The NICD is established from 2 predecessor institutions – the National Institute for Virology (NIV) for the virology component, and the public health microbiology laboratories of the South African Institute for Medical Research (SAIMR) for the microbiology component.

The Poliomyelitis Research Foundation (PRF) Training Centre officially opens in November 2006. The centre consists of a modern 230-seat auditorium, seminar rooms, training laboratories for virology, microbiology and molecular biology and supporting offices. The lecture theatre was named “The James H S Gear Auditorium” in honour of one of the great pioneers of infectious diseases in South Africa and the Institute’s first Director [1953-1976].

The Center for Disease Control and Prevention (CDC) approves an important training development to site a Field Epidemiology and Laboratory Training Programme (FELTP) at the NICD. The FELTP Programmes, established in 33 countries throughout the world, are modelled on the highly successful EIS (Epidemiology Intelligence Service) field epidemiology training programme of the CDC.

The NICD formally becomes a member of the International Association of National Public Health Institutes [IANPHI] in May 2007. The Association, launched in January 2006, consists of some 50 national public health institutes throughout the world. It aims to establish a collaborative network to facilitate scientific exchange and to assist under-resourced institutes to achieve their optimal potential.

The African Centre for Integrated Laboratory Training (ACILT) was established in 2008 on the Johannesburg campus of the NICD and the NHLS. This new centre aims to build a new generation of laboratory experts, particularly in the fields of HIV, TB and malaria throughout Africa.

The Outbreak Response Unit joins the National Health Operations Committee for the 2010 FIFA World Cup in South Africa.
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The National Institute for Communicable Diseases (NICD) is internationally recognised as an important public health resource in South Africa, and possibly the only one of its kind in Africa. The NICD in 2011/12 transitioned from the leadership of Professor Barry Schoub, who led the institute since its inception in 2002 until the appointment of Professor Madhi in April 2011.

Despite the financial challenges experienced by the NHLS during 2011, which created turbulence in the operations of the institute, there have nevertheless been some important developments and accomplishments. Foremost among these has been a consolidation in the spectrum of activities within the NICD, to enhance its primary objective of servicing public health needs of South Africans. The past year has therefore seen a re-structuring of the character of the institute. The motivation was to develop a more integrated approach in the manner in which the institute takes on its primary task of contributing and responding to priority communicable disease challenges in South Africa. In particular, the re-structuring has involved the integration of 23 previously separate units into seven centres which have a thematic focus, as illustrated below.

Included among these are the Centre for Tuberculosis and the Centre for HIV and Sexually Transmitted Infections. These two centres are expected to grow over the next few years and contribute substantially to the dominant communicable disease burden of HIV-TB in South Africa.

Despite the challenges of re-structuring, the NICD has been fortunate not to have lost any senior staff members during this transition period. Many of the past unit heads have admirably taken on collective leadership responsibility in various centres. In addition to pooling the expertise of virologist and microbiologist within centres, there has also been a focus on strengthening active epidemiology activities within each centre. As part of the restructuring, it is expected that each centre will develop a portfolio of activity which spans the breadth of activity from basic science research, laboratory-based surveillance, and molecular studies to that of field epidemiology.
As such, the previous Epidemiology Division has been integrated into centres, with the outlook of expanding the active epidemiology component within each centre to enable the NICD to be more timeous in detecting and responding to public health communicable disease threats and challenges. The role of the NICD in being a public resource and supporting the Department of Health is further supplemented with the establishment of the Division for Public Health Surveillance and Response. This division, has remained at the forefront of investigating communicable disease outbreak threats in South Africa and providing technical support to the Department of Health in this regard.

In the midst of this restructuring, there have been a number of other notable accomplishments within the NICD over the past year. Included is the official commissioning and accreditation of the level 4 biosafety laboratory by the Centre for Emerging and Zoonotic Diseases. This facility is the only one of its kind in Africa and places the NICD in a strategic position in dealing with established and emerging highly communicable infectious disease threats in South Africa, as well as being a resource for the rest of Africa. The opening of the facility was also marked with the centre hosting the first “One Health for All in Africa” conference at the NICD during 2011. This meeting of leading scientists in Africa focused on scrutinising the inter-dependency of humans and animals, and subsequent threats faced by each in the emergence of communicable diseases threats.

Another key development during 2011 was the establishment - in partnership with the national Department of Health and in collaboration with the Centers for Diseases Control and Prevention (CDC) (USA) - of the Southern African Regional Global Disease Detection Programme. This initiative in establishing a worldwide network of intelligence on communicable diseases is presently funded by the CDC, with South Africa being the eighth global disease detection (GDD) centre and the third in Africa to have been established. The SA-GDD programme activities have been successfully integrated within centres of the NICD and in the communicable diseases cluster division at Department of Health. Also, core to this initiative is the training of field epidemiologists to better equip South Africa in having at least one epidemiologist for every 200,000 of the population.

The next year (2012/13) is likely to witness a consolidation of the restructuring process at the NICD. Included among this, is the development of five-year strategic plans within each of the centres, including deliberation on how the NICD can substantially contribute toward the agenda of establishing an efficient National Health Insurance programme to help improve the life of all South Africans. Together with the talent of personnel that the NICD is privileged to have, it is a task which the institute relishes and looks forward to remaining an internationally acclaimed Institute.

"The National Institute for Communicable Diseases (NICD) is internationally recognised as an important public health resource in South Africa"
CENTRE FOR EMERGING AND ZOONOTIC DISEASES
EMERGING AND ZOONOTIC DISEASES

Zoonotic diseases are emerging or re-emerging at alarming rates throughout the world. While changes in climate, urbanisation and farming practices, coupled to increasing travel and trade, are shared drivers for infectious diseases globally, some have special relevance to Africa, including extensive human-wildlife interaction, increased land usage and current socio-economic conditions. The Centre for Emerging and Zoonotic Diseases (CEZD), which incorporates the previous Special Pathogens, Special Bacterial Pathogens and Electron Microscopy units, renders diagnostic expertise and investigatory capacity on highly dangerous bacterial and viral pathogens associated with zoonotic disease in South Africa and on the African continent. The CEZD functions as a resource for knowledge and expertise to the South African government, the SADC countries and the African continent. Its purpose is to assist in the planning of relevant policies and programmes, and to harness innovation in science and technology to support surveillance, detection and outbreak response systems. In observing this goal, the CEZD supports South Africa’s commitment to the international health regulations.

Surveillance and diagnostic services

The Special Viral Pathogens Reference Laboratory (SVPRL) provides laboratory investigation for viral haemorrhagic fevers (VHFs), arboviral disease, human rabies and rabies-related infections. Arbovirus serology and virus isolation assays have been accredited by the South African National Accreditation System (SANAS) under ISO 15189:2007 since 2000. The laboratory played a key role in the response to the 2008-2011 Rift Valley fever (RVF) outbreak in South Africa. A total of 302 cases were confirmed from a total of 2,262 suspected cases. In order to provide the diagnosis and perform research on class 3 and 4 viral pathogens, the laboratory manages high biocontainment facilities. In May 2011, the biosafety level 4 (BSL4) facility was commissioned and certified after extensive upgrading. This facility is the only positive-pressure-suit maximum biocontainment laboratory on the continent. SVPRL piloted its mobile capacity for diagnostic outbreak response during September 2011 by deployment of portable nucleic acid detection platforms at sites in the Free State and Mpumalanga provinces. The mobile laboratory includes portable biosafety equipment in the form of a vinyl glovebox under negative pressure which allows for safe processing and testing of dangerous pathogens under field conditions.

The Special Bacterial Pathogens Reference Laboratory (SBPRL) is primarily tasked with the laboratory confirmation and investigation of anthrax, plague, leptospirosis, cat scratch disease (Bartonella infection) and botulism. The laboratory operates a BSL3 facility and is accredited with the Department of Agriculture, Forestry and Fisheries and SANAS according to ISO15189:2007. In addition, SBPRL drives a regional surveillance project for plague (previously the RATZOOMAN project) and is recognised as the anthrax reference laboratory for human diagnostics in South Africa. During 2011, the diagnostic capacity for \textit{Leptospira} was improved through implementation and accreditation of a \textit{Leptospira} IgM ELISA.

The CEZD houses a transmission electron microscope which is a useful tool for diagnosis of complicated cases and for pathogen discovery.
RESEARCH

**Bartonella diagnostics and surveillance programme**

*Researchers:* AN Trataris-Rebisz, Prof JA Frean, Dr J Rossouw  
*Funding:* South African Global Disease Detection Initiative

*Bartonella* is an opportunistic pathogen transmitted from animals to human hosts. Aims of this study are to determine clinical importance of *Bartonella* infections, characterise circulating *Bartonella* species, investigate carriage of bartonellae by commensal and wild rodents, and compare molecular techniques with the objective of introducing a validated polymerase chain reaction (PCR) for routine diagnostic purposes. *Bartonella* diagnostic testing is offered to clinicians practicing at various HIV-clinics and government hospitals. Patient samples consistent with case definitions for *Bartonella* are tested. Following initial detection of *Bartonella*, by culture and PCR, the isolates are sequenced for phylogenetic analysis. Potential benefits include improved patient management of HIV-positive patients with chronic and/or acute *Bartonella* infections and increased knowledge on the prevalence and importance of *Bartonella* spp. as an emerging and zoonotic pathogen.

**Experimental inoculation of *Rousettus aegyptiacus* with Marburg virus**

*NICD researchers:* Prof JT Paweska, Dr P Jansen van Vuren, PA Leman, AA Grobbelaar, Dr M Birkhead, A Kemp  
*Collaborators:* Dr J Masumu (University of Kinshasa, Kinshasa, Democratic Republic of the Congo [DRC], National Institute for Biomedical Research, Kinshasa, DRC); Dr S Clift and Prof R Swanepoel (University of Pretoria)

Filoviruses cause fatal haemorrhagic fever in humans and non-human primates in Africa. The Egyptian fruit bat, *Rousettus aegyptiacus*, is currently regarded as a potential reservoir host for Marburg virus (MARV). Captive-bred *R. aegyptiacus* were exposed to MARV by different inoculation routes. Blood, tissues, faeces and urine from bats inoculated by a combination of nasal and oral routes were all negative for the virus and seroconversion could not be demonstrated for up to three weeks post inoculation. In bats inoculated by a combination of intraperitoneal/subcutaneous route, viraemia and the presence of MARV in different tissues were detected on days 2-9 post inoculation, and seroconversion on days 9-16 post inoculation. In bats inoculated subcutaneously, viraemia was detected on days 5 and 8 and virus was isolated from different organs. MARV could not be detected in urine, faeces or oral swabs in any of the three experimental groups. However, MARV was detected in tissues which might contribute to horizontal or vertical transmission, e.g. lung, intestines, kidney, bladder, salivary glands, and the female reproductive tract. Viraemia lasting at least five days could also facilitate MARV transmission by blood-sucking arthropods and infection of susceptible vertebrate hosts by direct contact with infected blood. All bats were clinically normal and no gross pathology was identified on post mortem examination. This work confirms the susceptibility of *R. aegyptiacus* to infection with MARV and contributes to establishing a bat-filovirus experimental model.

