improve patient care.

The chromatography unit has initiated a programme to assist the intensive care staff at both hospitals in the detection of poisons in critically ill patients. This has proven to be of enormous benefit to the management of these patients. In addition, the menu of tests being performed in this unit has increased significantly in keeping with international trends.

Senior staff within the department, in addition to their routine service work, serve on several NHLS and other committees such as the Expert Committee, Point-of-Care working group, Standardisation Committee and HPCSA. The pathologists are further involved in serving on university committees and teaching at the undergraduate and postgraduate levels.

Donald Gordon Medical Centre and Contract Laboratory Service

Registrars and consultants provide a consultative service to the Contract Laboratory Service and the Donald Gordon Medical Centre as well as routinely reviewing results and troubleshooting problems as they arise. This activity provides registrars with experience of working in a multidisciplinary private laboratory.

RESEARCH PROJECTS

The focus areas of research are obesity, diabetes, cardiovascular disease, metabolic consequences of HIV and its therapy and toxicology. The following projects were newly initiated or are ongoing.

Pathophysiology of obesity and type 2 diabetes

Genetics of obesity

Researchers: S Mnyosi, Dr C van Niekerk, Prof N Crowther

Mutations in the melanocortin 4 receptor (MC4R) have been shown to cause morbid, early-onset obesity in humans. The MC4R receptor resides in the hypothalamus and is bound by α-melanocyte stimulating hormone, a potent anorexigenic neurotransmitter. The study is investigating the occurrence of mutations in the MC4R gene in obese and lean white and black South Africans. Thus far, the study has shown that polymorphisms in the MC4R gene do not contribute to the high prevalence of obesity in the black population.

Ethnic differences in serum 25 hydroxy vitamin D levels and its relationship to insulin sensitivity and markers of the metabolic syndrome

Researchers: Dr J George, Prof S Norris, Prof N Crowther

Funding: MRC

Low levels of vitamin D have been associated with increased insulin resistance in a number of different studies. It is also known that the level of insulin sensitivity differs between ethnic groups. The study will therefore measure serum vitamin D levels in a large cohort of Indian, white and black South Africans to determine if vitamin D levels differ between these groups and whether this relates to ethnic differences in insulin sensitivity and the prevalence of cardiovascular disease risk factors. The study will also be used to develop reference ranges for vitamin D for the South African population.

Type 1 diabetes and maturity onset diabetes of the young (MODY)

Pathophysiology of type 1 diabetes in Africans

Researchers: Dr C Padoa, Prof N Crowther, Prof P Rheeder (Division of Clinical Epidemiology, University of Pretoria)

Funding: NHLS Research Trust, MRC.
Very little is known about the aetiology or the genetics of type 1 diabetes in black Africans. The main genetic input into the disease comes from the HLA region of the genome. This study is investigating HLA haplotypes and other gene polymorphisms and their association with diabetes in African type 1 diabetic patients as well as the prevalence of autoimmune antibodies to insulin and other islet beta-cell autoantigens. Present data show that in Africans the age of diagnosis of diabetes is older than in white patients with 20-30% of Africans being diagnosed with type 1 diabetes between 20-25 years of age. Differences in the prevalence of certain autoantibodies have also been observed between African and European diabetic subjects.

**HIV/AIDS**

**HIV-associated nephropathy**

*Researchers:* Dr J George; Prof S Naiker, Dr R Duarte (Department of Nephrology)

*Funding:* NHLS Research Trust

HIV-associated nephropathy is a leading cause of end stage renal disease among HIV-positive subjects. The aetiology of the disease will be studied in HIV-positive subjects using kidney biopsy samples. The infiltration of immune system cells into the renal tissue will be analysed as will the chemokine and CD4 receptor and chemokine co-receptor expression of renal tubular cells.

**The genetics of body fat re-distribution and lipid abnormalities in ART-associated lipodystrophy**

*Researchers:* T Tlomatsana, Dr N Naran, Prof N Crowther

Not all subjects who receive ART develop lipodystrophy and therefore it is possible that some subjects are more susceptible to this side effect of ART than others. Previous studies have investigated the possibility of gene polymorphisms that may account for this. Therefore, a number of candidate gene polymorphisms were studied in patients with and without ART-associated lipodystrophy to determine whether gene variation can increase the risk of lipodystrophy in subjects receiving HAART. The data show that a polymorphism in one of these candidate genes, the TNF alpha gene, does associate with lipoatrophy in this patient group.

**The role of cystatin C in the assessment of renal function for patients initiating HAART**

*Researchers:* Dr T Seape, Dr J George

The recent revision of ARV guidelines for the management of HIV-infected patients in South Africa has included the use of tenofovir, a nucleotide reverse transcriptase inhibitor. Tenofovir may be nephrotoxic and has been associated with the onset of acute renal failure following initiation of therapy. The guidelines suggest assessment of renal function before initiation of tenofovir. Cystatin C is a more sensitive marker of small changes in glomerular filtration rate (GFR) and overall is a better estimator of GFR than serum creatinine. The aim of this study is to assess the use of cystatin C as a better alternative to GFR for the determination of kidney disease in HIV patients initiating HAART.

**Neutrophil gelatinase-associated lipocalcin as a diagnostic marker of acute kidney injury in pre-eclampsia**

*Researchers:* Dr N Moyake, Prof E Buchman (Department of Obstetrics and Gynaecology, University of Witwatersrand); Dr J George

Pregnancy-induced hypertension contributes significantly to the number of still births and to neonatal morbidity and mortality. Patients who meet the criteria for diagnosis of pre-eclampsia can have fatal complications, notably abruptio placenta, disseminated intravascular coagulation, cerebral haemorrhage, hepatic failure and acute kidney injury (AKI) and if AKI is diagnosed early this will lead to more intensive monitoring and thus reducing morbidity and mortality. The current strategies used to diagnose AKI use creatinine measurement; however, serum creatinine is not an ideal marker for the early diagnosis of AKI as it rises slowly and there are several factors that change the creatinine concentration. Urinary neutrophil gelatinase-associated lipocalin (NGAL) could potentially be an early marker of AKI as it is excreted from the kidneys to the urine within minutes following kidney insult Therefore the aim of this study is to assess whether NGAL can be used as an early marker of acute kidney injury in women with pre-eclampsia.

**Toxicology and renal function**

**Toxic effect of plant extracts**

*Researcher:* T Snyman

*Funding:* NHLS Research Trust
Some plants used as herbal remedies in South African traditional medicines (muti) contain toxic agents known to cause liver pathology. The effect of these agents on apoptosis rate in two human hepatocyte cell lines – HepG2 and HUH7 – have been studied and methods developed to block their liver toxicity. Data show that these agents cause both apoptosis and/or necrosis.

TEACHING AND TRAINING

Technologists
The large variety of testing methods employed in the chemistry laboratories at both sites enables them to serve as ideal environments to provide teaching and training at all levels. In addition, routine tutorials, lectures and seminars form part of the weekly routine which further enhances the training capabilities of these laboratories.

Undergraduate
All medical staff, including the consultants and registrars, as well as the majority of the scientists participate in the GEMP 1 and 2 teaching programme by acting as block and case coordinators, presenting lectures, facilitating weekly cases, giving tutorials and setting, invigilating and marking exam papers. Teaching has also been extended to the final year medical students (GEMP 4) in the form of weekly tutorials.

Postgraduate
The department has a number of postgraduate students at MMed, MSc and PhD level. These students are taught research methodology within their chosen research area by experienced scientists and pathologists. Each student has to write a research protocol according to the guidelines of the University of the Witwatersrand and under the supervision of an appropriately qualified member of the department. Research funding is obtained from local funding agencies by the students, with guidance from their supervisors.

The department has eight registrar posts. Registrars rotate through all units of the laboratory, where they are exposed to a variety of clinical and technical disciplines. A consultant is in charge of each unit and oversees the training in that unit by means of tutorials and case discussions. Registrars are also obliged to attend related clinics where they manage patients. This further enhances their training, and helps them to develop insight into the clinically appropriate and cost-efficient work-up of patients as well as providing them with insight into the problems that clinicians experience regarding their interaction with the laboratory. Time has also been allocated to registrars during their rotation to do research and to complete their research report which is a prerequisite for the MMed degree.

PROFESSIONAL DEVELOPMENT

Postgraduate students graduated: 1 PhD
Postgraduate candidates enrolled:
17 (8 PhD, 3 MSc, 6 MMed)

RESEARCH OUTPUT

Publications


Ferris WF, Crowther NJ. Once fat was fat and that was that: our changing perspectives of adipose tissue. Cardiovasc J Afr 2011; 22: 147-154


Menezes CN, Maskew M, Sanne I, Crowther NJ, Raal F. A longitudinal study of stavudine-associated toxicities in a large cohort of African HIV infected subjects. BMC Infect Dis 2011; 11: 244


Waisberg R, Paiker JE, Crowther NJ. Adipokine serum concentrations, anthropometric measurements and socio-economic status in two ethnic groups with different prevalence levels for cardiovascular diseases and type 2 diabetes. *Horm Metab Res* 2011; **43**: 660-666

**Conference presentations**

**National:** 9

“A point-of-care service was successfully established in the diabetic clinic to improve patient care”
DEPARTMENT OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Head: Prof AG Duse

The Department of Clinical Microbiology and Infectious Diseases (CMID) supports the NHLS/Wits Academic Complex which includes four academic hospitals as well as the referral mycobacteriology and immunology/serology laboratories that are located on the Braamfontein NHLS campus. All sites, with the exception of the Helen Joseph laboratory, are fully accredited by SANAS.

CMID is organised to provide microbiology diagnostic and clinical consultative services as well as specialised infection prevention and control services. Infection prevention and control (IPC) activities are predominantly carried out by the Division of Hospital Epidemiology and Infection Prevention and Control. IPC is considered a key priority on the national Department of Health’s healthcare delivery agenda.

DIAGNOSTIC SERVICES

Infection control laboratory
The infection control services conducts tests for clinical microbiology, antibiotics, environmental microbiology (Legionella, air samples etc.), public health (water, milk and food), and molecular diagnostics. During the reporting period, test volumes amounted to 64,893 compared to 54,375 the previous year.

A comprehensive IPC support service and an outbreak response service for nosocomial outbreaks is offered to the CMJAH and all surrounding hospitals. This is supplemented by laboratory testing e.g. strain typing. Weekly microbiological and infection control rounds are done at the Wits Donald Gordon Medical Centre.

Diagnostic specialised microbiological assays are offered in the form of molecular diagnostics specialised identification of unusual isolates and enhanced antimicrobial susceptibility testing.

Legionella laboratory
The Legionella action group is made up of various interested parties from laboratory, industry and water treatment companies and meets quarterly. The group is currently adapting the British standard document (The Control of Legionella in Water Systems) for South African conditions.

Molecular diagnostics laboratory
During the past year, 600 Bordetella pertussis PCR, 339 vancomycin-resistant Enterococci PCR, 782 cytomegalovirus viral loads, 797 Clostridium difficile PCR, 121 respiratory virus PCR tests were conducted. The respiratory virus PCR is a newly introduced test which replaces the previous H1N1 PCR.

Oral microbiology laboratory
The laboratory provides microbiological diagnostic services to Wits Oral and Dental Teaching Hospital as well as to private dentists. Caries susceptibility tests are performed routinely and occasionally microbiology of periodontal diseases, infected root canals and pus from odontogenic abscesses. This year, 50 caries activity tests were performed.

Microbiology laboratory, CMJAH
Clinical specimens for bacterial and fungal culture, identification and antimicrobial susceptibility are processed. Selected serological testing, and bacterial, fungal and viral antigen detection assays are also offered. Approximately 466,990 specimens were processed for the year.

Microbiology laboratory, Chris Hani Baragwanath Hospital
This laboratory processes very large quantities of samples as it serves primary laboratories such as Natalsprite and Sebokeng. The laboratory started processing respiratory samples in April 2011, using line probe assays.

Microbiology laboratory, Helen Joseph Hospital
The laboratory serves several public healthcare centres namely: five district hospitals, two of which require routine microbiology diagnostic services, and the other three are referral centres for special identification. The laboratory also serves a step down facility and six primary healthcare clinics in and around the area and at the Leeuwkop prison.

Mycology referral centre
The laboratory processes clinical specimens for direct microscopy and culture of mycobacteria and drug susceptibility testing (DST) (first line drugs and second line TB drugs) of Mycobacterium tuberculosis. Eighty percent of the work received is referred through a network of other laboratories. The laboratory receives 800-900 specimens per day from hospitals and clinics in the City of Johannesburg, Sedibeng, West Rand and Ekurhuleni regions, as well as culture isolates from other TB culture laboratories in other provinces and neighbouring countries for first/second line DST.
Infection control
Three comparative evaluations of generic antimicrobial agents using reference methodology have been completed.