**Molecular characterisation of Rift Valley fever isolates, South Africa 2011**

*NICD researchers:* AA Grobbelaar, Dr J Weyer, A Kemp, PA. Leman, Prof JT Paweska  
*Collaborators:* Prof R Swanepoel (University of Pretoria)

RVF is an acute disease of domestic ruminants in Africa, Madagascar and the Arabian Peninsula and is caused by a mosquito-borne virus. Large outbreaks occur when heavy rains favour excessive breeding of the mosquito vectors. The aim was to monitor the viral genotypes circulating during outbreaks and to identify reassortant viruses. Partial sequencing of the M segment of RVF virus strains recovered from 11 of the 37 cases reported from the Eastern Cape, Free State, Northern Cape and Western Cape provinces during January-May 2011, revealed that they were infected with the genotype that prevailed during the 2010 outbreak in South Africa. The 2010 and 2011 isolates show little genetic diversity, with less than 1% difference between these isolates at the nucleic acid level, and were closely related to a 2004 Namibian isolate from the Caprivi Strip. They are phylogenetically distant to isolates from the 2008-2009 outbreaks in South Africa.
Evaluation of a recombinant Rift Valley fever virus nucleocapsid protein as a vaccine and an immunodiagnostic reagent

NICD researchers: Dr P Jansen van Vuren, Prof CT Tiemessen, Prof JT Paweska

Funding (partly): Polioymelitis Research Foundation.

RVF is capable of causing large outbreaks in domestic livestock and humans. Fears that RVF virus might spread to previously naïve regions have increased interest in development of safe diagnostic technologies and vaccines. The aim of this project was to evaluate a nucleocapsid protein generated by recombinant DNA technology as a safe antigen for producing novel immunodiagnostic techniques and vaccines. The study resulted in the development and validation of ELISAs for the detection of antibodies or antigen in different species, contributing to the array of available diagnostic tools for RVF. The nucleoprotein was evaluated as a vaccine in laboratory and host animal models, and a study was conducted to improve current knowledge on the role of anti-nucleoprotein responses in protection against viral challenge.

HONOURS

• Prof Janusz Paweska was awarded a prestigious statuette of Sapere Auso by the Rector of Wroclaw University of Environmental and Life Sciences, in Poland in October 2011. This award is conferred once a year on an outstanding alumnus whose professional achievements and position contribute greatly to the promotion of the university at home and abroad.

• Dr Petrus Jansen van Vuren was invited to join the prestigious Golden Key International Honour Society, reflecting his outstanding academic achievements.

• Anastasia Trataris-Rebisz and Dr Jacqueline Weyer were appointed as Secretariat/Treasurer and Vice-President of the South African Biorisk Association, respectively.

TEACHING AND TRAINING

Apart from extensive in-house training of staff in specialist techniques and the requirements for working in BSL3 and BSL4 biocontainment facilities, the CEZD contributes to human resource capacity development by supporting postgraduate studies in the fields of medical microbiology, medical virology and public health through collaborative projects with South African and international universities. A total of 10 PhD and 8 MSc students were supervised or co-supervised by CEZD staff during the review period. In addition, the CEZD is involved in the training of microbiology and clinical pathology registrars, intern scientists and technologists on an ongoing basis. International scientists frequent the laboratories for specialist diagnostic training, training related to working in biocontainment facilities and for collaborative research projects.

The CEZD co-ordinates a number of formal training programmes and is often requested to co-ordinate specialist diagnostic workshops. These included training environmental health officers from the City of Johannesburg on the dissection and storage of rodent organs for plague surveillance purposes. Centre staff members were involved in training courses, including the 2010-2011 rabies outbreak in Gauteng and diagnosis of RVF and rabies at the FAO/International Atomic Energy Agency (IAEA) Regional Training Course on Transboundary and Zoonotic Animal Diseases. The One Health concept training course was intended for laboratory scientists from throughout Africa involved in disease diagnosis in humans and animals. The training was held at the National Animal Disease Diagnostics and Epidemiology Center, Ministry of Agriculture, Animal Industry and Fisheries, Entebbe, Uganda. In addition, the centre co-hosted the 1st One Health Conference in Africa, an initiative of the Southern African Centre for Infectious Disease Surveillance programme which is seated in Morogoro, Tanzania. This conference was held at the Sandringham campus of the NICD/NHLS from 14 to 15 July 2011 and hosted nearly 100 South African and international delegates.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated:
2 (1 PhD, 1 MSc [Med])

Postgraduate candidates enrolled:
1 BSc (Hons)

RESEARCH OUTPUT

Publications

The CEZD published 10 papers in peer-reviewed journals and two book chapters during 2011-2012. The top publications were:


Synopsis: Phylogenetic relationships were examined for 198 Rift Valley fever virus isolates over a period of 67 years from various African countries, Madagascar and the Arabian Peninsula. Although the genetic diversity among viruses isolated is low, they sort into 15 genetic lineages. The large scale use of live, attenuated veterinary vaccine possibly contributed to the evolution of the virus.

**Synopsis:** A mobile laboratory unit (MLU) was deployed to Uige, Angola during the Marburg fever outbreak of 2005. The use of real-time PCR assays for MARV diagnostics in the field was illustrated. There was a high concordance in test results between the MLU and the reference laboratory in Luanda, operated by the US Centers for Disease Control and Prevention. The MLU was an important outbreak response asset, providing valuable support in patient management and epidemiological surveillance.


**Synopsis:** Zaire ebola virus re-emerged in the Democratic Republic of the Congo (DRC) after a quiescence of 12 years, causing two successive outbreaks in the Luebo region, Kasai Occidental province, in 2007 and 2008. In this study, full genome sequencing and phylogenetic analysis were used to study the viruses that caused the recent and previous outbreaks. This study highlights the fact that the Zaire Ebola outbreaks in the DRC in 2007 and 2008 were phylogenetically, temporally and spatially distinct from all previous outbreaks and suggests that bats became infected and allowed viral persistence and reemergence from year to year.


**Synopsis:** The nucleocapsid protein (NP) of the Rift Valley fever virus is the main immunogen but antibodies against the NP protein are not neutralising. Protection of NP immunised animals after lethal challenge has been shown and this study aimed to gain insight into the mechanism of this protection. The study found on a gene expression level that NP-immunised animals mounted an earlier innate immune response after challenge contributing to more efficient viral clearance, whereas non-immunised mice developed pro-apoptotic and pro-inflammatory responses during acute infection, contributing to the development of severe disease. The genes identified in this study as indicative of severe outcome could be targets for therapeutic interventions in future.

**Other publications:**

**Journal articles**


**Chapters in books**


Uejio CK, Kemp A, Comrie AC. Climatic controls on West Nile virus and Sindbis virus transmission and outbreaks in South Africa. *Vector Borne Zoonotic Diseases* 2011; 12: 117-125

**Conference presentations**

**International:** 26

**National:** 3
On 21 March 2012, the South African Human Rights Commission highlighted that 16 million South Africans still do not have adequate access to safe water and sanitation, compounding the problem of water-borne disease. Similarly, Article 25 of the United Nations Universal Declaration of Human Rights has recognised that “everyone has a right to a standard of living adequate for the health and well-being of himself and of his family, including food”. The United Nations Millennium Development Goal 4 of “reducing by two-thirds the under five mortality rate by 2015” emphasises that strategies to reduce the burden of disease attributable to diarrhoeal pathogens in children under 5 years need to become a major focus. In South Africa, the incidence of diarrhoeal disease doubled from 128.7 children below age 5 per 1,000 in 2004 to 268.7 per 1,000 in 2005. In addition, the 2006 South African Health Report attributed 15% of mortality in children below age 5 to gastroenteritis, second only to lower respiratory tract infections. The need to address these basic needs resulted in the formation of the Centre for Enteric Diseases (CED).

The CED was established through the amalgamation of the Enteric Diseases Reference Unit and the Viral Gastroenteritis Unit. The centre is tasked with developing strategies and providing information to combat diarrhoeal diseases in South Africa. In addition, the centre monitors trends in diarrhoeal pathogen incidence and identifies areas for the introduction of additional interventions.

The bacterial division of the CED collects data on patients presenting throughout South Africa with both invasive and non-invasive disease caused by Salmonella species (including Salmonella Typhi), Shigella species, Vibrio cholerae and diarrhoeagenic Escherichia coli. In order to make these data representative and reflective of disease burden in each province in the country, all diagnostic laboratories throughout the country are motivated to voluntarily submit limited demographic details and isolates to the CED. In exchange, the CED offers serogrouping and serotyping results free of charge (urgent results need to be requested telephonically), regular feedback (quarterly reports by province sent to every laboratory participating) and aggregated numbers are published in the NICD Bulletin.

In addition to serogrouping and serotyping, Etests are used to determine the minimum inhibitory concentration (MIC) of each isolate to antimicrobial agents, according to Clinical and Laboratory Standards Institute guidelines. The bacterial division also performs genotypic characterisation of isolates, should this be required, such as in outbreak situations. The molecular epidemiology of these bacterial pathogens is continually being elucidated, specifically that of outbreak or epidemic-prone pathogens such as S. Typhi, Shigella dysenteriae type 1 and V. cholerae. A multiplex PCR is used to elucidate the presence of toxin genes in diarrhoeagenic E. coli. The division is developing its molecular research laboratory involved with characterising the molecular basis for antimicrobial resistance in these pathogens and has plans to further characterise the mechanism of disease due to these pathogens at a molecular and cellular level.

The introduction of the rotavirus vaccine into the national expanded programme of immunisation (EPI) in August 2009 was a positive step in combating diarrhoeal disease burden in children below the age of 5 years in South Africa. The viral division of the CED has been tasked with monitoring the impact of the rotavirus vaccine, and surveillance is planned to continue into 2015. Projects investigating rotavirus vaccine safety, optimum vaccine use and improved vaccine efficacy are also being undertaken and will generate practical regional data for African countries considering introducing the rotavirus vaccine.

While rotavirus cases are being reduced by the introduction of efficacious vaccines, the remaining 70% of diarrhoeal cases need to be investigated. Stools collected through the rotavirus sentinel surveillance programme are examined via an integrated diagnostics platform within the divisions of the CED. Surveillance for enteric viruses, other than rotavirus, has previously only been conducted on an ad hoc basis and the contribution of mixed pathogen infections has never been studied.

**Heads**

**ENTERIC DISEASES**

Dr Karen Keddy  
Dr Nicola Page
in the South African population. Expansion of the current diarrhoeal surveillance programme to include more sentinel sites and offer a wider range of screening options will increase the quality and representativeness of the data generated.

SURVEILLANCE AND DIAGNOSTIC SERVICES

The CED currently has the responsibility for surveillance and characterisation of bacterial enteric disease and rotavirus in South Africa. Specifically, CED collects all human isolates from diagnostic microbiology laboratories in South Africa for surveillance and is an active member of the Group for Enteric, Respiratory and Meningeal diseases Surveillance in South Africa (GERMS-SA). Bacterial isolates are characterised at no charge to the laboratory of origin, irrespective of whether the laboratory functions in a private capacity or has a public role and includes those isolates that may represent carriage of an enteric bacterial pathogen, rather than disease due to that pathogen.

In addition, CED has a sentinel rotavirus surveillance programme, enrolling children under 5 years who present for the treatment of diarrhoea at selected clinics and hospitals. While the study’s main focus is the monitoring of the rotavirus vaccine impact, specimens are screened for bacteria, parasites and other enteric viruses, providing the most comprehensive look at diarrhoeal pathogens in the South African under 5 population. The centre is also involved in rotavirus testing and genotyping for the rotavirus case-control study examining real world vaccine effectiveness.

The CED participated in the investigation of outbreaks, including a water-borne outbreak in Verkeerdevlei, Free State province where *Shigella* spp, norovirus and astrovirus were detected in patient samples submitted for diagnoses. The CED also assisted in outbreaks due to foodborne bacteria, including the confirmation of organism identity, serotyping and molecular epidemiology of these pathogens.

RESEARCH

Genetic characterisation of rotavirus strains detected during the rotavirus vaccine efficacy trial and during vaccine introduction in South Africa

NICD researcher: Dr N Page
Collaborators: P Bos, Prof J Mphahlele (University of Limpopo, Medunsa Campus); Dr D Steele, Dr J Erickson (Program for Appropriate Technologies in Health [PATH]); Dr N Cunliffe (University of Liverpool); Dr M Gómara (Health Protection Agency [HPA]); Dr S Debrus, Dr H Han (GlaxoSmithKline [GSK])

Funding: PATH

The monovalent rotavirus vaccine showed >20% difference in vaccine efficacy in South Africa and Malawi. The aim of the project was to determine if differences in the genetic make-up of rotavirus strains circulating in the respective countries correlated with differences in vaccine efficacy. The project involved genetic characterisation of 11 rotavirus genes from specimens collected during the rotavirus efficacy trial (2005-2007) using Sanger sequencing. A sub-study investigated rotavirus strains collected from patients during vaccine introduction in 2009/2010, using 454 sequencing.

Investigation of other enteric viruses circulating during the rotavirus vaccine efficacy trial in South Africa

NICD researchers: Dr N Page
Collaborators: P Bos, Prof J Mphahlele (University of Limpopo, Medunsa Campus); Dr D Steele, Dr J Erickson (PATH); Dr N Cunliffe (University of Liverpool); Dr M Gómara (HPA); Dr S Debrus, Dr H Han (GSK)

The aim of the project was to determine if differences in the numbers of other enteric virus infections in South Africa and Malawi were responsible for differences in vaccine efficacy. Rotavirus-positive specimens from the rotavirus efficacy trial were tested for other enteric viruses using real-time detection. The centre is awaiting rotavirus-negative specimens for screening to complete the project.
Investigation of viral load in rotavirus-positive stool samples collected during the rotavirus vaccine efficacy trial in South Africa

**NICD researcher:** Dr N Page  
**Collaborators:** P Bos, Prof J Mphahlele (University of Limpopo, Medunsa Campus); Dr D Steele, Dr J Erickson (PATH); Dr N Cunliffe (University of Liverpool); Dr M Gómara (HPA); Dr S Debrus, Dr H Han (GSK)

The project aimed to determine if differences in the rotavirus viral loads of children in the respective countries were responsible for differences in vaccine efficacy. Real-time quantification has been completed and preliminary results indicated differences in the viral loads of South African and Malawian patients. Plans are underway to ensure that the observed results are accurate and not due to inter-laboratory variation.

The development of real-time detection techniques and increased surveillance of diarrhoeal disease viruses in the South African population

**NICD researchers:** Dr N Page, S Nadan, R Netshikweta  
**Funding:** Poliomyelitis Research Foundation (PRF)

Elucidating diarrhoeal disease burden in South Africa can only be achieved if researchers understand the cause of diarrhoeal episodes. The aim of the project was to develop and use real-time detection techniques to identify enteric viruses, other than rotavirus, and to determine the contribution to the overall diarrhoea burden. Stool samples collected through the sentinel rotavirus surveillance programme in 2009/2010 were screened for norovirus, astrovirus, adenovirus and sapovirus. Plans are to source additional funding to screen specimens from 2011-2015, genotype enteric virus strains, and to develop detection assays for human bocavirus and enteroviruses.

**PulseNet Africa**

**NICD researchers:** Dr KH Keddy; Dr AM Smith, H Ismail, N Tau  
**Collaborators:** Dr P Gerner-Smidt, N Maxwell (CDC, USA); K Kubota (Association of Public Health Laboratories, USA); PulseNet Africa member countries

PulseNet is an international molecular subtyping network for foodborne and waterborne disease surveillance. Pulsed-field gel electrophoresis (PFGE) analysis of bacteria is the primary subtyping technique used by PulseNet. The network consists of national and regional laboratory networks dedicated to tracking foodborne and waterborne infections worldwide. PulseNet Africa is the newest network and was launched in August 2010. The CED is the coordinating laboratory for PulseNet Africa, with member countries including South Africa, Kenya, The Gambia, Senegal, Cameroon, Malawi, Tanzania, Cote d’Ivoire, Ghana, Uganda and Mozambique. PulseNet Africa aims to build, increase and strengthen capacity for molecular surveillance of enteric diseases in sub-Saharan Africa. In 2011, the momentum of PulseNet Africa was maintained. Two major activities included a PFGE training course in June 2011 and the launch of a PFGE proficiency testing programme for African laboratories.

**Typhoid fever surveillance in sub-Saharan Africa: Burden of Disease Study**

**NICD researchers:** Dr KH Keddy, A Sooka, Dr B Harris  
**Collaborators:** Dr V Howell, Dr S Haffejee (NHLS); Dr H Dawood, Dr F Naby (Edendale/Greys hospitals, Pietermaritzburg, KwaZulu-Natal); Dr F Marks, J Im (International Vaccine Institute, Seoul, Korea)

The lack of credible data on typhoid fever in many African countries has also limited awareness of the disease among clinical and public health providers. Fever-related diseases are mostly diagnosed based solely on clinical signs and symptoms. As a result, typhoid fever may be indistinguishable from other serious infections common to Africa such as malaria, tuberculosis and infection due to invasive non typhoidal *Salmonella*. This is an international bacteraemia study, to identify which pathogens cause illness in 10 sentinel sites in Africa in patients presenting with fever. Data from South Africa on typhoid fever and other febrile illnesses are scanty and very much driven by outbreak or case reports, and information on emerging resistance in the pathogen or through laboratory-based surveillance systems is limited. Edendale Hospital is the site selected for this study; patients presenting with invasive disease due to various pathogens, including *Salmonella Typhi*, are identified and additional information on the patients’ history, including antimicrobial exposure, HIV status and outcome data are recorded by a surveillance officer.

**Group for Enteric Respiratory and Meningeal diseases Surveillance in South Africa - laboratory-based surveillance for enteric pathogens**

**NICD researchers:** Dr KH Keddy, A Sooka  
**Collaborators:** GERMS-SA principal investigators
The CED does laboratory-based surveillance and characterisation of bacterial enteric disease in South Africa; specifically on all human isolates from diagnostic microbiology laboratories. The case definition for the laboratory-based surveillance includes all Salmonella, Shigella, Vibrio cholerae (O1 and non-O1) and enterohaemorrhagic Escherichia coli isolates from all body sites, and diarrhoeagenic E. coli isolates from stool only. The case definition for enhanced surveillance isolates includes only those Shigella and Salmonella enterica isolates that are from normally sterile body sites in ‘in-patients’ only – that is, the patient should have been admitted to the hospital or enhanced surveillance site; CED currently receives specimens from over 4,000 human cases per annum, according to the definition above. In addition, the centre undertakes to serotype Salmonella, Shigella and diarrhoeagenic E. coli (DEC) isolates for commercial purposes and has in the past performed a multiplex PCR to diagnose DEC from veterinary specimens. Regular reports on the isolates received are extracted from the database for the purposes of information sharing. Molecular methods may be used to establish strain relatedness in outbreaks.

**Escherichia coli O104 associated with human diarrhoea in South Africa, 2004-2011**

**NICD researchers:** Dr KH Keddy, Dr AM Smith, N Tau  
**Collaborators:** Dr P Meidany (NHLS); GERMS-SA

*Escherichia coli* serotype O104 caused an outbreak of bloody diarrhoea and haemolytic uraemic syndrome in Germany over the period May to June 2011. From 2004-2011, more than 4,000 suspect DEC isolates were investigated. Seven isolates were identified as serotype O104; five were enteroaggregative *E. coli* O104:H4 and two were enteropathogenic *E. coli* O104:non-H4. Analysis of PFGE patterns determined that these isolates were unrelated to the 2011 German *E. coli* O104:H4 outbreak strain.

**Molecular characterisation of cholera outbreak isolates in South Africa, 2008-2009**

**NICD researchers:** Dr KH Keddy, Dr AM Smith, H Ismail  
**Collaborators:** GERMS-SA

The mechanism of antimicrobial resistance of 100 antimicrobial-resistant *Vibrio cholerae* O1 study isolates associated with outbreaks of cholera in South Africa for the period of 1 January 2008 to 31 May 2009 was investigated. PCR analysis showed that all 100 isolates were PCR-positive for the cholera toxin (CT) and the El Tor variant of the toxin co-regulated pilus. Nucleotide sequencing of the CT showed that isolates expressed the *ctxB-1* allele of the classical biotype and were defined as ‘altered El Tor’. All isolates harboured the SXT element, which conferred resistance to chloramphenicol (*floR*), sulfamethoxazole (*sul2*), trimethoprim (*dfrA1*) and streptomycin (*strA* and *strB*). Seventeen percent of isolates were PCR-positive for the tetracycline resistance determinant (*tetA*). Nucleotide sequencing of the quinolone resistance-determining region of DNA gyrase (*gyrA*,*gyrB*) and topoisomerase IV (*parC*,*parE*), showed that all 100 nalidixic acid-resistant isolates harboured the same amino acid mutations in GyrA (S83-I) and ParC (S85-L). Sixteen percent of isolates were PCR-positive for the *bla*TEM gene and carried a single plasmid of approximately 140 kilobase pairs in size. Southern blotting and DNA probing showed that this plasmid harboured the *bla*TEM gene, which produced the TEM-63 β-lactamase. This is the first incidence of TEM-63 β-lactamase-producing, antimicrobial-resistant, toxin-producing, *V. cholerae* O1 altered El Tor isolates in South Africa.