Enhanced surveillance of healthcare-associated infections at both the CMJAH and the Wits Donald Gordon Medical Centre has been initiated and data collection started mid-year 2011. Enhanced antimicrobial susceptibility surveillance performed on isolates from the CMJAH has thus far included vancomycin-resistant enterococci and pan-resistant Acinetobacter baumannii and Pseudomonas aeruginosa.

Molecular laboratory
The following collaborative projects are being undertaken:

- Active case finding for TB in contacts of children with TB with Dr N Martinson from the Perinatal HIV Research Unit; and
- Evaluation of the in vitro activity of an ‘active’ gel dressing on biofilms with Dr C Patel from the Department of Surgery, Division of Plastic and Reconstructive Surgery.

RESEARCH PROJECTS

Micro-organisms cultured from laryngoscope blades in a secondary level hospital theatre
Researcher: Dr W Lowman

Clinical outcomes in newly diagnosed patients with M/XDR-TB, a two-year prospective cohort study at Sizwe Hospital: a high-HIV prevalence setting
Researcher: Dr F Conradie
Supervisor: Prof AG Duse

The evaluation of rapid screening of M/XDR-TB patients within a dedicated M/XDR-TB hospital
Researcher: L Isherwood
Supervisor: Prof AG Duse

Lujo virus: a novel old world arenavirus - clinical and pathophysical aspects
Researcher: N Sewillal
Supervisor: Prof AG Duse

Molecular typing of drug-susceptible, mono-resistance and XDR Mycobacterium tuberculosis strains in the Johannesburg area
Researcher: PS Kamudumuli
Supervisor: Dr L Blann

Buffering capacity of saliva, salivary flow rates and cortisol levels in a patient with active caries
Researcher: Dr P Hira (Msc Dent)
Supervisor: Prof MM Coogan

A comparison of pathogenic characteristics of Candida albicans isolated from the saliva of healthy subjects, patients with denture-related stomatitis and cancer patients wearing oral prostheses
Researcher: V Mothibe (Msc)
Supervisors: Prof MM Coogan, Dr M Patel

Oral Candida in HIV-positive woman: influence of oral hygiene, clinical and social factors on the carriage rates and the influence of virulence of the organism on the development of clinical infection
Researcher: Prof F Owotade (PhD)
Supervisors: Dr M Patel, Dr L Blann

The effect of Dodonaea viscosa var. Angustifolia on oral pathogens
Researcher: R Naidoo (Msc)
Supervisors: Dr M Patel, Z Gulube

The effect of Dodonaea viscosa var. Angustifolia (L.F) on the ultrastructure of Candida albicans cell wall and biofilm formation
Researcher: S Naicker (Msc)
Supervisor: Dr M Patel

The prevalence of β-lactamase-producing anaerobic oral bacteria and the genes responsible for this enzyme production
Researcher: B Binta (Msc)
Supervisors: Dr M Patel, C Thorold
A prospective observational study of the programmatic implications of molecular testing for diagnosis of pulmonary forms of TB and drug-resistant TB in a cohort of patients at the Luthando neuropsychiatric HIV clinic

**Researchers:** G Jonsson, F Jeenah, B Gripsoolver, A Taege, J Wadula, J Furun, MYH Moosa, Y Jeenah

**EXpert MTB/RIF for the diagnosis of pulmonary and extrapulmonary TB in children – effectiveness study**

**Researchers:** S Sawry, A van Rie, L Scott, H Moultrie, C Verwey, G Reubenson, J Wadula, D Blaaw

**Audit of percutaneous drainage of abdominal abscesses or pyomyositis**

**Researchers:** A Karstaedt, S Ndlovu, A Sparaco, J Wadula

**Community-acquired pneumonia**

**Researchers:** Dr E Shaddock, Prof C Feldman (Department of Pulmonology, Wits); Dr T Nana, Dr N Bosman, Dr C Sriruttan, Dr N Beylis

The study aims to determine the clinical and bacteriological profile of patients with community-acquired pneumonia at CMJAH; evaluate the occurrence of secondary bacterial infection in patients with newly diagnosed active pulmonary TB; determine the accuracy of BinaxNOW® Streptococcus pneumoniae urine antigen testing in HIV-positive patients; and evaluate and determine TB LAM Ag urine test efficacy for the diagnosis of TB in a tertiary hospital setting, compared to sputum direct microscopy and culture.

**Comparison of two Clostridium difficile toxin immunoassays and a real-time PCR assay for C. difficile tcdC to toxigenic culture for detection of toxin-producing C. difficile in clinical samples**

**Researcher:** Dr T Nana

**Funding:** NHLS K Funding, Wits Faculty of Health Sciences MMed Grant

The study is comparing the performance of a real-time PCR assay and two enzyme inhibition assays (EIAs) to toxigenic culture for direct detection of toxin-producing C. difficile in clinical samples; and comparing the relative sensitivity, specificity, and positive and negative predictive value of the various methods. It further intends to establish the optimal stand alone test or testing algorithm using a combination of different tests in a step-wise manner provides superior results in terms of accuracy and cost-effectiveness, without increasing turnaround time excessively; and establish burden of C. difficile disease at CMJAH. CMJAH patients’ admission numbers are being used during the study period to estimate burden of disease.

**Evaluation of the GenoType MTBDRplus assay on smear-positive FNA specimens**

**Investigators:** M Molaudzi, L Jenkin, N Beylis

The usefulness of the assay on 100 smear-positive FNA specimens was assessed to determine the presence of M. tuberculosis as well as the presence of resistance to isoniazid and rifampicin. The assay was performed on the direct specimen and the result was compared to the results of the assay done routinely on the culture of the FNA specimen.

**BCG disease in children in Johannesburg**

**Researchers:** R Magobo, N Beylis, L Jenkin

A retrospective analysis of positive TB cultures from children was conducted to speciate M. bovis BCG. It was found that a proportion of 6% was positive for BCG disease.

**Drug-resistant TB profiles in Johannesburg**

**Researchers:** L Jenkin, N Beylis

The study describes the common mutations associated with rifampicin- and isoniazid-resistant TB from positive cultures of routine clinical specimens processed from August 2009 until January 2010; the discrepant results between the genotypic results and the phenotypic results; and the associated resistance profiles by second line drugs in identified MDR-TB isolates.

**HONOURS**

R Magobo, N Beylis and L Jenkin won the award for the best oral presentation at the laboratory technologist conference in July 2011.

**TEACHING AND TRAINING**

**Undergraduate**

All the registrars and consultants, and scientists are involved with undergraduate teaching and training as well as facilitating, which includes modular or full-time courses to the following categories of university students:
second year molecular medicine students, second year fundamentals of health and disease students, medical students in their first, second and third years of the their graduate entry medical programme study, and third year dental, pharmacy and nursing students.

The Oral Microbiology Department presents lectures and hand on practicals to dental students from first to third year and to oral hygiene students in their first year.

Ongoing training of intensive care staff at CMJAH and the Wits Donald Gordon Medical Centre was conducted and it encompassed surveillance of hospital-associated infections.

Postgraduate
The department offers programmes for IPC practitioners, intern medical scientists, Diploma in Tropical Medicine and Hygiene students and Masters and PhD candidates. An MSc (Med) with particular emphasis on infection prevention and control on a course-work and research report basis has been initiated.

The microbiology registrars and laboratory managers of Chris Hani Barawgwanath give training and lectures at external hospitals and laboratories on topics such as methicillin-resistant Staphylococcus aureus (MRSA), quality assurance, antibiotic stewardship, and approach to processing CSF and blood culture specimens. Most of the other laboratories conduct their own in-house training on a regular basis and all staff members are motivated to attend workshops offered i.e. TB microscopy, risk assessment, health and safety.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 6 (2 PhD, 4 MSc)
Postgraduate candidates enrolled: 7 (4 PhD, 3 MSc)

RESEARCH OUTPUT


Conference presentations
International: 5
National: 7
Local: 12

“Infection prevention and control is a key priority in healthcare delivery”

DEPARTMENT OF IMMUNOLOGY

Head: Prof AA Wadee

In 2011 Prof Wadee was appointed Dean of the Faculty of Health Sciences at Wits but retains the title of Professor of Immunology. The Immunology Department continues to form part of laboratory-based research and diagnostics.

DIAGNOSTIC SERVICES

As a standard service, the laboratory offers a wide range of immunological tests useful in the diagnosis of clinical disorders, including autoimmune antibody profiles and assessments for the diagnosis and management of disorders such as rheumatoid arthritis, systemic lupus erythematosus, Sjögren’s syndrome and scleroderma. Other services include assessments of immunoglobulin, immunoglobulin subclasses and both total and individual component complement levels for the diagnosis of primary immunodeficiencies and a wide range of tests for allergic states. Where specific tests are not available, samples are referred to other centres and particularly to the Immunology Division at Tshwane Academic Hospital.

RESEARCH

The research emphasis is on the immunology of TB. Research into areas of immune function/dysfunction with particular reference to the host’s ability to resist infection is also undertaken.

10 years of supporting academic excellence
TEACHING AND TRAINING

Undergraduate
Lectures, tutorials and practicals are presented to nursing, dental, pharmacy and medical students in the graduate entry medical programme. Staff also engages students as facilitators in problem-based learning sessions.

Postgraduate
Weekly journal clubs and monthly research presentations are held. Members of the laboratory staff are responsible for lecturing to postgraduate students and groups, including registrars in various clinical departments. Facilities and supervision are provided to MSc, MMEd, DMed and PhD students. Students receive immunological training throughout the year and tutorials are offered on a regular basis. Students’ work, dissertations and theses are closely supervised. Diagnostic and clinical immunology was taught at postgraduate level to Diploma of Tropical Medicine and Hygiene students and other groups including registrars and general practitioners.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled: 3 (2PhD, 1 MSc)

RESEARCH OUTPUT

Publication

“Prof AA Wadee was appointed Dean of the Health Sciences Faculty, Wits”

DIVISION OF HUMAN GENETICS

Head: Prof A Christianson

DIAGNOSTIC SERVICES

Up to 80% of the country’s diagnostic molecular genetic testing and a significant proportion of cytogenetic testing in the public service are undertaken in the division’s laboratories. The diagnostic services are divided into laboratory services and clinical genetic services. Within the laboratory there are three sections: molecular genetics, cytogenetics, and applied polymorphisms. During the past year, the molecular genetics laboratory processed 6,851 tests, cytogenetics 2,478 and applied polymorphisms 6,257. The clinical genetics services facility conducted 1,972 appraisals.

In addition, the MRC/NHLS/Wits Human Genome Diversity & Disease Research Unit is hosted within the division, and provides genetic ancestry testing to the public.

Molecular genetics
The laboratory conducts tests for more than 40 genetic conditions. The laboratory once again obtained full accreditation for the biannual external quality assurance programme through the American College of Pathologists. The laboratory is chronically and critically under-staffed with now only three staff members. This has resulted in significant delays in turnaround times. The single nucleotide polymorphism (SNP) genotyping facility is jointly funded by the NRF (Research Infrastructure and Support Grant), Wits University and the NHLS. The facility is available for research and collaboration with scientists locally, regionally and nationally. The facility has two major sections, namely:

- A semi-automated robotic system for DNA quantification and normalisation: the Tecan EVO150 robotic system is a modular platform that is used for semi-automated DNA pipetting related to quantification and normalisation. The system includes the Tecan Infinite 200 reader for both fluorescent and absorbance DNA quantification. The reader includes the Infinite 200 NanoQuant plate, which allows for the direct quantification of nanoquantities (2μl) of DNA.

- A medium-throughput genotyping platform. The Illumina BeadXpress Reader is a medium-
throughput; dual-colour laser detection system that enables scanning of a broad range of multiplexed assays developed using the VeraCode digital microbead technology. This platform supports DNA-, RNA-, and protein-based assays. The VeraCode technology utilised by this system allows from 48 to 384-plex assays per well in a standard 96-well format. The assay enables the specific amplification of the sequence around a particular SNP, and the two amplified alleles are then labelled with two different fluorescent dyes (Cy3 and Cy5) for detection purposes.

The genotyping facility also includes a resource dedicated to the secure storage of DNA samples for present and future research projects. All DNA samples stored in the biobank are bar-coded, anonymised and catalogued in the biobank database.

During the reporting period, data were generated for research studies related to obesity, pharmacogenomics, population genetics, bone mineral density and abalone genetic diversity.