**TEACHING AND TRAINING**

Laboratory and scientific staff are involved in postgraduate training. The staff has a specialised programme for the training of microbiology registrars, and over a two-week period, registrars are exposed to a range of biochemical, serotyping and molecular techniques in the identification of bacterial and viral enteric pathogens. The senior staff members are experienced in postgraduate supervision of scientists and have recently started projects with epidemiology students who are examining the extensive database.
The CED assisted in the training supervision of SA-FELTP students.

**PROFESSIONAL DEVELOPMENT**

Postgraduate candidates enrolled:
6 (2 PhD, 4 MSc)

**RESEARCH OUTPUT**

**Publications**
Staff authored/co-authored 13 peer-reviewed publications during 2011/2. A synopsis of the key manuscripts follows:

Jere KC, Mlera L, Page NA, Van Dijk AA, O’Neill HG. Whole genome analysis of multiple rotavirus strains from a single stool specimen using sequence-independent amplification and 454®pyrosequencing reveals evidence of intergenotype genome segment recombination. Infection, Genetics and Evolution 2011; 11: 2072-2082

**Synopsis:** The study investigated the genetic composition of a mixed strain rotavirus infection using a sequence-independent amplification strategy and 454 sequencing. The specimen contained rotaviruses from both Wa and DS-1 genogroups and four different rotavirus strains were identified. Intergenogroup recombination events were observed in the NSP2, VP4 and VP6 genes. The study illustrated how infection with multiple strains of different rotavirus genotypes may result in novel progeny through genome recombination.


**Synopsis:** The aim of the study was to investigate the genetic composition of G2, G8, G9 and G12 strains which are emerging globally and may not be covered by the monovalent vaccine. Strains were amplified using a sequence-independent method and sequenced by 454 technology. Genotype G12 strains belonged to the Wa genogroup while G2, G8 and G9 strains belonged to the DS-1 genogroup. Amino acid substitutions were also identified in six genes. These data provide a genetic database of African rotavirus strains pre-vaccine introduction and will allow identification of changes in genetic reassortment of rotavirus strains post-vaccine introduction.


**Synopsis:** This study highlighted the advantages of using internationally standardised protocols for the molecular epidemiology and investigation of enteric pathogens in international outbreaks, as well as the importance of international collaboration for these outbreaks.


**Synopsis:** This study showed the strong relationship between the cholera outbreak in Haiti after the earthquake there and those in other parts of the world, including in West Africa and South Africa in 2008-2009.

Other journal articals:


Conference presentations
International: 8
National: 6

“The lack of credible data on typhoid fever in many African countries has also limited awareness of the disease among clinical and public health providers”
The Centre for HIV and Sexually Transmitted Infections (STIs) was created through the amalgamation of five separate NICD sections:

- The Virology Laboratory (head: Professor Lynn Morris) and Cell Biology Unit (head: Professor Caroline Tiemessen) of the former AIDS Virus Research Unit;
- The HIV Molecular and Serology Laboratories of the former Specialised Molecular Diagnostics Unit (head: Professor Adrian Puren);
- The former STI Reference Centre (head: Professor David Lewis); and
- The World Health Organization-linked HPV LabNet Laboratory (head: Professor Anna-Lise Williamson).

The first four sections are based on NICD’s Sandringham campus and the last section is based at the University of Cape Town. The Centre for HIV & STIs has six portfolios, which are led by different senior centre staff for two-year periods at a time, namely administration/head of centre (Prof Lewis), surveillance (Prof Lewis), diagnostics (Prof Puren), World Health Organization (WHO) HPV LabNet (Prof Williamson), research (Prof Morris), teaching and training (Prof Tiemessen).

The Centre for HIV and STIs is a resource of knowledge and expertise in HIV and other regionally relevant STIs to the South African government, SADC countries and the African continent at large, in order to assist in the planning of policies and programmes related to the control and effective management of HIV/STIs. The centre has a strong track record in the research disciplines of HIV virology, HIV immunology, HIV/STI epidemiology, HIV/STI diagnostics and HIV-STI interactions, as well as in successful supervision of MSc and PhD students.

**SURVEILLANCE/DIAGNOSTIC SERVICES**

**HIV prevalence and incidence surveillance**
The serology laboratory coordinates the HIV prevalence testing for the annual Department of Health (DoH) antenatal survey and has also completed HIV incidence testing up to 2010 using the BED capture ELISA. In addition, the laboratory has introduced a limiting antigen avidity index to determine whether the assay alone or in combination or as part of an algorithm will improve incidence estimates. The serology laboratory will apply this approach to a microbicide study and the Human Sciences Research Council’s general population HIV prevalence and incidence study.

**HIV drug resistance surveillance**
The Centre for HIV and STIs is a designated WHO Regional Laboratory for HIV drug resistance and performs surveys for transmitted HIV drug resistance in South Africa. This survey makes use of specimens from the annual antenatal clinic survey focusing on young women in their first pregnancy. The HIV drug resistance surveillance laboratory is equipped to perform genotyping of specimens, processing of data and analysis of data according to threshold levels. The centre also performs surveillance for neighbouring southern African countries including Swaziland, Namibia, Zimbabwe and Malawi. Plans are in place to implement the early warning indicators for HIV drug resistance at a national level as well as conduct surveys of acquired resistance among treated patients.

**STI clinical syndrome and aetiological surveillance**
The Gauteng STI surveillance project, run by the centre in collaboration with the Gauteng provincial health department, continued to collect STI syndrome data from public clinics in 2011-12. In collaboration with the national and provincial health departments, Alexandra Health Centre and NHLS laboratories, the centre undertook aetiological surveillance of three major STI syndromes (male urethritis [MUS], vaginal discharge [VDS], genital ulceration [GUS]) in Johannesburg, Polokwane and Rustenburg. Molecular, serological and microscopy methods were employed to test for a variety of STI pathogens. The aetiological surveillance confirmed that gonorrhoea continues to be the main cause of the MUS, that STIs account for less than half of VDS presentations in women, and that genital herpes accounts for the majority of GUS cases. The centre secured Global Disease Detection funding from the CDC to support aetiological surveillance activities in 2012.
**Gonococcal antimicrobial resistance surveillance**
The centre undertook surveillance to determine the prevalence of resistance to ciprofloxacin, cefixime and ceftriaxone among gonococci isolated in men with MUS enrolled in surveys conducted in Johannesburg, Polokwane and Rustenburg in 2011. These surveys confirmed continued effectiveness of cephalosporins but ciprofloxacin resistance was prevalent at different levels (Johannesburg 23%, Polokwane 51%, and Rustenburg 13%). During the year, the centre supported international aetiological and antimicrobial susceptibility STI surveys in Harare, Zimbabwe and in three sites within Madagascar. The centre co-ordinated the WHO Gonococcal Antimicrobial Susceptibility Programme (GASP) within Africa and participated in a global GASP meeting at WHO headquarters in June 2011.

**Evaluation of the effectiveness of the national PMTCT programme**
Internationally and nationally, prevention of mother-to-child transmission of HIV (PMTCT) has been recognised as an essential intervention. In 2011, the molecular and serology laboratories participated in the second round to evaluate the effectiveness of the national PMTCT programme. Additional follow-up of HIV-exposed infants has also been introduced at six, nine, 12 and 18 months to determine infection rates during breastfeeding and post-weaning.

**Reference testing and diagnostic services**
The centre is SANAS-accredited and provides key support for HIV/STI diagnostics for other NICD surveillance programmes, NHLS, other organisations and the DoH in Lesotho. Primary HIV-related diagnostic tests include CD4 counts, HIV DNA PCR for early infant diagnosis and HIV viral load monitoring and can be performed on standard matrices including plasma and dried blood spots. The HIV serology component of the centre similarly provides specialised testing including HIV Western blot testing in support of clinical trials and also an increase in demand for serology testing on dried blood spots. In addition, several new platform/technology evaluations for HIV viral load and HIV DNA PCR including HIV viral load using dried blood spots were performed. Dried tube sera were evaluated as an alternative HIV testing method. During 2011-12, HIV drug resistance testing was performed on a Next Generation Sequencer, the Roche GS Junior. A number of specialised molecular assays as well as conventional serological and microscopy-based tests are available for diagnosis of STIs and other genital tract conditions.

**External quality assessment and quality assurance for HIV**
HIV rapid test quality programmes, supported through PEPFAR, have supported the national and provincial health departments to train and mentor 876 staff as part of the provider-initiated HIV testing in various settings. An additional 493 participants were also trained on HIV quality management procedures. As part of the programmes, materials and manuals were distributed. The serology laboratory performs post-marketing surveillance testing on HIV rapid test kits. New batches are not released into the field until they have met the centre's criteria for successful evaluation. During 2011-12, an external quality assurance (EQA)/proficiency testing molecular programme for HIV viral load was developed for NHLS laboratories that form part of the National Priority Programme within South Africa. Six distributions of HIV RNA EQA panels were distributed to 17 HIV viral load NHLS testing laboratories nationwide and all were trained as to the importance of EQA, result interpretation and troubleshooting. Executive reports have been generated and distributed to regional and national QA managers on a quarterly basis. The serology laboratory provided an EQA programme for 205 NHLS-participating laboratories as well as for 76 African country laboratories. In keeping with its supraregional laboratory responsibilities, the centre assists the Lesotho government with laboratory-based training, external auditing of laboratories and evaluation of quality assurance activities.

**Support for HIV vaccine trials**
The centre serves as the South African Immunology Laboratory for the HIV Vaccine Trials Network performing validated end-point humoral antibody and molecular HIV assays for human clinical trials. For this purpose, the centre is equipped to perform both the TZM-bl neutralisation assay and an ELISA against two HIV antigens to Good Laboratory Practice standards. This allows for an assessment of the immunogenicity of candidate vaccines and whether it induces HIV-specific binding and neutralising antibodies in vaccinated individuals. Evaluation of these antibodies is a crucial step in the vaccine development process as these laboratory data are used to make decisions regarding whether a vaccine progresses to the next stage.
RESEARCH

The Orange Farm part 2 study: a community study of male circumcision

**NICD researchers:** Prof D Lewis, Prof A Puren, E Cutler, B Singh, V Maseko

**Collaborators:** Prof B Auvert (INSERM, University of Versailles [PI]); Dr D Taljaard (Progressus/CHAPS)

This project, which commenced in the latter part of 2007, has overseen the roll-out of male circumcision intervention in Orange Farm in order to evaluate its impact on knowledge, attitudes and practice regarding male circumcision, existing means of prevention (behaviour change, condom use, STI treatment-seeking behaviour and HIV counselling and testing), and the spread of HIV and other STIs. By the end of 2011, just over 30,000 circumcisions had been undertaken at the Bophelo Pele Male Circumcision Centre. The project is now considered a model for the roll-out of comprehensive adult male circumcision services, and could be tailored for implementation in other rural and urban low-income settings in eastern and southern Africa. The Centre for HIV and STIs supported the laboratory component of this high impact HIV prevention project, testing for HIV incidence using a modified method of the BED capture ELISA, HSV-2 antibodies, gonorrhoea, and chlamydial infection. Data demonstrating a significant effect of the male circumcision programme on lowering HIV prevalence among those circumcised were presented at the 6th International AIDS Society conference in Rome in July 2011.