Cytogenetics
The cytogenetics laboratory provides a pre- and post-natal laboratory service. This laboratory processes samples of peripheral blood, amniocenteses, chorionic villi, products of conception, and skin fibroblasts. FISH studies are performed on samples for microdeletions or other chromosomal rearrangements. Cytogenetics has been severely affected by continuing staff losses of trained individuals who are difficult to replace as there are few trained cytogeneticists in the country. Prenatal samples from women of advanced maternal age, products of conception and blood from infants with suspected Down syndrome, trisomy 13 and 18 are diverted to the applied polymorphism section for rapid detection of Down and other ‘common’ chromosomal aneuploidies by quantitative fluorescent (QF)-PCR.

Applied polymorphisms
The laboratory’s main activities include parentage testing, QF-PCR for the detection of chromosomal aneuploidies and tests for certain inherited biochemical abnormalities. Test numbers have decreased by approximately 7% since last year and most of the decrease can be attributed to a decline in the demand for parentage tests (a fall-off of about 10%). Overall, QF-PCR saw a 3% increase, with state testing increasing by 13.6%. However, private testing saw a decline of 11.5%. This section remains critically short staffed with only three out of seven posts being occupied. This has had a significant adverse impact on turnaround times and progress towards accreditation.

Clinical genetic services
The clinical diagnostic service consults patients with a wide spectrum of genetic disorders, including antenatal and clinical cases. Weekly clinics are held at CMJAH, Chris Hani Baragwanath Hospital, Rahima Moosa Hospital and at Donald Gordon Medical Centre. Regular clinics are also held in specialist clinics in the teaching hospitals, including haemophilia (at CMJAH and Chris Hani Baragwanath Hospital), breast cancer clinic and a haematology/oncology genetic clinic at Chris Hani Baragwanath Hospital. Fewer clinics are being serviced due to significant staff losses of medical geneticists and genetic counsellors.

Outreach clinics were held in East London and Port Elizabeth which provided local patients with genetic services and local doctors with CPD in medical genetics.

The clinical section provides many educational talks at the academic teaching hospitals, at a number of private hospitals and other venues to specialists and lay people on a wide array of medical genetics topics. These continue to be well received and have a positive impact on the section’s interaction with health professionals and patient referral.

Genetic ancestry testing
A major translation of the division’s research is the service provided to the public in the form of genetic ancestry testing. During 2012, 222 genetic ancestry tests were conducted. This activity contributes to public education and understanding of science.

RESEARCH
Research is being undertaken on policy issues related to the implementation of medical genetic services, particularly laboratory services in middle- and low-income countries. Research and development on medical genetic tests for locally relevant medical genetic disorders continue. Members of the academic staff are part of the Wits Molecular Biosciences Research Thrust: Health for Africa and participate in workshops and research forums organised under the umbrella of the Thrust. Further, the Human Genetics Diversity and Disease Research Unit makes use of population and evolutionary genetics to understand how changes in the human genome are associated with the epidemiology of disease, and reconstruct human history and human origins.
RESEARCH PROJECTS

An audit of thyroid function tests in South African children with Down syndrome

Researchers: Dr S Moosa (for MMed), Dr D Segal, Prof A Christianson, Dr N Gregersen

Thyroid dysfunction is the most common endocrine abnormality associated with Down syndrome. Due to the paucity of data from sub-Saharan Africa regarding thyroid function in African children with Down syndrome, this study aimed to document the range of thyroid function in a cohort of 391 South African children with Down syndrome, seen at the genetic clinics from 2003 to 2008. The majority (84%) of children had at least one thyroid function test (TFT) performed, and the most common form of thyroid dysfunction encountered was subclinical hypothyroidism (28.7%). Notably, up to one-third of patients with abnormal TFT results were not referred to the endocrine clinics for evaluation, and were thus not receiving the necessary treatment. Difficulties in interpretation of results obtained from different biochemical machines and populations, as compared to those used to derive the reference ranges, were raised. Problems with regular follow-up of patients and annual thyroid surveillance were also highlighted.

Inherited breast and ovarian cancer: a review of the available genetic counselling and testing services in Johannesburg

Researchers: M Jefferies (for MSc Med), T Wessels, S Macaulay

Familial breast and ovarian cancers account for between 5-10% of all breast cancer and 10-15% of all ovarian cancer cases. In South Africa, testing for these familial cancers is limited to those individuals with a high risk family history of breast and/or ovarian cancer and those relatives of individuals with identified mutations in the predisposing genes, BRCA1 and BRCA2. This study aimed to assess the genetic counselling service offered to patients in terms of BRCA1/2 testing and to assess patients' experiences of the genetic counselling service. Seventy-four percent of patients attended genetic counselling for predictive or diagnostic BRCA mutation testing. Testing was offered to 78% of patients, with an uptake of 81%, 91% of which was performed nationally. There was 100% participation in the telephonic questionnaire and 94% of respondents stated they would refer the genetic counselling service. Results from this study will assist in improving the genetic counselling service offered by the NHLS and Wits.

Pregnant women's perceptions and knowledge regarding alcohol use during pregnancy

Researchers: S Macaulay, T Wessels, Ms C le Roux

Foetal alcohol syndrome (FAS) is a major cause of intellectual disability throughout the world, with the highest rates reported in South Africa. Little is known about the perceptions and knowledge of pregnant women in South Africa regarding the effects of alcohol on an unborn baby. No information is available on whether these ideas differ between patients in the state hospital system, versus the private setting. To investigate pregnant women's perceptions and knowledge regarding alcohol use during pregnancy and its effects on the foetus, randomly selected participants at CMJAH and two private antenatal centres in Randburg and Bryanston are being asked to complete a structured questionnaire. Information from this study can be used in the development of preventative strategies and awareness campaigns. Data collection has commenced.

South African women's experience of genetic counselling

Researchers: M Morris, M Glass, T Wessels

Funding: NHLS Research Trust

Client satisfaction is a commonly researched topic in clinical settings as it has aided in obtaining desirable outcomes for patient care. There has been limited research on client satisfaction in genetic counselling. Some people may perceive heritability to be influenced by culture, community and family values. South Africa provides a unique situation for genetic counselling because of the multicultural and linguistic diversity. This qualitative study explores black South African women's experiences of genetic counselling in Johannesburg. The study makes use of interpretative phenomenological analysis and voice-recorded focus groups. The groups are conducted in a suitable African language and facilitated by an experienced psychologist. All recordings will be transcribed and translated into English. During data analysis, themes will be extracted, inter-connected and interpreted to create a comprehensive picture of the informants' experiences.

The characteristics of the genetic counselling process in an antenatal multicultural setting

Researchers: Prof C Penn, T Wessels (for PhD)

Funding: NRF, Thuthuka
Results of this study have shed some light on the structure of the sessions and the interventional features. Genetic counselling is a complex interaction where the different phases of the interaction have very distinct features. The phases of the session include opening, information gathering, information giving, decision-making and life world. The nature of the genetic counselling interactions is such that there is an inherent tension between ‘education’ and ‘counselling’. This is apparent from examining the overall structures as well as detailed analysis of different phases.

**Family history and risk assessment in black South African women with breast cancer**

*Researchers:* T Wainstein, Prof A Krause, C Van Wyk  
*Collaborators:* Dr H Cubasch

Black South African women with breast cancer are generally diagnosed at a younger age, have a more aggressive disease and a poorer prognosis than their Caucasian counterparts. Little is known about the manner in which breast cancer is inherited in black South African families or whether these individuals harbour deleterious mutations in the most common breast cancer predisposing genes (BRCA1 and BRCA2). The aim of this study was to determine whether or not black South African women who have breast cancer, have significant family histories of cancer. Results indicated that there were few affected women with significant family cancer histories. Results also suggested that age at diagnosis may not be an appropriate predictor of inherited breast cancer risk in this population. The study highlighted the necessity of molecular genetic screening in this population to delineate individuals who are truly at increased risk of developing inherited breast cancer. Information obtained from this study will direct future research in the South African black population concerning genetic counselling and testing for inherited breast cancers.

**The molecular aetiology of inherited breast cancer in the South African black population**

*Researchers:* W Chen, Dr R Kerr, Prof A Krause  
*Funding:* NHLS Research Trust, University of the Witwatersrand – Faculty Research Trust

Hereditary breast and ovarian cancer caused by mutations in the BRCA1 or BRCA2 genes has been well studied in many populations but little is known about the genetic basis for the disease in African and South African black populations. Black South African women with breast cancer are characterised by earlier age of onset and more rapid progression of disease compared to their white counterparts. It is possible that founder mutations exist within the BRCA1 and/or BRCA2 genes in the South African black population. The majority of mutations within the BRCA1 and BRCA2 genes are sequence variants (90%), with large gene rearrangements contributing to only 10% of all cases. This study will investigate 30 South African black individuals presenting with breast cancer. These individuals will be selected based on either: early age of onset (<50 years of age), without a family history of cancer or positive family history (aged under or over 50 years). Sanger dideoxynucleotide chain termination sequencing analysis and multiplex ligation-dependent amplification analysis will be performed to screen for mutations present in either the BRCA1 or BRCA2 genes.

**Genotype-phenotype correlation in black and Afrikaans individuals with Fanconi anaemia**

*Researchers:* Prof A Krause, Dr C Feben, T Haw  
*Collaborators:* Dr L Wainwright, Prof J Poole, Prof D Stones, Dr C Sutton  
*Funding:* MRC

Fanconi anaemia (FA) is an inherited disorder which leads to premature death from bone marrow failure or malignancy. FA is caused by allelic mutations in one of at least 13 different genes. The Afrikaans and black populations in South Africa both have founder FA-causing mutations. This unique genetic homogeneity provides opportunities to establish whether genotype-phenotype correlations exist in FA. The aim is to describe the clinical features of patients homozygous for black and Afrikaans founder mutations, and to assess whether they correlate with the disease course, particularly the age of onset of bone marrow failure and/or malignancy. A retrospective file review of deceased patients will also gather longitudinal information about the disease course. Awareness of the clinical features of FA in South African populations will lead to more efficient diagnosis of FA. Knowledge of the clinical markers for prognosis of patients with founder mutations will allow appropriate medical monitoring and interventions. Thirty-three black patients have been examined and six Afrikaans patients have been seen to date.
Phenotypic consequences in black FA patients, homozygous for a FANCG637-643 deletion mutation

Researchers: Dr C Feben (for MMed), Prof A Krause, T Haw
Collaborators: Dr L Wainwright, Prof J Poole, Prof D Stones, Dr C Sutton
Funding: MRC

A founder mutation (FANCGdel), identified in 82% of affected black patients, has been identified. The aim of this study was to investigate a genotype-phenotype correlation in a cohort of affected individuals. Thirty black patients, homozygous for the FANCGdel and recruited from haematology/oncology clinics in Johannesburg and Bloemfontein, were subjected to a comprehensive examination to document growth measurements, congenital anomalies and phenotypic variability. Descriptive statistical analysis of the data showed significant growth abnormalities in 50% of patients and a high frequency (96.7%) of skin pigmentary anomalies. Anomalies of the eyes, ears and hands occurred more frequently than has been reported in other FA cohorts. Apart from abnormalities of the kidney (in ≥40% of the cohort) and gastrointestinal malformations (in 10%), congenital malformations of other major organ systems were infrequent (<5%).

Investigating the molecular aetiology of hereditary nonpolyposis colorectal cancer

Researchers: K Davison, Prof A Krause, Dr R Kerr
Funding: NHLS Research Trust

Colorectal cancer (CRC) has a heritable component in some families. Hereditary non-polyposis colorectal cancer (HNPCC) accounts for about 5-6% of all CRC and is the most common inherited condition. HNPCC is characterised by deficiencies in the DNA mismatch repair pathway and four major genes have been implicated: MSH2, MLH1, MSH6 and PMS2. No genetic diagnostic service for HNPCC is available in South Africa. The aim of this project was to establish mutation screening for HNPCC using two mutation detection strategies: multiplex ligation-dependent probe amplification (MLPA) testing for large deletion or duplication mutations in MSH2, MLH1, MSH6 and PMS2, and, sequencing of the MSH2 and MLH1 genes looking for single nucleotide changes or smaller insertions or deletions. In a cohort of 19 patients, 12 of whom were Amsterdam-positive and seven of whom were Bethesda-positive, four disease-causing mutations were identified (21%) using Sanger sequencing. Three of these mutations were in MLH1 and one in MSH2. This detection rate is comparable to the literature, where rates of between 11% and 30% have been reported. No mutations were identified using MLPA analysis.

Testing for microsatellite instability in tumour cells of hereditary nonpolyposis CRC

Researchers: M Lazarus, Dr R Kerr, K Davison, Prof A Krause
Funding: FRC

As DNA mutation screening is laborious and expensive, it is an international recommendation that a step-wise approach to genetic testing for HNPCC be adopted – before undertaking DNA mutation screening, tumour DNA should be tested for microsatellite instability (MI). Testing for genomic instability was undertaken by analysis of five random microsatellite loci, constituting a panel known as the Bethesda panel, looking for inconsistencies in repeat length between tumour tissue and normal (blood) tissue. Inconsistencies suggest that one or more MMR proteins are non-functional in the tumour tissue. If MI is found, this would be strongly suggestive of the presence of a MMR mutation and would support inclusion of the patient in the mutation screening step.

Eight patients were chosen (a sub-set of the patient cohort included in the study described above). DNA was extracted from both blood and tumour (paraffin-embedded block) tissue. Repeats were scored at the random microsatellite markers. Of the eight patients chosen for this study, no results were obtained for one patient due to non-amplification of the tumour DNA; two patients showed microsatellite stability; one patient exhibited a microsatellite-low phenotype (only one microsatellite was unstable); four patients were found to show microsatellite instability-high (MSI-H) phenotypes (two or more microsatellites were unstable). Germline mutations were found in two of the four MSI-H patients (in the study described above). These results lend strong support to the hypothesis that, in the South African population, microsatellite instability is a good predictor of finding a germline DNA MMR mutation.

Genetic factors influencing inhibitor development in haemophilia A patients

Researchers: Dr A Lochan, Prof A Krause, S Macaulay, Prof J Mahlangu
Funding: NHLS Research Trust, Wits
A critical complication of factor VIII (FVIII) concentrate replacement therapy in haemophilia A (HA) treatment is inhibitor development. Inhibitor-predisposing genetic factors are multiple and include F8 gene mutations, ethnicity, a family history of inhibitor development and FVIII haplotype mismatch. Knowledge of genetic risk factors for inhibitor development is important not only in predicting inhibitor risk but also in planning exposure to rapidly evolving replacement therapy. The study aims to characterise and correlate HA disease severity, inhibitor development, intron 22 inversion mutation status, ethnicity and FVIII haplotype in a South African severe HA (sHA) cohort. Of the 249 sHA records reviewed, 238 were included for analysis in the study; 124 (52%) were black and the remainder were white. Ninety (38%) patients had the intron 22 inversion mutation (of which 52 were black) and 30 (12%) had inhibitors (of which 22 were black). The H2 haplotype was the commonest in blacks. Preliminary data suggest a correlation exists between this haplotype and inhibitor development.

**Screening for a novel FKRP-related muscular dystrophy mutation and identifying a possible founder haplotype in Afrikaners with a DMD/BMD phenotype**

**Researchers:** F Essop, Prof A Krause, C Prentice

Muscular dystrophy incorporates a wide range of inherited disorders that involve muscle weakness, caused by the loss of muscle tissue that progresses over time. Muscular dystrophy diseases can be divided into several classes based on the specific type of skeletal muscle involved, the age of onset, mode of inheritance and overall disease progression. Duchenne and Becker muscular dystrophy (DMD/BMD), collectively referred to as the dystrophinopathies, are the most common forms of muscular dystrophy diseases. The **FKRP**-related muscular dystrophies involve mutations in the fukutin-related protein (**FKRP**) gene and are often misdiagnosed as dystrophinopathies. This is due to the clinical similarity associated with the disease phenotypes of both forms of muscular dystrophy. The **FKRP** gene encodes the fukutin-related (**FKRP**) protein which is widely believed to be a glycosyltransferase. A previously unreported **FKRP** mutation (c.1100T>C) was found in exon 4 of the **FKRP** gene in two Afrikaner patients, clinically diagnosed with DMD/BMD. A total of 39 white South African patients (clinically diagnosed with DMD/BMD, but negative for common DMD/BMD mutations) and 100 Afrikaner controls were screened for the c.1100T>C mutation using amplification refractory mutation system (ARMS)-PCR, the results of which were confirmed by RFLP analysis and Sanger sequencing. Six Afrikaner patients were found to be homozygous for the mutation and five were heterozygous for the mutation. The carrier frequency of the c.1100T>C mutation was estimated to be one in every 50 individuals in the general Afrikaner population. Microsatellite analysis showed a possible founder haplotype for the c.1100T>C mutation, suggesting that the c.1100T>C mutation is likely to be a founder mutation in the Afrikaner population and could be an important contributor to the aetiology of muscular dystrophy in the South African population.

**Fc gamma receptor polymorphisms as risk factors for systemic lupus erythematosus in black South Africans**

**Researchers:** J Cunniffe, Prof M Ramsay, J Frost

**Collaborator:** Prof M Tikly (Division of Rheumatology, Wits)

**Funding:** The Connective Tissue Disease Research Fund, Wits

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterised by immune-mediated injury to and chronic inflammation of various tissues. The disease is more prevalent, more severe, as well as associated with a higher mortality, in black South African SLE patients. Multiple genes and environmental factors are involved in the aetiology of SLE, with more than 30 susceptibility loci already identified. One of the strongest candidate regions is the Fc gamma receptor (**FCGR**) gene cluster at chromosome 1q21-24. These genes encode the Fc gamma (Fcγ) receptors that recognise antibody-antigen immune complexes containing immunoglobulin G and aid in their clearance. These receptors are essential mediators of the immune response and several SNPs in the Fcγ receptor genes have been proposed to influence susceptibility to and severity of SLE. This study concerned two such SNPs in the **FCGRIIA** and **FCGRIII A** genes, respectively. **FcγIIIA** is classified as an activating receptor and is involved in initiation of immune responses, whereas **FcγIIIB** is an inhibitory receptor which down-regulates immune responses; the opposing roles of these receptors control the balance between tolerance and autoimmunity. The two SNPs in this study are rs1050501, c.695T>C in the **FCGRIIIA** gene, and rs396991, c.526T>G in the **FCGRIII A** gene. Both cause mis-sense mutations and affect the ability of these receptors to perform their normal functions. To date, no studies on these SNPs have been conducted in South Africa.
The main aim of this study was to genotype 100 black SLE patients and approximately 200 ethnically and geographically matched healthy controls for rs1050501 and rs396991, and to establish their roles as susceptibility and severity risk factors for SLE in this population. The two SNPs were genotyped by either pyrosequencing or high resolution melt (HRM) analysis and both were confirmed to be polymorphic in this population. For rs1050501, genotype frequencies were approaching Hardy-Weinberg equilibrium, and significant differences in genotype frequencies and allele frequencies were seen between patients and controls. Further statistical investigation established a significant association of this SNP with SLE in the black population, with the C allele being associated with an approximate four times higher risk of developing the disease than the T allele. This SNP was also confirmed to be a severity risk factor in the black population, with the CC genotype being associated with a statistically significantly higher disease activity than either CT or TT. Assessment of Hardy-Weinberg equilibrium revealed a significant deviation of observed genotype frequencies from expected genotype frequencies for rs396991, suspected to be the result of genotyping errors associated with inaccurate interpretation of HRM analysis results. This prevented establishing an association of rs396991 with SLE in black South Africans and warrants future investigation of this SNP in this population.

Genetic factors influencing bone health in the black population

Researchers: A May (for MSc Med), R Potgieter, Dr Z Lombard, Prof M Ramsay
Collaborators: Prof SA Norris, Prof J Pettifor (MRC/Wits Developmental Pathways for Health Research Unit)
Funding: Carnegie Corporation NRF, Wits FRC

Maintaining suitable bone health is emerging as a serious concern worldwide, as the prevalence of skeletal disorders threatens to reach unmanageable proportions. Despite unfavourable environmental factors, black South Africans demonstrate elevated bone mass, especially at the femoral neck, when compared to population-matched whites. Genetic factors are thought to mediate this effect, which may have clinical or therapeutic value. Using a candidate gene approach, this study investigated associations of six candidate genes (ESR1, TNFRSF11A, TNFRSF11B, TNFSF11, SOST and SPP1) with bone mass among pre-pubertal black children that formed part of the longitudinal Birth to Twenty cohort. The GoldenGate genotyping assay with VeraCode microbeads was used to genotype 151 black children at 366 polymorphic loci, including 112 previously associated and 254 tagging SNPs. A linear regression approach was implemented to highlight significant associations while adjusting for height, weight, sex and bone area. Twenty eight markers were found to link to either femoral neck (19) or lumbar spine (9) BMC. These signals derived from three genes, namely ESR1 (18), TNFRSF11B (9) and SPP1 (1). One marker (rs2485209) maintained its association with the femoral neck after correction for multiple testing. These results fully support the existence of a strong genetic effect acting at the femoral neck in African ancestry individuals. Tagging SNP signals suggest the presence of a number of population specific variants that require further investigation. Combined, these markers may help to account for increased bone mass among black South Africans.

Analysis of genetic variation and obesity-related traits in the Birth to Twenty cohort

Researchers: Prof M Ramsay, Dr Z Lombard, P Pitamber
Collaborators: Prof S Norris (MRC/Wits Developmental Pathways for Health Research Unit); Prof N Crowther (NHLS/Wits Dept Chemical Pathology)
Funding: NRF, NHLS and Birth to Twenty study

Heritability studies have demonstrated a genotype-phenotype correlation in obesity, with whole-genome association studies linking several genes and regions to obesity risk. Some of these associations are being examined in a collaborative project with the Birth to Twenty study group. This cohort is the largest and longest running study of child and adolescent health and development in Africa, and one of the few large-scale longitudinal studies in the world. Over 900 participants were evaluated for genetic variation within 13 candidate genes previously shown to be associated with obesity, including FTO, MC4R, LEP and NPY2R, as well as several ancestry-informative SNPs. Genotyping was performed using the Illumina BeadXpress genotyping platform. Four SNPs in the FTO, LEP and MC4R genes were shown to be associated with body mass index in their black African cohort, following adjustment for sex, sex-specific pubertal stage and age.

Transgenerational inheritance of DNA methylation alterations at the h19 imprinting control region following chronic maternal ethanol exposure in mice

Researchers: M Ungerer, Prof M Ramsay
Funding: NHLS Research Trust, Wits

Prenatal alcohol exposure leads to a wide range of developmental abnormalities. Upon prenatal alcohol exposure, only between 5% and 10% of offspring
display alcohol-related developmental anomalies. However, this is probably greatly underestimated with common behavioural disorders, such as attention deficit hyperactivity disorder. Likely to be linked to the transgenerational effects of in utero alcohol exposure. The transgenerational effects of alcohol exposure remain poorly understood. Attention has focused on epigenetics, more specifically DNA methylation, as a potential mechanism. The H19 imprinting control regions (ICRs), a paternally imprinted locus is vital for embryonic growth and development and there is evidence to suggest that it may be susceptible to alteration upon alcohol exposure. If this occurs in the germ-line it may be transmittable to future generations. By use of a mouse model this study aims to investigate the transgenerational inheritance of DNA methylation alterations at the imprinted locus, H19, following chronic maternal ethanol exposure.

**Epigenetic modification at imprinted loci following alcohol exposure during prenatal development**

**Researchers:** Prof M Ramsay, Dr Z Lombard, M Masemola  
**Collaborator:** Prof D Viljoen (Foundation for Alcohol-related Research)  
**Funding:** NHLS Research Trust, FRC, NRF, Mellon Postgraduate Mentoring Programme

Foetal alcohol syndrome (FAS) is a serious developmental disorder resulting from in utero alcohol exposure. Although environmentally induced, twin concordance studies and animal models suggest that there are genetic and epigenetic susceptibility factors. Imprinted genes are known to play a role in growth and development. Since DNA methylation is thought to play an important role in regulation of gene expression during embryogenesis, ethanol-associated alterations in foetal DNA methylation may contribute to developmental abnormalities seen in FAS. The specific aim of this study is to examine the effects of alcohol on methylation of ICRs of specific imprinted genes in FAS children and ethnically matched unaffected individuals.

The following loci are being investigated: IGF2/IGF2R, KCNQ1O1/CDKN1C ICR (KvDMR), PEG3 DMR and DLK1/MEG3 (IG-DMR), H19ICR and KvDMR1 were not significantly different in average or site specific methylation when FAS cases were compared to controls. There was a significant difference in methylation (average and at different CpG sites) at IG-DMR and PEG3DMR, after adjusting for age and gender. The reduction of methylation at IG-DMR and PEG3 DMR may contribute to some aspects of the phenotypes seen in FAS offspring.

**Determining genetic variants associated with rheumatoid arthritis in black South Africans**

**Researchers:** Prof M Ramsay, J Frost, Dr N Govind  
**Collaborators:** Prof M Tikly  
**Funding:** MRC, Connective Tissue Disease Research Fund (Wits)

Rheumatoid arthritis (RA) is a chronic multisystem disorder affecting 1% of adults worldwide. Genetic factors play a major role in RA disease susceptibility. HLA class II genes carrying the ‘shared epitope’ contribute only an estimated 30% of the genetic risk for RA. Other susceptibility genes are beginning to shed light on the complex genetics of RA. In persons of European and Asian ancestry, PTPN22 and STAT4 have been reported to play a role in RA pathogenesis, but these results have proved to be population-specific, with no consistent or reproducible findings across different populations. It was recently shown that the PTPN22 R620W polymorphism does not occur in black South Africans and is therefore not a risk factor for RA in this population. The objective of the study is to genotype 96 SNPs in 400 RA patients and 400 control samples, to determine their role in the various candidate genes identified in other populations (PTPN22, PAD4, STAT4, CTLA4 and BANK1) as risk factors for susceptibility and severity in black South Africans with RA. The genotyping of the samples has been done using the Illumina BeadXpress with VeraCode bead technology and data are being analysed.