Neutralising antibody research for HIV vaccine development

**NICD researchers:** Prof L Morris, Dr P Moore, Dr B Lambson, Dr N Mkhize

**Collaborators:** Dr C Williamson (University of Cape Town); Dr S Abdool Karim (University of KwaZulu-Natal); Dr B Haynes (Duke University); Dr J Mascola (Vaccine Research Centre, National Institutes of Health)

A major focus is the study of the evolution of anti-HIV envelope antibodies, particularly those that are broadly cross-neutralising. The investigators have mapped the specificities of plasma neutralising antibodies in a subset of HIV-infected women and isolated the corresponding B cell through antigen-specific sorting and single B cell PCR. These monoclonal antibodies have been expressed and characterised. Work is now ongoing to back-track and study the ontogeny of these antibodies. These studies will provide insights as to how neutralising antibodies develop which will guide HIV vaccine design. On the flip side, centre staff have undertaken extensive analyses of how HIV escapes neutralising antibody pressure through genetic escape. More recently the centre started examining antibody responses in mucosal tissues and undertaking research on the interaction of HIV with cellular receptors.

Correlates of protective immunity to HIV

**NICD researchers:** Prof C Tiemessen, Dr L Damelin, Dr M Paximadis, Dr D Schramm, Dr S Shalekoff

**Collaborators:** Prof L Kuhn (Columbia University); Prof G Gray, Dr N Martinson (Perinatal HIV Research Unit); Dr D Spencer (Right to Care); Dr P Ive (Clinical HIV Research Unit); Prof G Sherman (NHLS); Dr A Coovadia (Coronation Hospital)

The aim is to identify and understand immune and host genetic correlates of protection to HIV, both in the context of acquisition of infection (using maternal-infant HIV transmission as a model) and in disease progression
The centre’s staff collaborated with scientists at the Wellcome Trust’s Sanger Institute in Cambridge, UK, to investigate aspects of the molecular biology of \textit{Chlamydia trachomatis}. Whole genome sequences from representative strains of the trachoma (ocular and genital tract serotypes) and lymphogranuloma venereum biovars from temporally and geographically diverse sources were used to reconstruct a detailed genome-wide phylogeny of the species. Data analysis showed that predicting phylogenetic relationships using the small number of genetic targets traditionally used to classify \textit{Chlamydia} is misleading because it masks true diversity due to extensive recombination of these markers. Moreover, the research team provided evidence for both exchange of and recombination within the cryptic plasmid, also an important diagnostic target.

**Human papillomavirus research**

**NICD researchers:** Prof A-L Williamson, Prof D Lewis, Prof A Puren  
**Collaborators:** Prof L-G Bekker, Dr D Coetzee, Prof L Denny, Prof E Rybicki (University of Cape Town); Prof Q Abdool Karim (University of KwaZulu-Natal); Dr J Burger (University of Stellenbosch); Dr C Finnhaber (University of the Witwatersrand); Dr S Delaney-Moretlwe (Wits Reproductive Health & HIV Institute); Dr D Adler (University of Rochester); Prof B Auvert (INSERM, University of Versailles)

Human papillomavirus (HPV) typing has been undertaken at the centre’s HPV LabNet Laboratory as part of collaborative studies with a number of different groups. Some of these studies have been published and concentrate on HIV-positive women where the prevalence of HPV is extremely high and this correlated with a high prevalence of cervical disease. In a study done on a couple cohort, it was confirmed that HIV co-infection increases HPV prevalence, multiple infections and viral load in both women and men. HIV co-infection in couples increased type-specific HPV concordance and transmission. In men, type-specific HPV concordance with their female partner is influenced by their own HIV status and that of their male partner while in women type-specific HPV concordance is influenced by their own HIV status but not that of their male partner. Couples with type-specific HPV concordance were found to have higher HPV viral load compared to couples with no type-specific HPV concordance, suggesting that high HPV viral load may play a role in HPV transmission between partners. Men demonstrated a higher HPV acquisition and clearance during follow-up compared to women. The data from this study add to the very limited data on the natural history of HPV in men and sexually active couples and assist to inform the government on HPV vaccine policy.

**Evolutionary genetics of \textit{Chlamydia trachomatis}**

**NICD researchers:** Prof D Lewis, F Radebe  
**Collaborators:** Prof N Thomson, Dr S Harris, Dr H Seth-Smith (Wellcome Trust Sanger Institute)

The centre is involved in studying the emergence and impact of HIV drug resistance on clinical outcomes in adults and children on antiretroviral therapy. In addition to using standard genotyping, new methodologies were developed to detect minority populations of resistant variants, which have been shown to have important clinical consequences. This includes the use of allele-specific PCR, high resolution melting analysis and ultra-deep sequencing. Studies are also underway to validate dried blood spots for HIV drug resistance testing to determine if equivalent data are obtained to that of plasma. This will be important particularly for paediatric resistance surveillance. The centre is also equipped to perform phenotypic drug resistance testing to determine the effect of genotypically identified HIV drug resistance mutations on virus replication capacity and drug susceptibility. This assay is being used in drug discovery and to determine whether new drugs are active against circulating resistant viruses.

**STIs in men-who-have-sex-with-men**

**NICD researchers:** Prof D Lewis, Dr E Müller, F Radebe, V Maseko  
**Collaborators:** Dr K Rebe (Ivan Toms Centre for Men’s Health & ANOVA Health Institute); Prof J McIntyre (ANOVA Health Institute)

The centre is collaborating with the ANOVA Health Institute to determine the prevalence of gonococcal and chlamydial infections in symptomatic and asymptomatic men-who-have-sex-with-men (MSM) attending the Ivan Toms Centre for Men’s Health in Cape Town. There are no data on the prevalence of these two STIs among MSM in South Africa and first pass urine, pharyngeal and ano-rectal swabs were tested for \textit{Neisseria gonorrhoeae} and \textit{Chlamydia trachomatis} infection.

**HIV drug resistance research**

**NICD researchers:** Prof L Morris, Dr G Hunt, Dr A Basson  
**Collaborators:** Dr L Kuhn (Columbia University); Dr C Hoffman (Johns Hopkins); Dr J Johnson (CDC); Dr B Korber (Los Alamos); Dr P Cane/D Pillay (UK Health Protection Agency)
Members of the centre’s Sandringham site are collaborating with the Wits Reproductive Health and HIV Institute to provide laboratory diagnostic support for a European Union-funded project entitled ‘Evaluation and impact of screening and treatment approaches for the prevention of cervical neoplasia in HIV-positive women in Burkina Faso and South Africa’. The purpose of this study is to improve cervical cancer prevention programmes for HIV-infected women in Africa, by evaluating the performance and cost-effectiveness of alternative screening strategies, and by developing algorithms leading to earlier detection and management of cervical cancer in high-risk populations.

**GRANT FUNDING**

Funding to support the centre’s work was obtained from the following organisations:

- CDC, (CDC, PEPFAR and Global Disease Detection funds);
- Canadian HIV Vaccine Initiative;
- Elizabeth Glaser Pediatric Foundation;
- Medical Research Council;
- Merck Serono;
- NHLS Research Trust;
- National Institutes of Health;
- National Research Foundation Professional Development Programme;
- Poliomyelitis Research Foundation, Research Foundation Incentive Funding for Rated Researchers;
- Technology Innovation Agency (SHARP funding: South African HIV/AIDS Research and Innovation Platform); and the
- World Health Organization (WHO).

In addition, research collaborators obtained funding to support centre activities from the Agence Nationale de Recherches sur la SIDA et les hépatites virales (collaboration with INSERM at the University of Versailles and Progressus), European Union (via 3rd party agreement with Wits Reproductive Health and HIV Institute), Population Services International, United States Agency for International Development (collaboration with ANOVA Health Institute) and the World Bank. Members of the centre also participate in major networks such as the Centre for HIV Vaccine Immunology, Bill and Melinda Gates Centre for AIDS Vaccine Discovery and the HIV Vaccine Trial Network.

**HONOURS**

Anabela Picton, Dr Nonohlana Mkhize, Cathrine Mitchell and Jinal Bilman were all awarded Columbia University – Southern Africa Fogarty AITRP Traineeship Fellowships for 2011–2012.

Dr Kabamba Alexandre was awarded the University of the Witwatersrand’s Faculty of Health Sciences prestigious PhD award for 2011.

Prof David Lewis was elected World President-Elect for the International Union against STIs.

Dr Penny Moore was elected as a founding member, and nominated to serve on the Executive Committee, of the South African Young Academy of Science.

Prof Caroline Tiemessen was awarded a grant through the professional development programme of the NRF, aimed to retain capacity at the level of PhD and post-doctoral scientists.

**TEACHING AND TRAINING**

During the past year, undergraduate and postgraduate lectures were delivered to medical, nursing, pharmacy and dental students at the University of the Witwatersrand and to medical students at the University of Cape Town. In addition, postgraduate lectures were delivered to practising doctors on the Right to Care Doctor’s HIV course.

The centre offers a thorough and comprehensive training programme for interns and technologists in line with Health Professions Council of South Africa (HPCSA) guidelines. Seven intern medical scientists and one technologist were trained during the review period. The centre also participated in the long and short NICD training course for microbiology registrars, and in the QA department’s continuing education training course on laboratory quality management for NHLS staff. Staff members also assisted with the National Priority Programmes’ training courses for HIV viral load and early infant diagnosis. In 2011, the NICD was selected as the Supranational Reference Laboratory for HIV for the SADC region and the Centre for HIV and STI is developing specific laboratory training courses for this purpose. HIV/STI training was provided to laboratory staff from Burkina Faso, Tanzania and Madagascar. Staff undertook STI microbiological survey training of several clinical and laboratory staff in Madagascar, Tanzania, Zimbabwe, and participated in a dedicated STI training course in Zimbabwe and a dermatology training course in Tanzania.
PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated:
7 (3 PhD, 3 MSc, 1 BSc [Hons])

Postgraduate candidates registered:
19 (13 PhD, 2 MMed, 4 MSc)

RESEARCH OUTPUT

Publications

During 2011-12, staff authored/co-authored 45 journal articles and one book chapter. The following publications are highlighted as they present work that has advanced public health and/or laboratory science within South Africa:


Synopsis: In a study utilising centre data, HIV seroconversion among female sex workers was shown to be associated with genital high risk human papillomavirus infection.


Synopsis: This study describes the factors associated with the development of anti-viral antibodies in a group of HIV-infected women followed for up to five years in Durban. While these antibodies afforded no protection against HIV disease progression, such antibodies are protective if present prior to exposure. Thus a better understanding of how these antibodies are elicited is needed in order to assist HIV vaccine design. In addition, this research provides insights into the targets of broadly neutralising antibodies on the HIV envelope glycoprotein.