**Epigenetic effect of alcohol on sperm DNA – potential risk for foetal alcohol spectrum disorders**

**Researchers:** Prof M Ramsay, P Pitamber, S Patel, Dr Z Lombard  
**Funding:** NRF, NHLS Research Trust, FRC

Exposure to alcohol in utero is the main attributable cause of foetal alcohol spectrum disorders (FASD). Paternal preconception drinking is not considered to be a significant risk factor, even though animal studies have demonstrated that chronic paternal alcohol consumption has a detrimental effect on the physical and mental development of offspring even in the absence of in utero alcohol exposure. Alcohol can reduce the levels and activity of DNA methyltransferases resulting in DNA hypomethylation and that reduced methyltransferase activity can cause activation of normally silenced genes. It is hypothesised that, should these epigenetic changes in imprinted genes be transmitted through fertilisation, they would alter the critical gene expression dosages.
required for normal prenatal development resulting in offspring with features of FASD. Global DNA methylation was examined in response to preconception paternal alcohol consumption using the luminometric methylation assay (LUMA).

There was no significant correlation between alcohol exposure and global DNA methylation, but a trend towards decreased global DNA methylation was observed in alcohol exposed spermatozoa. Bisulphite modification and quantitative pyrosequencing technology was used to assess locus-specific levels of methylation. There was also no significant correlation in sperm between alcohol exposure and average H19 ICR DNA methylation, but one CpG site, CpG3, showed a significant increase in DNA methylation in the drinking group. At the IG-DMR locus an overall reduction in methylation was noted in males who consumed alcohol after adjusting for confounding variables, and when analysed by individual CpG sites, alcohol consumption was found to correlate with demethylation at CpG 3 while alcohol-dosage preferentially correlated with demethylation at CpG 7. These data demonstrate subtle alterations in DNA methylation, both increased and decreased, in a locus-specific manner.

Exploring the role of genetic variation at the leptin and the leptin receptor genes in obesity and hypertension in a black South African cohort

Researchers: T Ngcugcu, Prof M Ramsay
Collaborators: Prof A Woodiwiss, Prof G Norton
Funding: MRC, NHLS Research Trust

Obesity and hypertension are common non-communicable disorders that are increasing in South Africa and throughout the world. They often occur together and are risk factors for other cardiometabolic disorders including stroke. Leptin regulates appetite by binding leptin receptors in the hypothalamus to signal satiety. The LEP and LEPR genes which encode leptin and its receptor, respectively, are compelling candidate genes. The African Programme on Genes in Hypertension (APOGH) cohort comprises small family groups who have been phenotyped for height, weight, skinfold thickness, waist and hip circumference and brachial, aortic (central) and 24-hour ambulatory blood pressure. DNA from 905 individuals is available for genotyping for SNPs associated with the LEP and LEPR genes. The LEP SNP, rs7799039, has been shown to be associated with elevated systolic and diastolic blood pressure in Tunisian men and American women, but was not significantly associated in the APOGH cohort with blood pressure. Other LEP and LEPR SNPs will be genotyped using the BeadXpress platform.

Computational identification of synonymous SNPs in the human genome and their potential role in disease

Researchers: L-A Sharma, Prof M Ramsay
Funding: NRF

The potential phenotypic effects of synonymous SNPs (sSNPs) have long been overlooked. Although several sSNPs are no longer thought to be silent, no one has identified which sSNPs may contribute to phenotypic variation on a genome-wide scale. sSNPs that cause a change in codon-usage frequency or mRNA secondary structures may alter translational and protein folding kinetics. In addition, sSNPs that alter splice-site consensus sequences may cause aberrant slicing, which could change the protein product. A sSNP that contributes to any of these molecular mechanisms may thus potentially alter protein structure and function. To computationally identify sSNPs with a potential impact, SynSNP was created. SynSNP is a text-based tool written in Python. SynSNP uses established bioinformatics tools to determine which of the sSNPs may potentially result in a molecular effect.

The potentially functional sSNPs are then assessed to determine whether any have previously been associated with a trait or disease in genome-wide association studies (GWAS) and/or occur within genes known to be associated with disease in OMIM (Online Mendelian Inheritance in Man). Of the 90,102 identified sSNPs, 23.4% were predicted to potentially have a functional impact, through one or more of the three molecular mechanisms investigated. Of the sSNPs predicted to potentially have a functional impact, 14 (0.07%) had previously been associated with a trait or disease in GWAS and 4,057 (19.2%) of the potentially functional sSNPs were within genes known to be associated with disease in OMIM. This study demonstrates that a significant proportion of sSNPs may have a functional impact and their potential role in disease should therefore not be underestimated or neglected.

Genetic predisposition to kidney disease in HIV-positive South African blacks

Researchers: AN Kasembeli, Prof M Ramsay
Collaborators: Dr R Duarte, Prof S Naicker (Department of Clinical Medicine)
Funding: NIH Training Grant
The burden of renal disease associated with HIV infection among black patients is on the increase. The mechanism for the development of end stage renal disease (ESRD) once an HIV-positive patient has been diagnosed with kidney disease is not well understood but since this phenomenon is regional, with a higher prevalence in Africans, it is possible that genetic factors play a role in susceptibility. Polymorphisms in and around the MYH9 and APOL1 genes have been identified as genetic factors influencing progression to kidney disease among black Africans, but no studies have been conducted in South Africa. This study is being undertaken to investigate the genetic impact on kidney disease among HIV-positive South African blacks focusing on the two gene regions (MYH9 and APOL1). South African patients (with kidney disease) and ethnically and geographically matched HIV-positive and healthy controls (without kidney disease) will be genotyped for SNPs. An assay has been designed for SNP genotyping on the Illumina BeadXpress genotyping platform.

**Genetics of primary open-angle glaucoma in black southern Africans**

**Researchers:** Dr S Williams, T Zwane, Prof M Ramsay  
**Collaborators:** Prof T Charmichael (Department of Ophthalmology, Wits)  
**Funding:** Carnegie Foundation

The purpose of this research is to determine the contribution of genetic variation at candidate genes to the pathogenesis of primary open-angle glaucoma (POAG) in black southern African patients using a case-control association study. POAG is most common in people of African descent where in some groups the prevalence is estimated to be six times as high as that in Caucasians. In South Africa, glaucoma was present in 2.8% of the population sampled, and more than 33% of the patients with POAG were bilaterally blind. Only 10% of the urban population with POAG had received prior treatment for glaucoma. Little is known about the genetics of POAG in black southern Africans, including the proportion of POAG index cases with a family history of the disease.

A detailed family history and pedigree analysis of POAG patients will allow an estimate of the extent to which glaucoma is familial in this population. A group of candidate genes with some previous association with POAG or a related phenotype was identified and the contribution of genetic variation to susceptibility to POAG will be tested in black southern Africans. Identifying the genetic variation implicated in the disease could lead to the development of DNA-based diagnostic tests which could be used to detect a genetic predisposition for developing POAG. Novel insights into the molecular mechanisms of POAG could alter therapeutic approaches and lead to the development of targeted treatments for the condition.

**Genetic risk factors for painful antiretroviral toxic neuropathy in HIV-positive individuals of African ancestry**

**Researchers:** Dr Z Lombard, L Hendry, J Mellet  
**Collaborators:** Prof P Kamerman, A Wadley (School of Physiology, Wits); Prof P Price (School of Pathology and Laboratory Medicine, University of Western Australia); Dr C Cherry (Burnet Institute, Melbourne, Australia)  
**Funding:** Wits URC, Wits FRC, Belgian Embassy Masters fellowship

HIV-associated sensory neuropathy (HIV-SN) is a common complication associated with HIV-infection. Two distinct forms of neuropathy exist: i) a viral-mediated HIV-distal symmetrical polyneuropathy (HIV-DSP) and ii) an iatrogenic antiretroviral toxic neuropathy (ATN). The two forms often occur together and are difficult to distinguish. The study will focus on ATN, as the patient group consists of individuals who have been receiving ARV treatment. A common symptom of HIV-associated neuropathy is pain. Variation at specific loci within certain candidate genes has been suggested to alter susceptibility to developing ATN, as well as the intensity of neuropathic pain. The aim of the current research is to conduct an in depth study, in a black African population, of genes previously associated with susceptibility to developing ATN (TNFA) and variations in pain intensity and sensitivity (GCH1 and KCNS1) in other neuropathic pain states. Ninety-six SNPs were selected from within the candidate genes chosen. Genotyping of these genes was carried out using the GoldenGate genotyping assay with VeraCode microbeads and data were read on the Illumina BeadXpress Reader. A couple of SNPs which failed using the GoldenGate genotyping assay are being genotyped using a TaqMan assay. Preliminary analysis suggests an association between several haplotypes constituting the four SNPs selected in the KCNS1 gene and the presence of pain, as well as with differences in pain sensitivity. Following completion of the genotyping, analysis will be completed on all SNPs to determine associations with susceptibility to neuropathy and pain intensity/sensitivity.
Mapping the modelling genetic variation among sub-Saharan African populations

Researchers. A Hobbs, D Deveredicis, S-R Harris, T Hughes, Prof H Soodyall

Funding: MRC, National Genographic Project

Sequencing of hypervariable regions I and II of mtDNA were completed in samples from Central African Republic, Democratic Republic of Congo, Uganda and Zambia for the Genographic Project and various southern African Khoe-San and Bantu-speaking populations. The mtDNA haplogroups were deduced and used to examine population structure and population affinities among sub-Saharan African populations. This study emphasised the antiquity of the Khoe-San gene pool among living people like the Karretjie people in the vicinity of Colesberg.

Reconstructing the maternal ancestry of the Malagasy

Researchers: P Patel, A Hobbs, Prof H Soodyall

Collaborators: Prof T Jenkins, G Campbell (Montreal, Canada)

Funding: MRC, National Genographic Project

The sample consisted of 981 unrelated males, sampled throughout Madagascar between May 1992 and November 1996. Phylogenetically informative SNPs were screened for using Taqman assays and the non-coding hypervariable regions (HVR I and II) were sequenced using previously published protocols. The intergenic COII/tRNALys 9-bp deletion which has multiple independent origins, used in conjunction with sequence data, was particularly useful at distinguishing sequences derived from African and Asian sources. In addition, sequencing of two coding region SNPs (1473C-T and 3423T-A) definitive of the ‘Malagasy motif’ were sequenced. SNP data in conjunction with control region sequence data delineated 296 unique sequences. Using the recommended nomenclature for mtDNA haplogroups, 41.28% of the mtDNA lineages were traced to African sources, 58.51% to Asian and 0.21% to Eurasian origins. The 9-bp deletion occurred at a frequency of 22.66%. Control region sequence data in conjunction with SNP variation resolved the origin of the 9-bp deletion to African (17.04%), Taiwanese (0.45%) and Polynesian (21.08%) motifs. Further analysis revealed that the Malagasy motif represented 74.05% of the Asian form of the 9-bp deletion. This study corroborates historical, linguistic and archaeological data concerning the parental origins of the Malagasy. However, these preliminary findings suggest that the female gene pool has an appreciably higher contribution from non-African sources of origin compared with African origins. This is in contrast to what is observed using Y chromosome data.

Rain forests to deserts: the phylogeography of haplogroup B2b

Researchers: T Naidoo, Prof H Soodyall

Collaborators: Dr C Schlebusch (Uppsala University, Sweden)

Funding: MRC, NRF, National Geographic Project

Haplogroup B2b has previously been resolved into nine subclades through the discovery of their defining markers. This study provided population data on haplogroup B2b, defined by seven bi-allelic markers and 17 microsatellites. After screening almost 5,000 male, primarily sub-Saharan African individuals, and an extensive literature survey, 371 Y chromosomes belonging to haplogroup B2b were identified. Phylogeographic analysis was conducted using the above data in an attempt to elucidate the complex patterns involved in the evolution of haplogroup B2b and its spread across sub-Saharan Africa. Overall, while haplogroup B2b was found throughout most of sub-Saharan Africa (including Madagascar), the highest frequencies were observed in hunter-gatherer populations. Most of the subclades of haplogroup B2b exhibited substantial population specificity: B2b1 and B2b4a occurred among the Khoe-San and their descendent populations of southern Africa; B2b2 was found only in the Mbuti of north-eastern DRC; while B2b3 and B2b4b were restricted primarily to the Western Pygmy groups of Cameroon, Central African Republic and Gabon. Paragroups B2b* and B2b4*, however, displayed wider distribution, and were present in populations across Central, East and southern Africa. The distribution of haplogroup B2b and its subclades appears to be characterised by the dispersal of, and/or continued gene-flow between hunter-gatherer groups. Though the origins of many of the subclades of B2b could be attributed to the populations they were found in, locating the origin of the original B2b chromosomes is made difficult by the presence of the uncharacterised B2b* throughout sub-Saharan Africa, and its high diversity across these regions.
Phylogeographical insights into haplogroup A in southern Africa

Researchers: T Naidoo, Prof H Soodyall
Collaborators: Dr C Schlebusch (Uppsala University, Sweden)
Funding: MRC, NRF, National Geographic Project

Haplogroup A is the oldest of the Y chromosome haplogroups and is restricted almost entirely to the African continent. While it is distributed widely across Africa, it also displays strong geographical structuring, with its subclades A1 in Central and West Africa, A3b2 in East Africa, while A2 and A3b1 are found usually in southern Africa. The present study provided population data on haplogroups A2 and A3b1, defined by bi-allelic and microsatellite markers. After screening almost 5,000 male, primarily sub-Saharan African individuals, and an extensive literature survey, 155 A3b1 Y chromosomes and 64 A2 chromosomes were identified. While the highest frequencies of haplogroup A3b1 were found among the Khoe-San populations of Angola, Namibia and South Africa, it was also found at low to moderate frequencies among south-eastern Bantu-speakers and South African coloureds, with one chromosome found as far as Mozambique. This distribution points to the origin of haplogroup A3b1 in the Khoe-San population of southern Africa, before it developed a presence in other neighbouring populations through gene flow. With regard to haplogroup A2, most chromosomes within its subclades were found among the northern Khoe-San populations of Angola and Namibia, and a Khoe-San/Bantu-speaker admixed population in Botswana. However, an ancestral clade of haplogroup A2, paragroup A2-M14*, was found at low frequencies among the Pygmy and Ubangian populations of Central Africa, and in the Malagasy of Madagascar. This occurrence of an ancestral A2 has extended the range within which haplogroup A2 was previously found, as well as raising the possibility that it did not originate in the Khoe-San.

Reconstructing the origins of the Mlunga people from the Eastern Cape

Researchers: D Deveredicis, Prof H Soodyall
Collaborator: J Kalis (Walter Sisulu University)
Funding: MRC, National Geographic Society

Since Y chromosome DNA is inherited patrilineally, like surnames, we have used Y chromosome DNA markers to test the oral history of the Mlungu clan, that they are descended from ‘two brothers’ of European origin. If their claim is correct, then we would expect to find the same Y chromosome DNA haplotype in their descendants. In addition, this haplogroup would be placed on a branch of the human Y chromosome phylogenetic tree that has non-African (either European or Asian) origins. So far, a high proportion of non-African Y chromosomes has been found among men tested, but the female gene pool examined using mtDNA is very sub-Saharan African. Overall, this study brings together anthropological and genetic studies in refining the oral narrative of local populations.

TEACHING AND TRAINING

Undergraduate
In the MBCh course, teaching is provided to medical students in molecular medicine in the second year and in medical genetics in the graduate entry medical programme 1, 2 and 3. Human and medical genetics lectures are also given to undergraduates in physiotherapy, speech therapy, pharmacy and occupational therapy.

Postgraduate
Postgraduate teaching is given to registrars in psychiatry, paediatrics, pathology, internal medicine, family medicine and MSc students in occupational therapy, medical ethics and neuro-developmental paediatrics and midwives and nurses. The division teaches the BHSc (Hons) and MSc (Med) in Genetic Counselling. MSc and PhD degrees by research continue to be undertaken. The division remains the largest national training unit for medical genetic specialists and genetic counsellors. In 2007 medical genetics became a primary specialty. Primary specialty training in medical genetics was initiated in January 2009, and the first three registrars are into the fourth year of their training and scheduled to write Part 2 exams in August 2012 or March 2013.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 7 (4 BSc [Hons], 2 MSc [Med] Genetic counselling, 1 PhD)
Postgraduate candidates enrolled: 40 (8 BSc [Hons], 14 MSc [Med], 6 MSc [Med] Genetic Counselling, 3 MMed, 8 PhD, 1 postdoctoral fellow)
RESEARCH OUTPUT

Publications


Dandara C, Lombard Z, du Plooy I, McClellan T, Norris A, Ramsay M. Genetic variants in CYP (-1A2, -2C9, -2C19, -3A4 and -3A5) VKORC1 and ABCB1 genes in a black South African population: a window into diversity. Pharmacogenomics 2011; 12(12): 1663-1670


Kinsley N. We Are ‘Woman’, after All. Journal of Genetic Counseling 2012; 21(2): 203-204


Those of Other Modern Humans Is Supported by an ABC-Based Analysis of Autosomal Resequencing Data. *Molecular Biology and Evolution* 2011; [Epub ahead of print]


**DEPARTMENT OF MOLECULAR MEDICINE AND HAEMATOLOGY**

**Head:** Prof W Stevens

**DIAGNOSTIC SERVICES**

In the period under review, work volumes increased by 16%, partly due to centralisation of the HIV viral load/ HIV PCR early infant diagnosis (EID) testing in the NHLS’ Central Region. The average turnaround time for all test methods improved significantly to 97% of set targets. The overall performance on EQA for all test methods in the department improved from 84% to 91%. All diagnostic service laboratories within the department retained their SANAS accreditation status for the 11th consecutive year in 2011.

Renovations and expansion were undertaken for the viral load testing facility and the genotyping unit, and the flow cytometry divisions were amalgamated into a new facility.

**TEACHING AND TRAINING**

The department teaches and trains in the fields of medical technology, haematological pathology, clinical haematology and molecular medicine. Students taught and trained in the department included those doing graduate entry medical programme I and II, trainee medical technicians and technologists, intern science students, trainee medical scientists, haematopathology, clinical pathology and clinical haematology registrars. The biomedical engineering course shared with the Department of Information and Electrical Engineering is yielding fruit for both the Wits and the NHLS. This year, two of the graduates did projects that assisted the National Priority Programme activities in many ways including geographic information system mapping services, better matching of patients for the warehouse and the development of a number of software programmes for the GeneXpert programme.

The department coordinates the molecular medicine course for 280 MBBCh and BHSc students. This course aims to prepare students for the new paradigms of personalised and molecular medicine which are becoming increasingly vital in modern medical practice. A shorter molecular medicine course is delivered to the Biomedical Engineering 2 students.

**Conference presentations**

**International:** 16

**National:** 5

**Local:** 1
PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 22 (4 MMed, 3 PhD, 8 MSc, 7 BSc Hons)
Postgraduate candidates enrolled: 38 (2 MMed, 17 PhD, 19 MSc)

FUNCTIONAL UNIT ACTIVITIES
The department has a diverse range of activities that is separated and managed in functional units that are described alphabetically, as follows.

ANTIVIRAL GENE THERAPY RESEARCH UNIT

RESEARCH

Previous research has been aimed at development of effective antiviral sequences, and highly effective RNA interference activators that inhibit HBV, HIV-1 and Rift Valley fever virus have been identified and thoroughly characterised. Although the sequences have therapeutic potential, a major challenge to realising the potential of nucleic acid-based treatment for viral infections remains the difficulty of delivering DNA and RNA molecules to target cells. To address this hurdle, the unit’s work has shifted to nucleic acid vectorology. Much of the research has been channelled into the topic of using viral, virus-like and non-viral vectors to deliver therapeutic sequences. New technologies are being developed and the unit is now well positioned to take advantage of a broad technological base.

Nurturing of strategic collaborations is important to this research and a new partnership has been established with Prof Injae Shin of Yonsei University in South Korea. This relationship will be particularly important for the work on the use of modified recombinant capsid particles as vectors. Highlights of completed and ongoing work areas follows:

African Network for Drugs and Diagnostics Innovation (ANDI) Centre of Excellence
During 2011, the unit was recognised as one of the 32 continent-wide centres of excellence by ANDI. The programme is being run under the aegis of the WHO and the European Commission for Africa. This initiative is aimed at advancing the creation of solutions to African health problems by research carried out by African research institutions.

Acquisition of IVIB kinetic in vivo imaging system
This major equipment purchase was made possible through a grant from the NRF National Equipment Programme and support from the university that totalled R2.7m. Access to the IVIS kinetic instrument is particularly important as it enables the convenient real-time tracking in vivo in animals that have received nucleic acid vector formulations. The equipment has highly sensitive cameras that enable detection of bioluminescence and fluorescence signals within organs of anaesthetised mice. Analysis of organ distribution and kinetics of vector decay is convenient with this instrument. It is very accurate and eliminates the need for time-consuming tissue sectioning and in vitro reporter gene assays. Moreover, fewer animals need to be sacrificed to carry out animal studies.

AUTOMATED HAEMATOLOGY LABORATORY

DIAGNOSTIC SERVICES
The laboratory forms part of a totally automated and amalgamated clinical pathology service serving CMJAH, and serves as a national reference laboratory for both the private and public sectors. The laboratory provides a 24-hour service for full blood and differential counts. In the year of review, the diagnostic test volumes for full blood count testing increased by 4%.

RESEARCH AND DEVELOPMENT

During the reporting period, the laboratory was involved in the implementation and validation of modified International Standards of Laboratory Haematology guidelines with the aim of decreasing the number of peripheral smears that require manual review, as well as to standardise the criteria for checking results. The diagnosis of haematological malignancies in the era of total laboratory automation comparing the Advia 2120 to immunophenotyping and morphology has been investigated as a project for an MMed degree.
CHRIS HANI BARAGWANATH
HAEMATOLOGY LABORATORY

DIAGNOSTIC SERVICES

A comprehensive laboratory service is provided to the hospital and surrounding clinics. Most specialised tests have been centralised to the CMJAH/Wits Medical School complex as a cost-saving measure. CD4 counts and selected special coagulation tests are now done on site. Registrars rotate through the department on a monthly basis. The medical staff in the laboratory consult with clinicians in the hospital, where an excellent rapport exists between the two groups.

Construction of the automation laboratory has been completed. Construction of the day ward and INR (anticoagulation) clinic is also complete and is well-positioned to serve all adult clinics and wards. Two nursing sisters man the clinic and are under supervision of the haematology medical staff. The day ward is designed for procedures such as FNA, bleeding time, glucose tolerance tests etc. Focus group meetings between the laboratory, hospital and clinical staff are held bimonthly; this forum provides a two-way communication channel to discuss laboratory service issues.

Clinical haematology training forms a large part of the registrar’s activities. This includes participation in haematology outpatient clinics (oncology and non-oncology), grand ward rounds and clinical case presentations.

RESEARCH AND DEVELOPMENT

Projects include examining the new morphology instrument to assess the extent of reliance for morphological assessments and the potential for tele-pathology; the role of α thalassaemia in the South African population (for an MSc thesis); an investigation into pure red cell aplasia in the HIV setting; and the development of guidelines for management of sickle cell anaemia.

EVOLUTIONARY MEDICINE
LABORATORY

This unit’s main research focus is the emergence of biocomplexity in disease.

FLOW CYTOMETRY AND HIV/
IMMUNOLOGY UNIT

DIAGNOSTIC SERVICES

Several flow cytometric-based assays including immunophenotyping of leukaemias/lymphomas and CD34 enumeration as well as flow cytometric crossmatching, Luminex-based HLA antibody testing and virtual crossmatching for deceased donors are provided.

The CD4 reference laboratory processes in excess of 20,000 samples per month, including the workload referred as a consequence of the consolidation of the Helen Joseph laboratory into CMJAH during February 2012. Further activities include non-HIV immune deficiency testing and clinical trial study support for groups such as the Wits Health Consortium partners including Contract Laboratory Services, as well as various local and international collaborators including the US-based Adult AIDS Clinical Trials Group, Pediatric AIDS Clinical Trials Group, and International AIDS Vaccine Initiative. Ongoing studies within the unit reflect the various research interests and include T-Reg cell insights, TB-related assay development and CD38 monitoring in the context of treatment for HIV.

HAEMOPHILIA COMPREHENSIVE
CARE CENTRE

The centre provides clinical service, research and teaching in the context of haemostasis and thrombosis with emphasis on bleeding disorders. The patient database continued to grow with currently over 750 patients in the local register. The centre collaborates with a number of clinical disciplines including surgery, internal medicine, orthopaedic surgery and dentistry.