Lewis D, Chirwa T, Msimang V, Radebe F, Kamb M, Finnhaber C. Screening asymptomatic individuals for genital discharge pathogens at a HIV treatment centre in Johannesburg: patient characteristics and pathogen prevalence. Sex Transm Dis. Published online February 2012, doi 10.1097/OLQ.0b013e31824cbecc

Synopsis: This STI screening study of HIV-infected patients, asymptomatic for genital discharges, demonstrated a high prevalence of asymptomatic urethral, endocervical and vaginal STI pathogens which may elevate the risk of HIV transmission to HIV seronegative sexual partners.


Synopsis: This study reports data from a cross-sectional biomedical survey conducted in 2007-2008 among a random sample of 1,198 men aged 15 to 49 from Orange Farm. Men without foreskins had lower HIV incidence and prevalence than men with foreskins. The uptake of adult male circumcision was almost 60%.

Tiemessen CT, Paximadis M, Minevich G, Winchester R, Shalekoff S, Gray GE, Sherman GG, Coovadia AH, Kuhn L. Natural killer cell responses to HIV-1 peptides are associated with more activating KIR genes and HLA-C of the C1 allotype. JAIDS 2011; 57: 181-189

Synopsis: This study showed that HIV-infected individuals whose natural killer cells responded to HIV-1 peptides in whole blood assays (‘responders’), when compared to nonresponders, possessed a more activating KIR gene profile and possessed HLA-C1 alleles which bind only certain KIR receptors.

Other journal articles:

Alexandre KB, Gray ES, Mufhandu H, McMahon JB, Chakauya E, O’Keefe BR, Chikwamba R, Morris L. The lectins griffithsin, cyanovirin-N and scytovirin inhibit HIV-1 binding to the 2 DC-SIGN receptor and transfer to CD4+ cells. *Virology* 2012, **423**: 175-186


Fayemiwo S, Müller EE, Gumede L, Lewis DA. Development of a duplex PCR assay for the detection of plasmid-mediated resistance to penicillin and tetracycline in *Neisseria gonorrhoeae*. *Sex Transm Dis* 2011; **38**: 329-333


Jansen van Vuuren P, Tiemessen CT, Paweska JT. The role of anti-nucleocapsid protein immune responses in counteracting pathogenic effects of Rift valley fever virus infection in mice. *PLOS One* 2011; **6**: e25027

follow-up of a randomised, open-label trial. *Lancet Infect Dis*. Published online March 16, 2012


Lewis DA. HIV/STI epidemiology, management and control in the IUSTI-Africa Region: focus on sub-Saharan Africa. *Sex Transm Infect* 2011; 87 : ii10-ii13

Lewis DA, Lukehart SA. Emerging drug resistance in *Neisseria gonorrhoeae* and *Treponema pallidum*. *Sex Transm Infect* 2011; 87: ii39-ii43

Lewis DA. Cefixime is first-line treatment for gonorrhoea in South Africa. *South Afr J Epidemiol Infect* 2011; 26: 103-104


Mufhandu HT, Gray ES, Madiga MC, Tumba N, Alexandre KB, Khoza T, Wibmer CK, Moore PL, Morris L, Khati M. UCLA1, a Synthetic Derivative of a gp120 RNA Aptamer, Inhibits Entry of the Human Immunodeficiency Virus Type-1 Subtype C. *J Virol* 2012 Feb 29 (Epub ahead of print)


Ross JDC, Lewis DA. Cephalosporin resistant *Neisseria gonorrhoeae*: time to consider gentamicin? *Sex Transm Infect* 2012; 88: 6-8


Tiemessen CT. Commentary: The quest to understand protective immunity to HIV-1 through studies on maternal-infant HIV-1 transmission. *South Afr J Epidemiol Infect* 2011; **26**: 205-209


Venter JME, Müller EE, Maseko VD, Lewis DA. Is the new variant *Chlamydia trachomatis* present in South Africa? *South Afr J Epidemiol Infect* 2011; **26**: 36-37


**Book chapter**


**Conference presentations**

*International*: 35  
*National*: 18
CENTRE FOR OPPORTUNISTIC TROPICAL AND HOSPITAL INFECTIONS
OPPORTUNISTIC, TROPICAL AND HOSPITAL INFECTIONS

The Centre for Opportunistic, Tropical and Hospital Infections (COTHI) was formed by the amalgamation of the former Parasitology, Vector Control, Mycology and Microbiology External Quality Assessment and Antimicrobial Resistance Reference units of the NICD, and also includes the satellite Molecular Epidemiology Unit located in Cape Town. The functional thrusts of the centre are embodied in its name: opportunistic infections, particularly those that are HIV-related; tropical infections, especially malaria and its vectors; and nosocomial infections, concentrating on antimicrobial resistance, molecular epidemiology and outbreak investigations in the hospital setting.

SURVEILLANCE AND DIAGNOSTIC SERVICES

Specialised vector control reference services include the identification of medically important arthropods for entomologists, medical practitioners and health inspectors. Malaria vector mosquitoes were routinely identified by PCR for the Mpumalanga Province Malaria Control Programme. Advice and expertise were provided to the Department of Health both at the national and provincial levels, with participation on the National Malaria Advisory Group. Other specialised diagnostic services were offered by the Parasitology and Mycology Reference laboratories in the fields of opportunistic or unusual parasitic and fungal infections. Molecular tests, particularly PCR and pathogen sequencing, are increasingly being offered. Surveillance functions encompassed national and regional monitoring of cryptococcal meningitis, candidaemia, cryptosporidial infection, and antibiotic-resistant hospital infections. Phenotypic and genotypic characterisation of mechanisms of resistance especially focused on Staphylococcus aureus and Klebsiella pneumoniae, but a reference service was offered for all multidrug-resistant organisms, such as carbapenem-resistant Enterobacteriaceae.

Quality assessment (QA) services provided by the COTHI contributed to monitoring diagnostic laboratory proficiency in South Africa and other African countries. The external QA programmes provided schemes for malaria microscopy, bacteriology, tuberculosis microscopy and culture, and syphilis serology. The centre has played an active role in reporting on laboratory capacity in the WHO AFRO region for the past 10 years, and has supported QA for laboratories for international malaria vaccine trials (GSK Biologicals) and Global Vaccine Preventable Invasive Bacterial Diseases sentinel sites. The centre also houses the National Stock Culture Collection as well as one of the world's largest collections of medically important arthropods.

OUTBREAKS

A lymphocutaneous sporotrichosis outbreak occurred at a gold mine in Mpumalanga province and investigations by the centre identified 17 confirmed and probable cases. Environmental specimens collected from rotting wood and soil samples from underground levels tested positive for Sporothrix schenckii. It is likely that the source of infection was contaminated soil and untreated, rotting wood.

Members of the outbreak response team enter the gold mine affected by an outbreak of lymphocutaneous sporotrichosis to observe practices and collect environmental samples.
An outbreak of malaria in Gauteng province residents without recent travel history was explored. Entomological investigations revealed no evidence of local transmission by anophelines and it is likely that infections were acquired via infected mosquitoes imported from endemic areas in vehicles, containers and other means. The Molecular Epidemiology Unit in Cape Town investigated a hospital outbreak of multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections in immunocompromised patients. Ten cases (of which eight were fatal) were detected between January 2010 and January 2011. No source or reservoir of infection was detected despite intensive environmental sampling. The original outbreak strain has now spread more widely, both within the original hospital and also in a second local hospital. The outbreak strain belongs to sequence type ST 233 and carries a VIM-2 metallo-β-lactamase gene. Other molecular epidemiology services were provided for the investigation of clusters of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in paediatric cardiac surgery patients, and nosocomial extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* infections. Following identification of a particular MRSA strain in a local maternity hospital, a South African Field Epidemiology Training Programme (SA-FELTP) trainee studied risk factors associated with MRSA infection among maternity patients. The study will help inform routine practices at caesarean section deliveries at the hospital.

**Public health programmes**

Cryptococcal disease is distinct from many other opportunistic infections in that subclinical disease can be detected a median of three weeks prior to the development of meningitis. Early targeted screening of HIV-infected patients with CD4+ T-cell count <100 cells/mm3 by detection of cryptococcal antigen (CrAg) followed by pre-emptive antifungal treatment is a cost-effective means of reducing cryptococcal meningitis-related mortality. Phased implementation of laboratory-based cryptococcal screening is expected to begin in May 2012 at clinics in Gauteng and Free State provinces. Asymptomatic CrAg-positive patients will be started on fluconazole therapy, provided through the South African Diflucan Partnership Programme, before initiation of antiretroviral treatment.

**RESEARCH AND SPECIAL PROJECTS**

**Insecticide resistance studies in Anopheles funestus and An. arabiensis**

**NICD researchers:** Dr B Brooke, Dr R Christian, Prof M Coetzee, Prof R Hunt, G Kloke, Prof L Koekemoer, Dr G Munhenga, B Spellings

**Collaborators:** Prof H Ranson, Dr C Wondji, Dr C Strode (Liverpool School of Tropical Medicine, UK); Dr N Coetzer (University of Pretoria); Prof S Hanrahan (University of the Witwatersrand)

**Funding:** Multinational Initiative on Malaria/WHO; NHLS Research Trust, National Research Foundation; DST/NRF Research Chair Initiative and the Medical Research Foundation (MRC); African Population and Health Research Centre; Carnegie

*Anopheles funestus* and *An. arabiensis* are the major malaria vectors in southern Africa and several populations in this region are resistant to pyrethroid insecticides. A novel microarray and quantitative real time approach was used to identify genes associated with pyrethroid resistance. This was the first study of its kind in Africa and provided important information on how these mosquitoes develop insecticide resistance. Expression of these genes is not significantly affected by mosquito age, suggesting that other physiological factors account for changes in tolerance to pyrethroids in association with age. Sequencing of the *An. funestus* transcriptome using cDNA libraries significantly advanced understanding of the genetics and genomics of this species.

**Novel vector control studies in South Africa**

**NICD researchers:** Dr B Brooke, Dr R Christian, Prof M Coetzee, C Kikankie, Prof L Koekemoer, J Mouatcho, G Munhenga

**Collaborators:** Dr N Jarvis (Nuclear Energy Corporation of South Africa); Prof R Maharaj (MRC); Dr F Chirwa (University of the Witwatersrand); Dr D Govender (South African National Parks); Dr W Focke (University of Pretoria); Dr A Kilian (Malaria Consortium-International, UK); Dr S Blanford (Penn State University, USA)

**Funding:** Bill & Melinda Gates Foundation; Nuclear Technologies in Medicine and Biosciences Initiative, Nuclear Energy Corporation of South Africa, IAEA, and NRF; DST/NRF chair; Innovative Biological Control of Mosquitoes Consortium; MRC
“Owing to the increasing prevalence of insecticide resistance, additional malaria control interventions are urgently needed”

Owing to the increasing prevalence of insecticide resistance, additional malaria control interventions are urgently needed. The use of the sterile insect technique (SIT) is currently under investigation and it was recently shown that the target *An. arabiensis* population is genetically indistinct and therefore compatible with a laboratory-reared colony. This study concluded that there is a unique opportunity within South Africa for assessing the feasibility of SIT as a malaria vector control option. Other malaria control alternatives are also being investigated including the use of bacterial larvicides and entomopathogenic fungi. Manuscripts detailing the progress of these projects to date have recently been published.