CLINICAL SERVICE

The clinical and diagnostic service is provided primarily by clinical haematologists and haematology trainees with input from CMJAH paediatricians, internists, orthopaedic surgeons, dentists, physiotherapists, occupational therapists and specialist haemophilia nurses. During the year under review, 1,430 clinic visits were undertaken, and 55 hospital admissions and 25 new patients were managed. Procedures supported include 12 yttrium synoviorthesis procedures, knee and hip arthroplasties as well as general surgical interventions.
TEACHING AND TRAINING

The centre gives a clinical and practical dimension to the laboratory-based haemostasis and thrombosis training of the haematology trainees. All haematopathology, clinical haematology and clinical pathology registrars rotate through and are trained at the centre. During the year, a basic haemophilia course was presented to 40 nurses.

RESEARCH

Several clinical, basic and translational research studies were conducted during the year, including phase I, II, III and IV studies of current and new generation therapeutic agents in the management of haemostatic defects and specific bleeding disorders.

HIV PATHOGENESIS RESEARCH LABORATORY

RESEARCH

Basic research is conducted into the molecular biology of HIV, work that is directly applicable to the design and development of novel antiviral therapies and vaccine immunogens. In 2011, work continued on several major projects funded by the Department of Science and Technology (South African HIV/AIDS Research Platform (SHARP), Indo-South Africa Research Initiative), NRF, Carnegie Foundation, Poliomyelitis Research Foundation and the African-European HIV Vaccine Development Network, which collectively contributed approximately R3 million to research during this year. Collaborative research partnerships were established or maintained with both local and international institutions such as the International AIDS Vaccine Initiative (USA), Karolinska Institute (Sweden), and MINTEK (RSA). Other highlights during the year were the receipt of an NRF National Equipment Programme award (approximately R3.5 million) for the procurement of a label-free, high-throughput protein interaction analysis instrument (Bio-Rad Proteon XRS). This state-of-the-art system, the first of its kind in South Africa, uses Surface Plasmon Resonance (SPR) technology to characterise the kinetics of 36 independent receptor-ligand interactions simultaneously, a critical resource that will have a major impact on efforts to develop new antiviral drug and vaccine candidates.

Key research projects included:
- An investigation into the ability of HIV-1 founder virus envelope-CD4 complex immunogens to elicit broadly neutralising anti-HIV antibodies in immunised rabbits (SHARP);
- The genotypic, phenotypic and biochemical characterisation of envelope glycoproteins cloned from a panel of circulating Indian HIV-1 isolates (Indo-SA Initiative);
- The development of CD4-mimetic mini-proteins as novel therapies against HIV (PRF);
- The development of novel antiviral drugs targeted to HIV-1 integrase (MINTEK);
- Biochemical characterisation of redox exchange reactions which occur in CD4 and viral envelope proteins during HIV entry into host cells, their functional importance and the host factors that participate in mediating these exchanges (NRF, Carnegie); and
- The provision of technical support to HIV vaccine clinical trial sites established by the AFREVacc network in Tanzania and Mozambique through the development of assays to quantify anti-HIV-1 specific IgG and IgA responses in individuals vaccinated with novel candidate HIV-1 vaccines (AFREVacc).

HONOURS

Dr A Capovilla was the recipient of the Faculty of Health Sciences Research Prize - this is the most prestigious prize offered by the faculty, and is awarded in recognition of excellence in research.

HIV AND HAEMATOLOGY MOLECULAR DIAGNOSTICS UNIT

DIAGNOSTIC SERVICES

The laboratory provides HIV diagnostic and monitoring services through early infant diagnosis PCR and viral load quantification (HIV PCR laboratory), HIV genotyping for drug resistance diagnosis and molecular support for a set of haematological disorders (haematology PCR laboratory).

HIV PCR laboratory

This laboratory is one of the busiest NHLS PCR laboratories in the country - currently processing approximately 30,000 viral load samples and 2,000 early infant diagnosis HIV qualitative samples per month, reaching close to
310,000 and 23,500 samples per annum, respectively. The laboratory supports numerous clinical trials from several networks including HIV Prevention Trials Network, AIDS Clinical Trials Group (ACTG), International AIDS Vaccine Initiative (IAVI) through Contract Laboratory Services.

**Genotyping laboratory**
The laboratory currently focuses on HIV ARV drug resistance testing for the public sector which provides clinicians with valuable knowledge on HIV-1 drug resistance and guides for patient management. The laboratory processed 1,225 samples during the last financial year, which is a three-fold increase. A similar service is provided for various international clinical trial research network groups such as ACTG, PharmAccess African Studies to Evaluate Resistance (PASER) and IAVI. Staff were involved in writing the guidelines for ARV drug resistance testing with the Southern African HIV/AIDS Clinicians Society.

In September 2011, a new state-of-the-art facility at CMJAH was commissioned; the facility has several dedicated areas to ensure quality sequences are obtained. These include a sample receiving, nucleic acid isolation, pre-PCR and post-PCR area.

**RESEARCH AND DEVELOPMENT**

**In-house assay development and evaluation**
In order to meet the needs of the public sector, a new in-house assay that will give more robust results was validated. Additionally, a more cost-effective one-step in-house assay is being validated as well. At the same time, these assays will also be validated on dried blood spots, which is a more convenient sample matrix for remote clinics.

**Affordable viral load and HIV drug resistance test**
A novel affordable two-step approach, using either plasma or dried blood spots as the sample input, was assessed to identify virological failure and subsequently detect key HIV drug resistance mutations in order to improve quality of HIV care in resource-poor settings. This two-step approach included a RT-PCR-based viral load test and a short real-time sequencing step and was evaluated in the genotyping laboratory. A good concordance with reference assays was noted, with these assays being simple to perform and are more affordable, viable options to commercial alternatives. Both tests may be used and adapted in either regional or reference laboratories, and their compatibility with dried blood spot sampling extends the access of HIV-1 virological monitoring to more remote settings.

**Ultra deep sequencing**
An NIH-PEPFAR-CIPRA-funded grant project to establish and use an HIV-1 subtype C specific ultra deep sequencing genotyping assay to evaluate the impact of ARV drug resistant minority variants on treatment outcome, and the feasibility of using pooled patient samples to establish more affordable resistance testing platforms was initiated in May, 2011. An HIV-1 subtype C specific protocol, encompassing three amplicons (1PR and 2RT) was set up in consultation with Roche and Dr D Dudley from Wisconsin University, USA. Samples from patients enrolled on the CIPRA trial were initially run on the 454 FLX. To date, ultra deep sequencing data and ARV drug resistance profiles have been obtained for RT from 627 samples with coverage down to 1% from 563 patients, and for PR, down to 1% coverage for 468 sequences from 416 patients. The same patient samples were pooled in batches of 48, with unique identifiers, and run on the Roche 454 Junior. The data are currently being analysed to evaluate the feasibility of this methodology as a more affordable resistance testing platform.

**Bioinformatics collaborations**
The laboratory is part of the South African Treatment Resistance Network, which has been set up to perform surveillance testing on HIV-1 drug resistance in the public sector. This laboratory will perform resistance testing for the network as well as be involved in data analysis of the resistance data. The laboratory is collaborating with Prof S Travers from the South African National Bioinformatics Institute, University of Western Cape for the development of a fast, accurate bioinformatics programme for the analysis of 454 ultra deep sequencing data for research purposes. The laboratory is collaborating with TherapyEdge and using their Deepcheck programme for data analysis for diagnostic purposes.

**Global Fund**
In collaboration with Right to Care, a grant was obtained in 2011 from the Global Fund. The HIV genotyping laboratory is involved in two components of this project, namely conducting a cross-sectional HIV drug surveillance study and expanding the HIV drug resistance testing capacity in South Africa.
HAEMOSTASIS AND THROMBOSIS LABORATORY

DIAGNOSTIC SERVICES

This laboratory conducts on average 5,500 tests per month.

Anticoagulation clinic
Services include monitoring of warfarin therapy of 100+ patients per day (serving in excess of 5,000 patients); assessing blood results and patients, adjusting warfarin doses and dispensing medication, and training clinic nursing sisters on a six monthly basis.

RESEARCH

Projects include:

The relationship between HIV infections and deep vein thrombosis
Researcher: Dr S Louw (MMed)

Haemostatic management system: guiding blood product replacement and anticoagulation therapy in cardiac bypass surgery
Researcher: T. Ramatsui (MSc)

Need to dose adjust prophylactic low molecular weight heparin therapy in trauma patients
Researcher: J Vlok (MMed)

Coagulation profile in HIV-infected children undergoing dental surgery
Researcher: A Zeijlstra (MMed)

MORPHOLOGY AND SPECIAL HAEMATOLOGY UNIT

DIAGNOSTIC SERVICES

A diagnostic service is provided for assessment of peripheral smears, bone marrow samples, haemoglobin electrophoresis and other tests. Samples are received from both local and international institutes.

RESEARCH PROJECT

Hemocue haemoglobin point-of-care testing
Ten Hemocue point-of-care haemoglobinometers were installed in various wards at CMJAH. More than 300 healthcare workers from this hospital have been trained to operate and maintain these instruments. During the reporting period, 7,000 such tests were performed. Stringent quality assurance and stock control managed by staff in this unit. The next step in this project is the development of an interface to the laboratory information system (TrakCare) for real-time transfer of test results.

HELEN JOSEPH HOSPITAL LABORATORY

DIAGNOSTIC SERVICES

In addition to routine haematological tests, the laboratory is a referral centre providing consultative services to the outlying, smaller NHLS laboratories by reporting on referred peripheral smears and bone marrow samples. The pathologists provide a consultative service to hospital clinicians, report on bone marrow samples (~80-100/month) and oversee a large anticoagulation clinic (~500-600 patients) located on the laboratory premises. About 16,000 tests are performed per month.
domains of four invasion proteins in various combinations have been created by overlap extension PCR. These will be cloned into pARL-GFP and pARL-mCherry vectors to create transgenic parasites. The pARL-GFP and pARL-mCherry vectors have been engineered to contain an ama-1 promoter to ensure that invasion proteins cloned into these vectors will be expressed at the correct phase of the parasite intraerythrocytic cycle. Cloning is currently underway.

Gene regulation
PfMyb2 is a DNA binding protein previously identified in the unit. Attempts to knock out the gene by double homologous recombination have been unsuccessful, implying that the gene is essential, but further studies to verify this are ongoing.

Kinases
The P. falciparum glycerol kinase gene was successfully knocked out and these parasites grow at a much slower rate than wild type parasites. Blot overlay assays have revealed an interaction between recombinant PfPK8, a parasite protein kinase, and two red cell membrane proteins.

Programmed cell death
Functional domains of P. falciparum homologues of p53, MDM2 and IAP have been cloned and expressed as recombinant GST-tagged proteins. Investigations into their function are underway. Assays to evaluate biomarkers of programmed cell death in P. falciparum have been established and the response of parasites to physiologically relevant stress factors, including febrile temperatures and high population density, has been analysed in in vitro cultures.

SOMATIC CELL GENETICS UNIT
Highly specialised tests to detect, characterise and monitor genetic alterations in the context of disease are performed. The unit also develops, evaluates and implements molecular diagnostic techniques with an emphasis on carcinogenesis.

DIAGNOSTIC SERVICES
Conventional cytogenetic analyses, FISH, reverse quantitative real-time PCR, reverse transcription PCR and sequencing are performed. Diagnostic volumes grew by 81% over the past eight years. This has not been followed by an equivalent increase in staff, mostly due to the extreme scarcity of skills in this highly specialised field. New tests implemented in the past year include sequencing of c-KIT mutations and platelet-derived growth factor receptor alpha in gastrointestinal stromal tumours; FISH probes for 18q11 aberrations seen in synovial carcinoma; FISH probe for the COL1A1/PDGFB gene rearrangement seen in translocation t(17,22)-positive dermatofibrosarcoma protubersans; and a panel of FISH probes including probes spanning CEP3, CEP7, CEP17 and 9p21 for bladder cancer diagnosis.

RESEARCH AND DEVELOPMENT
New molecular methods of cancer diagnostics are continuously explored and implemented. The unit is also studying the molecular pathogenesis of oesophageal squamous carcinoma and HIV-associated lymphomas. DNA copy number analysis is done to identify genes affected by DNA amplification or deletion in the respective malignancy. In the next step, expression levels of key genes identified by comparative genomic hybridisation arrays and functional studies are performed. The unit has an ongoing collaboration with Ithemba laboratory in studying DNA damage response to ionising radiations. The unit also collaborates with Pretoria University in the study of B cell lymphoma. International collaboration is currently with the Irvin Cancer Institute at Columbia University Funds were received from the following sources: NHLS Research Trust, Griffin Research Trust, CANSA and the University of Columbia (New York).

RESEARCH OUTPUT
Arbuthnot P. Micro RNAs-like antivirals. Biochimica Biophysica Acta 2011; 1809


Durand P, Michod R. What is life and why, how and when did it begin? *Journal of Cosmology and Astroparticle Physics* 2011; **16**: 7050-7055


Papasavvas E, Azzoni L, et al. Increased microbial translocation in <180 days old perinatally human immunodeficiency virus-positive infants as compared with human immunodeficiency virus-exposed uninfected infants of similar age. Pediatric Infectious Disease Journal 2011; 30(10): 877-882


Price M, Wallis CL, et al. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in east and southern Africa. AIDS Research and Human Retroviruses 2011; 1: 5-11


Willem P. Frequent Chromosome 1q Aberrations in HIV Associated B-Cell Lymphoma Unclassifiable with Intermediate Features Between Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma. Blood 2011; 118(21): P3572


Conference presentations
International: 46
National: 39
Four pathology departments are contained within the Health Sciences Faculty, namely Anatomical Pathology, Chemical Pathology, Haematology and Medical Microbiology. All four departments adhere to the NHLS’ mandates of providing laboratory services, performing research and undertaking teaching and training of students in the Health Sciences Faculty.

DEPARTMENT OF CHEMICAL PATHOLOGY

Head: Prof E Blanco-Blanco

RESEARCH PROJECTS

- Hypertension, proteinuria and obesity in high school students in Mthatha, Eastern Cape;
  - Researcher: Z Gqweta (for MSc);
- Students’ perceptions of the role and utility of the formative assessment feedback on problem-based learning tutorial;
  - Researcher: Prof E Blanco-Blanco (for MPhil);
- MBChB students’ perceptions on feedback and integration on PBL tutorial;
- Hypoadrenalism associated with HIV/AIDS.

TEACHING AND TRAINING

The department is involved in undergraduate teaching (MBChB programme, postgraduate diploma in Chem Path, MSc Chem Path and BTech) as well as in discipline-based teaching/consultancy in the laboratory at Nelson Mandela Tertiary Hospital.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduating: 1 MSc

RESEARCH OUTPUT

Publications

Garcia-Jardon M, Del Rio Romero A, Blanco Blanco E. Community Based Education and Service at the Faculty of Health Sciences of Walter Sisulu University. JIAMSE 2011; 21(15):104-106


Larrea Fabra ME, Garcia Jardón ME, Blanco Blanco, E. Ethical values in medical education. General considerations and comparison of two different medical schools in different countries. Revista Habanera de Ciencias Médicas 2011; 10(2): 246-253


Conference presentations

National: 6
DEPARTMENT OF HAEMATOLOGY

Head: Prof BA Ogunsanwo

DIAGNOSTIC SERVICES

The department offers comprehensive laboratory services to the Nelson Mandela Academic Hospital, Mthatha General Hospital, Bedford Orthopaedic Hospital, as well as to various secondary hospitals and clinics in the region. Expert opinion and care is offered for patients with haemophilia at the Haemophilia Clinic of the Nelson Mandela Academic Hospital. Telephonic consultations on haematology matters with medical officers throughout the catchment area of the region is routine.

RESEARCH PROJECTS

Ongoing research includes:
- Prevalence of deep vein thrombosis in patients with HIV/AIDS;
- Prolonged remission in chronic myeloid leukaemia patients treated with a tyrosine kinase inhibitor;
- Leukaemia in evolution: cytogenetic evaluation as a tool for early diagnosis;
- Retroviral induced aplastic anaemia and other cytopenias in Mthatha;
- Factor VIII inhibitors in patients with haemophilia A in the Transkei region; and
- Immune thrombocytopenic purpura in patients with AIDS: prevalence and response to standard therapy.

TEACHING AND TRAINING

Undergraduate
Members of the division are engaged in the problem-based learning and community-based education and service curriculum for third year medical students. This entails the identification of core material; student facilitation; lectures; and assessment of student performance. A total students 116 enrolled in 2011 of which 103 (88.8%) passed.

CONFERENCE PRESENTATIONS

National: 2

DEPARTMENT OF MEDICAL MICROBIOLOGY

Acting head: Prof SD Vasaikar

DIAGNOSTIC SERVICES

The department provides comprehensive laboratory services to the Nelson Mandela Academic Hospital, Mthatha General Hospital, Bedford Orthopaedic Hospital, as well as to various secondary hospitals and clinics in the region. Services in bacteriology include a TB laboratory utilising the GeneXpert technology, mycology, parasitology and serology. The epidemiological reports for antibiotic sensitivity patterns in Nelson Mandela Academic Hospital are presented to three clinical departments.

RESEARCH PROJECTS

Characterisation, antibiograms and activity of medicinal plants against *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates from clinical samples of patients in the Eastern Cape
Researchers: I Morobe (PhD student), CL Obi, SD Vasaikar, A Oyedeji, JN Eloff, T Hattori

Phenotypic and molecular characterisation and activity of medicinal plants against local isolates of *S. aureus* and *S. epidermidis* in the Eastern Cape
Researchers: NS Mthethwa (PhD student), CL Obi, SD Vasaikar, A Oyedeji, JN Eloff, T Hattori

Genes encoding antibiotic resistance, pathogenicity and phylogenetic profiles of local isolates of *Klebsiella* species
Researchers: S Vasaikar, CL Obi

Immune response to specific *Mycobacterium tuberculosis* antigens among parasite-infected school children in Mthatha: role of vitamin D and deworming
Researchers: N Nxasana (MSc student), SD Vasaikar, K Baba
Isolation and characterisation of enteric bacteria from water and clinical specimens in Mthatha and surrounding areas  
**Researcher:** K Bidla (BSc Hons student)

Isolation and characterisation of *Pseudomonas aeruginosa* and *Shigella* species from clinical specimens from various clinics in Mthatha  
**Researcher:** C Mabotja (BSc Hons student)

The aetiological characterisation of pathogens of sexually transmitted infections that cause MUS, VDS, and GUD  
**Researcher:** L Faye (BSc Hons student)

Enhancement of surveillance for invasive respiratory, meningeal and diarrhoeal diseases in South Africa, GERMS-SA  
This is collaborative research with the NICD.

National sexually transmitted infections surveillance programme in Mthatha  
This is collaborative research with STIRC-NICD.

### RESEARCH OUTPUT

**Publications**

Bisi-Johnson MA, Obi CL, Vasaikar SD, Baba KA, Hattori T. Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. *Gut Pathog* 2011; 3: 9


**Conference presentations**

**National:** 5

### TEACHING AND TRAINING

**Undergraduate**

Members of the division are engaged in the problem-based learning and community-based education and service curriculum for the MBCChB degree. This entails the identification of core material, student facilitation, lectures, and assessment of student performance. The total number of students taught were 116 of which 103 (88.8%) passed their exams.

Nursing science was taught to 44 BCUR II students; 41 (93.1%) passed their exams.

**Postgraduate**

Courses offered include PhD (Health Sciences), MSc (Medical Microbiology), BSc Honors (Med Micro).

### PROFESSIONAL DEVELOPMENT

**Postgraduate students graduated:** 1 PhD

**Postgraduate candidates enrolled:**

8 (4 PhD, 1 MSc, 3 BSc (Hons))
### GLOSSARY

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTG</td>
<td>AIDS clinical trials group</td>
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<tr>
<td>ARMS-PCR</td>
<td>amplification refractory mutation system PCR</td>
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<td>ART</td>
<td>antiretroviral therapy</td>
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<tr>
<td>ARV</td>
<td>antiretroviral</td>
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<td>CANSA</td>
<td>Cancer Association of South Africa</td>
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<td>CAPRISA</td>
<td>Centre for the AIDS Programme of Research in South Africa</td>
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<tr>
<td>CCHF</td>
<td>Crimean-Congo haemorrhagic fever</td>
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<td>CCMT</td>
<td>Comprehensive Care Management and Treatment</td>
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<tr>
<td>CDW</td>
<td>Corporate Data Warehouse</td>
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<tr>
<td>CMJAH</td>
<td>Charlotte Maxeke Johannesburg Academic Hospital</td>
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<td>CMV</td>
<td>cytomegalovirus</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CPD</td>
<td>continuing professional development</td>
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<td>CPUT</td>
<td>Cape Peninsula University of Technology</td>
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<tr>
<td>CRC</td>
<td>colorectal cancer</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>CSIR</td>
<td>Council for Scientific and Industrial Research</td>
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<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
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<tr>
<td>DST</td>
<td>drug susceptibility testing</td>
</tr>
<tr>
<td>EQA</td>
<td>external quality assurance/assessment</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>DGGE</td>
<td>denaturing gradient gel electrophoresis</td>
</tr>
<tr>
<td>DGM</td>
<td>Dr George Mukhari Hospital</td>
</tr>
<tr>
<td>DST</td>
<td>Department of Science and Technology</td>
</tr>
<tr>
<td>EID</td>
<td>early infant diagnosis</td>
</tr>
<tr>
<td>ESBL</td>
<td>extended-spectrum beta-lactamase</td>
</tr>
<tr>
<td>FA</td>
<td>Fanconi anaemia</td>
</tr>
<tr>
<td>FBC</td>
<td>full blood count</td>
</tr>
<tr>
<td>FISH</td>
<td>fluorescence in situ hybridisation</td>
</tr>
<tr>
<td>FNA</td>
<td>fine needle aspiration</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography mass spectrometry</td>
</tr>
<tr>
<td>GEMP</td>
<td>graduate entry medical programme</td>
</tr>
<tr>
<td>GERMS-SA</td>
<td>Group for Enteric, Respiratory diseases and Meningitis of South Africa</td>
</tr>
<tr>
<td>GSH</td>
<td>Groote Schuur Hospital</td>
</tr>
<tr>
<td>HA</td>
<td>haemophilia A</td>
</tr>
<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HBV</td>
<td>hepatitis B virus</td>
</tr>
<tr>
<td>HBC</td>
<td>hepatitis C virus</td>
</tr>
<tr>
<td>HEU</td>
<td>HIV-exposed uninfected</td>
</tr>
<tr>
<td>HHV 8</td>
<td>human herpesvirus 8</td>
</tr>
<tr>
<td>HLA</td>
<td>human leucocyte antigen</td>
</tr>
<tr>
<td>hMPV</td>
<td>human metapneumovirus</td>
</tr>
<tr>
<td>HPV</td>
<td>human papillomavirus</td>
</tr>
<tr>
<td>HVTN</td>
<td>HIV Vaccine Trials Network</td>
</tr>
<tr>
<td>IALCH</td>
<td>Inkosi Albert Luthuli Central Hospital</td>
</tr>
<tr>
<td>ICU</td>
<td>intensive care unit</td>
</tr>
<tr>
<td>IMD</td>
<td>inherited metabolic disease</td>
</tr>
<tr>
<td>IPC</td>
<td>infection prevention and control</td>
</tr>
<tr>
<td>IRMA</td>
<td>immunoradiometric assay</td>
</tr>
</tbody>
</table>
KEH  King Edward VIII Hospital
KIDCRU  Children's Infectious Diseases Clinical Research Unit
LTI  Laboratory for Tissue Immunology
MDR-TB  multidrug-resistant tuberculosis
MIC  minimum inhibitory concentration
MGIT  mycobacterium growth indicator tube
MLPA  multiplex ligation-dependent probe amplification
MRC  Medical Research Council
MRSA  methicillin-resistant *Staphylococcus aureus*
MSSA  methicillin-susceptible *Staphylococcus aureus*
NAAT  nucleic acid amplification test
NIAID  National Institute of Allergy and Infectious Disease
NICD  National Institute for Communicable Diseases
NIH  National Institutes of Health
NRF  National Research Foundation
PBMC  peripheral blood mononuclear cell
PCR  polymerase chain reaction
PFGE  pulsed-field gel electrophoresis
PRF  Poliomyelitis Research Foundation
QF-PCR  quantitative fluorescent polymerase chain reaction
RA  rheumatoid arthritis
RCCH  Red Cross Children's (Memorial) Hospital
RFLP  restriction fragment length polymorphism
RIA  radioimmunoassay
RSV  respiratory syncytial virus
RT-PCR  real-time polymerase chain reaction
SAAVI  South African AIDS Vaccine Initiative
SABMR  South African Bone Marrow Registry
SADC  South African Development Community
SANAS  South African National Accreditation System
SARI  severe acute respiratory infection
SCC  staphylococcal cassette chromosome
SLE  systemic lupus erythematosus
SME  sub-acute measles encephalitis
SNP  single nucleotide polymorphism
STI  sexually transmitted infection
TB  tuberculosis
TMS  tissue microarray analysis
T-RFLP  terminal restriction fragment length polymorphisms
UCT  University of Cape Town
UFS  University of the Free State
UKZN  University of KwaZulu-Natal
US  University of Stellenbosch
WHO  World Health Organization
Wits  University of the Witwatersrand
Notes