Malaria vector control and transmission dynamics

NICD researchers: Dr B Brooke, Dr R Christian, K Choi, Prof M Coetzee, Prof R Hunt, M Kaiser, Prof L Koekemoer, M Lo, G Munhenga, R Norton, S Oliver, B Spillings

Collaborators: S Knowles (Malaria Control Programme, AngloGold/Ashanti); M Edwardes (Bayer Environmental Sciences, South Africa); Prof G Bouwer (University of the Witwatersrand)

Malaria vector control programmes that rely on insecticide-based interventions such as indoor house spraying with residual insecticides or insecticide treated bed nets need to base their decision-making processes on sound baseline data. An increasing number of commercial entities in Africa, including mining companies, are realising the value to staff productivity by controlling malaria in their areas of operation. Specialised malaria control consultations have been undertaken at localities in Ghana and the Republic of the Congo. Research has also been conducted to increase the stability of insecticide formulations that are used for indoor residual spraying as well as to assess effectiveness of long lasting insecticide treated nets after three years of field use. Field research was facilitated through AngloGold/Ashanti and Gold Fields Limited.

Resolution of mixed dihydropteroate synthase genotypes in respiratory specimens from patients with *Pneumocystis jirovecii* pneumonia in Gauteng

NICD researchers: B Poonsamy, Prof J Frean

Collaborators: Prof M Wong, Prof A Karstaedt (Chris Hani Baragwanath Hospital and University of the Witwatersrand)

Funding: NHLS Research Trust

*Pneumocystis jirovecii* is an unconventional opportunistic fungal pathogen which causes the important AIDS-defining infection, *Pneumocystis* pneumonia (PcP). Point mutations in the *fas* gene, which codes for the dihydropteroate synthase (DHPS) enzyme, have been associated with resistance to trimethoprim-sulphamethazol, used for treatment and prophylaxis of PcP. Sixty-four percent (110/173) of PCR-positive specimens contained *P. jirovecii* with mutant DHPS genotypes and 24% of these were mixed genotypes. Secondary sequencing showed that 13 different genotypes were present. Associations between genotype and clinical outcome are being analysed as part of a larger study.

PcP pneumonia in hospitalised patients with severe acute respiratory infections using an existing surveillance network in South Africa

NICD researchers: B Poonsamy, Prof J Frean, Dr S Walaza, Dr C Cohen

Collaborators: Severe acute respiratory infections (SARI) Study Group

Funding: Global Disease Detection, CDC

Early in the HIV epidemic in Africa, PcP was rarely diagnosed. More recent studies show that PcP is an increasingly important contributor to pneumonia in...
Africa. This is in contrast with industrialised nations, where the number of PCP cases has fallen since the early days of AIDS. The proposed surveillance at selected SARI sites in South Africa may give a better understanding of the prevalence of PCP in both hospitalised adults and children with pneumonia.

Analysis of the strain types of Toxoplasma gondii prevalent in humans and animals in South Africa

Researchers: Dr du Plessis, Prof J Frean
Funding: Global Disease Detection, CDC

The obligate intracellular protozoan parasite Toxoplasma gondii is a significant cause of congenital disease and an increasingly important AIDS-defining opportunistic pathogen. This project is investigating the genotypes and virulence markers of Toxoplasma prevalent in food animals, primary hosts (cats) and high-risk humans, and how they compare to strain types in the rest of Africa and the world.

Genetic characterisation of Cryptosporidium spp. in children from four provinces in South Africa

NICD researchers: B Poonsamy, D du Plessis, KB Mogoye, Prof J Frean
Collaborators: Dr N Samra, P Thompson, F Jori (University of Pretoria); L Xiao (CDC)
Funding: Faculty of Veterinary Science, University of Pretoria

The diversity of Cryptosporidium at species, subtype family and subtype level in diarrhoeic children was investigated in four provinces in South Africa. A total of 442 stool samples from under-5 children were collected under a rotavirus surveillance programme. Fifty-four (12.2%) were positive for Cryptosporidium. The majority were C. hominis (76%) and a high genetic diversity was found with five different C. hominis subtype families. C. parvum was found in 20% of the isolates with three subtype families. One specimen was C. meleagridis, subtype family IIId. This study is the first report of C. meleagridis, various subtypes of C. parvum, and the subtype family of C. hominis in South Africa.

Human cystic echinococcosis in South Africa

NICD researchers: KB Mogoye, Prof J Frean
Collaborators: Dr P Kern, K Wahlers (University Hospitals, Ulm, Germany); Dr T Romig (Hohenheim University, Germany); Prof M Grobusch (Amsterdam Medical Centre and University of the Witwatersrand); Dr C Menezes, Prof M Wong (University of the Witwatersrand)
Funding: German Research Foundation

Cystic echinococcosis (hydatid disease) is a zoonosis caused by the tapeworm Echinococcus granulosus. It affects various herbivore intermediate hosts (sheep, cattle, goats, camels etc) as well as humans that serve as accidental intermediate hosts. The disease is especially prevalent in pastoral communities, where there is close contact between humans, dogs and livestock. The study is investigating the molecular epidemiology, risk factors, geographical distribution as well as the impact of co-infections like HIV, hepatitis B and tuberculosis on the clinical course, treatment and outcome of hydatid disease.

Evaluation of the cryptococcal antigen test as a diagnostic and screening tool

NICD researchers: Dr N Govender, TG Zulu, Dr V Quan, Dr S Walaza, Dr C Cohen, GERMS-SA
Collaborators: Dr C Sriruttan, Dr T Nana (NHLS, Charlotte Maxeke Johannesburg Academic Hospital and University of the Witwatersrand)
Funding: NHLS/CDC CoAg-GERMS-SA; NHLS/CDC CoAg-SARI

To evaluate the new lateral flow assay as a diagnostic tool, specimens from 200 patients with and without cryptococcal meningitis at Charlotte Maxeke Johannesburg Academic Hospital were prospectively enrolled and collected. In another study, adult patients who were hospitalised for pneumonia and had suspected or confirmed tuberculosis for cryptococcal antigenaemia at Tshepong Hospital were prospectively screened. Approximately 200 patients were enrolled into the latter study. The objective was to determine the prevalence of antigenaemia among patients with severe pneumonia.

Molecular epidemiology of cryptococcal disease

NICD researchers: M van Wyk, Dr N Govender
Collaborators: Dr A Litvintseva, Prof TG Mitchell (Duke University Medical Centre)
Funding: NHLS Research Trust, American Society for Microbiology Fellowship, Duke University

The molecular epidemiology of incident cryptococcal disease in South Africa was determined using recently published, consensus guidelines for multilocus sequence typing (MLST), to determine the genotype
and mating type of 250 selected, clinical isolates of Cryptococcus neoformans obtained through national, population-based surveillance, 2005-2009. In another study, MLST was used to compare molecular genotypes between strains of Cryptococcus isolated from the incident and recurrent episodes of cryptococcosis that were separated by at least 120 days. For this study, 185 isolates from 89 cases were included.

**Clinical epidemiology of candidaemia at sentinel hospitals**

**NICD researchers:** Dr NP Govender, GERMS-SA  
**Funding:** SA Global Disease Detection Regional Centre

Species distribution and antifungal drug resistance profiles for isolates causing candidaemia were determined in the first phase of surveillance in 2010. To determine the risk factors associated with candidaemia and antifungal drug resistance, enhanced surveillance at 11 sentinel hospitals in Gauteng and Western Cape was re-initiated in 2011 through the GERMS-SA surveillance programme.

**Emergence of a novel Emmonsia species among HIV-infected persons**

**NICD researchers:** Dr NP Govender, M van Wyk, J Patel.  
**Collaborators:** Dr CR Kenyon, Dr C Bamford, Dr N Rivera, M Lockett, R Leehloena, S Dlamini (Groote Schuur Hospital and University of Cape Town [UCT]); Dr K Bonorchis (NHLS Greenpoint); Dr C Corcoran (Ampath); Dr HF Vismer, G Imrie (MRC); Dr P Naicker (NHLS Tygerberg and Stellenbosch University); H Prozesky (Stellenbosch University); Dr AM Borman (HPA UK, Bristol); Dr G Meintjes (GF Jooste Hospital, UCT); Dr R Colebunders, C Yansouni (University of Antwerp); Dr M Mendelson (UCT)

A novel species of Emmonsia, a dimorphic pathogenic fungus, was detected from a cluster of ~15 HIV-infected patients with disseminated fungal disease. In 2011, the isolates were characterised by culture-based phenotypic methods, DNA sequencing and electron microscopy.

**Surveillance of antimicrobial susceptibility patterns among nosocomial pathogens isolated in tertiary public hospitals**

**NICD researchers:** Dr O Perovic, Prof H Koornhof  
**Collaborators:** Dr P Nyasulu (University of the Witwatersrand); Dr J Murray (NIOH, NHLS)

This project provides information on the current landscape of antimicrobial resistance monitoring on selected organisms at a national level in South Africa. Such information will provide basic support for enhancement of an antimicrobial resistance surveillance system. It is believed that such system could effectively monitor patterns and distribution of antimicrobial resistance.

**Laboratory-based antimicrobial resistance surveillance for nosocomial bacterial pathogens**

**NICD researchers:** Dr O Perovic, Dr A Singh-Moodley  
**Collaborators:** GERMS-SA

Laboratory surveillance for antimicrobial resistance provides the platform for future coordination with the generation of reliable data on the occurrence of antimicrobial resistance in different geographical regions. A limited number of nosocomial bacterial pathogens such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Acinetobacter baumannii* will be identified to monitor trends in resistance.

**HONOURS**

Professor Lizette Koekemoer was second runner-up in the Distinguished Young Women in Science category.

Dr Basil Brooke was elected Fellow of the Royal Entomological Society, London and will act on the editorial boards of the journals *PloS One* and *African Entomology*.

Professor Maureen Coetzee received the following recognitions: African Union Kwame Nkrumah Regional Women Scientists Award; Vice-Chancellor’s Research Award; first runner-up, Distinguished Women in Science Award; elected Fellow of the Royal Society of South Africa; appointed to the Editorial Board of the Transactions of the Royal Society of Tropical Medicine & Hygiene.

Dr Nelesh Govender was nominated for the International Conference on Emerging Infectious Diseases 2012 Leaders Programme.

Dr Olga Perovic received a certificate of appreciation from Ministry of Health from Refik Saydam National Public Health Agency in Turkey.

PhD students M Dhoogra and C Lyons won the best poster and oral presentation awards, respectively, at the 39th Annual Parasitological Society of Southern Africa Conference in Stellenbosch.
PhD student S Vezenegho received a prestigious Carnegie Global Change Fellowship award.

MSc student Bhavani Poonsamy received an award for the best oral presentation at the 4th Federation of Infectious Diseases Societies of Southern Africa Congress in Durban.

**TEACHING AND TRAINING**

Teaching and training in various aspects of general microbiology, parasitology, mycology, entomology and communicable diseases were provided to students at postgraduate level, medical students, technicians, medical technologists, intern medical scientists, pathology registrars, SASTM travel medicine course participants as well as students enrolled for a Diploma in Tropical Medicine and Hygiene. The centre assisted with development of laboratory and clinical training materials for the relevant disease programmes.

**PROFESSIONAL DEVELOPMENT**

Postgraduate candidates graduated:
6 (1 PhD, 3 MSc, 1 MMed, 1 BSc [Hons])

Postgraduate candidates enrolled:
29 (3 Postdoctoral fellows, 9 PhDs, 11 MSc, 1 BSc [Hons] and 5 MMed)

**RESEARCH OUTPUT**

Publications
A total of 39 scientific publications were produced, and the following are highlighted as they present work that has advanced public health and/or laboratory science within South Africa:


**Synopsis:** Surveillance for communicable diseases is dependent on reliable laboratory data. This article summarises eight years of external quality assessment of African public health microbiology laboratories. Antimicrobial susceptibility and serotyping performance were persistently weak.


**Synopsis:** To detect the emergence of *Cryptococcus neoformans* with reduced fluconazole susceptibility, minimum inhibitory concentration testing using a reference broth microdilution method was used to determine if isolates with reduced susceptibility to fluconazole or amphotericin B had emerged among cases of incident disease.

Munhenga G, Brooke BD, Chirwa TF, Hunt RH, Coetzee M, Govender D, Koekemoer LL. Evaluating the potential of the sterile insect technique for malaria control: relative fitness and mating compatibility between laboratory colonized and a wild population of *Anopheles arabiensis* from the Kruger National Park, South Africa. *Parasites & Vectors* 2011; 4: 208

**Synopsis:** This article described the potential of using South African malaria vector populations as part of an additional vector control intervention called 'sterile insect technique.' This is the first of its kind in South Africa and illustrated that a wild vector population will successfully mate with a laboratory-reared population and laid the first step for testing this technology in South Africa.


**Synopsis:** This article describes an outbreak caused by a multidrug-resistant *P. aeruginosa* clone in a haematology unit of a tertiary academic hospital in Cape Town, South Africa. The majority of the patients were severely neutropenic following stem cell transplants.


**Synopsis:** This article describes an external quality assessment pilot programme using dried culture spots for the Xpert MTB/RIF (Cepheid, Sunnyvale, CA) assay for the diagnosis of *Mycobacterium tuberculosis*. This programme was developed at the NHLS.
Books/chapters


Conference presentations
International: 9
National: 23
Local: 2

“An outbreak of malaria in Gauteng province residents without recent travel history was explored”
10 years of supporting public health to South Africans

CENTRE FOR RESPIRATORY DISEASES AND MENINGITIS
Heads

RESPIRATORY DISEASES AND MENINGITIS

The Centre for Respiratory Diseases and Meningitis (CRDM) is a resource for surveillance, diagnostics, expertise and research in the field of communicable respiratory diseases and meningitis for South Africa and the African continent. The centre generates data and provides expertise related to respiratory diseases and meningitis of public health importance to the South African national Department of Health, healthcare providers and regional and international collaborators, to assist with the planning of public health policies, programmes and response to respiratory disease and meningitis outbreaks. The CRDM is also a source of capacity building and formal training within South Africa and the African region. The CRDM includes bacteriology and virology laboratories, and a team of epidemiologists and surveillance field staff.

SURVEILLANCE AND DIAGNOSTICS

Influenza surveillance

The CRDM co-ordinates the influenza and respiratory virus surveillance activities of the NICD which includes the Viral Watch surveillance programme for outpatient influenza-like illness, the Enhanced Viral Watch Programme for hospitalised patients with lower respiratory tract infection and the SARI sentinel surveillance programme. The centre houses the National Influenza Centre, a regional WHO reference laboratory for influenza. Key advances and outputs from the influenza surveillance programmes are detailed below.

New assays and laboratory surveillance function

NICD researchers: M Pretorius, F Treurnicht, O Hellferssee, A Buys, R Lassauniere, M Venter
Funding: NHLS, CDC Co operative agreement for influenza surveillance

A multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) for the 10 most common respiratory viruses developed in the centre was optimised for use on both the Roche Lightcycler and ABI machines, while a more economical 14-plex for common and recently described viruses developed for use on the Lightcycler machines was packaged in kit format and commercialised for use by collaborators. To facilitate rapid identification of a switch in the circulation of influenza B Brisbane vs Yamagata lineage strains and oseltamivir-resistant H275Y signature mutation that may result in vaccine or antiviral failures, respectively, allelic discrimination-based assays were implemented for the influenza HA and NA genes. To improve the efficacy of influenza A(H3N2) viral isolation, the use of MDCK cells transfected with the gene of 2,6-sialyltransferase (MDCK-SIAT1 cells) was implemented.

The virology laboratory was accredited by SANAS in May 2011 as well as the Department of Agriculture, Forestry and Fisheries to support diagnostic testing of influenza in ostriches during the HSN2 avian influenza outbreak.

Respiratory viruses associated with SARI

NICD researchers: M Pretorius, M Venter, C Cohen, S Walaza, J Moyes, J Mcanerney, F Treurnicht, S Madhi, D Naidoo, A Buys
Collaborators: SARI investigators
Funding: CDC Co-operative agreement for influenza surveillance

The newly developed multiplex assay was used to investigate 10 respiratory viruses including influenza A and B, parainfluenza, respiratory syncytial virus (RSV), enterovirus, human metapneumovirus (hMPV), adenovirus (AdV) and rhinovirus (RV) as causes of SARI during 2009-2010. Out of the 8,173 patients tested in this period, 3,240 (40%) had single-infections, 1,426 (17%) had co-infections and 3,507 (43%) were negative. The most common viruses were: RV (2,034, 25%), RSV (1,169, 14%), and influenza A (704, 9%). RSV, hPMV and influenza had seasonal patterns while AdV and RV were detected throughout the year. RV and RSV were associated with most single infections in children 0-1 years.
Evolutionary dynamics of 2009 pandemic influenza A(H1N1) in South Africa from 2009-2010

**NICD researchers:** M Venter, D Naidoo, M Pretorius, A Buys, J Mcanerney, L Blumberg, S Madhi, C Cohen, B Schoub

**Collaborators:** SARI investigators group.

**Funding:** CDC Co-operative agreement for influenza surveillance

Totals of 9,792 and 6,915 specimens from patients with influenza-like illness or SARI symptoms were investigated across South Africa for pandemic influenza A(H1N1) in 2009 and 2010, respectively, and molecular epidemiological investigations of 96 strains were conducted. The pandemic strain occurred as a second epidemic peak following seasonal H3N2 cases in 2009 and in 2010. Progressive drift away from the A/California/7/2009 vaccine strain was observed at both the nucleotide and amino acid level with 2010 strains clustering separate to 2009 strains, although antigenically these strains were still similar to the vaccine strain. No resistance or known pathogenicity mutations were detected.

Risk of death among TB patients hospitalised with influenza in South Africa, 2009-2010

**NICD researchers:** S Walaza, C Cohen, J Moyes, M Pretorius, M Venter, F Treurnicht, S Madhi, D Naidoo, A Buys

**Collaborators:** SARI investigators group

**Funding:** CDC Co-operative agreement for influenza surveillance

TB cases were defined as patients with either a laboratory-confirmed diagnosis of TB, or currently receiving or started on TB treatment at the current admission. The influenza detection rate was similar in patients with (8% (94/1,162) and without (9%) (618/6,935) TB; 76% of the 862 TB patients tested were HIV-positive compared to 50% (2,257/4,512) of those without TB. The case-fatality ratio was 10% (114/1,175) in TB cases as compared to 5% (319/7,004) in non TB cases. On multivariable analysis among TB cases, patients who were co-infected with influenza were more likely to die than patients who tested influenza-negative.

Increased risk of death among HIV-infected persons hospitalised with influenza-confirmed illness

**NICD researchers:** C Cohen, J Moyes, S Walaza, M Pretorius, M Venter F Treurnicht, S Madhi, D Naidoo, A Buys

**Collaborators:** SARI investigators group

**Funding:** CDC Co-operative agreement for influenza surveillance

Among 1,022 patients hospitalised with influenza-associated SARI, HIV infection status was available for 731 (72%). On multivariable analysis controlling for age-group and sex, HIV-infected patients were more likely to have confirmed pneumococcal (36/323, 11% vs 16/379, 4 %,) or TB (52/325, 16% vs 14/393, 4%) co-infection, be infected with influenza type B (vs A) (131/331, 40% vs 108/400, 27%) and have prolonged duration of hospitalisation.

On multivariable analysis controlling for receipt of mechanical ventilation and other underlying conditions; HIV-infection and TB co-infection were independent risk factors for death. HIV-infected individuals experienced a four to five times greater age-adjusted incidence of hospitalisation than HIV-uninfected individuals.

**HIV infection and influenza co-infection increase the risk of elevated blood pneumococcal loads and associated mortality in hospitalised pneumonia patients**

**NICD researchers:** N Wolter, A von Gottberg, M du Plessis, C Cohen, J Moyes, S Walaza, M Pretorius, M Venter F Treurnicht, S Madhi, D Naidoo, A Buys

**Collaborators:** SARI investigators group

**Funding:** Pfizer South Africa, CDC

The prevalence of pneumococcal DNA in blood and factors associated with high bacterial load and death in patients with hospitalised pneumonia was determined. Overall, 7% (372/5,130) tested lytA-positive. On multivariable analysis, the lytA-positive patients with higher blood pneumococcal loads had a higher prevalence of HIV, influenza co-infection and were more likely to be treated with supplemental oxygen. Among lytA-positive patients, increased risk of death was associated with pneumococcal loads of ≥10,000 DNA copies/ml, controlling for oxygen treatment and late presentation to the hospital.
Carriage of high pneumococcal load is associated with an increased risk of developing invasive pneumococcal disease

**NICD researchers:** N Wolter, A von Gottberg, M du Plessis, C Cohen, J Moyes, S Walaza, M Pretorius, M Venter, F Treurnicht, S Madhi, D Naidoo, A Buys

**Collaborators:** SARI investigators group.

**Funding:** Pfizer South Africa, CDC

Factors associated with pneumococcal carriage and high density of nasopharyngeal colonisation (NPC) in hospitalised pneumonia patients were investigated. The prevalence of pneumococcal carriage was 55% (584/1,065), 67% (320/4,81), 79% (19/24) and 44% (245/560) in patients <5, 5-12 and >12 years, respectively. On multivariable analysis, controlling for hospital and age, HIV, influenza, adenovirus and rhinovirus co-infections were associated with an increased risk of carriage while NPC was less prevalent in patients with current underlying TB. Controlling for age, high density of NPC was associated with increased blood lysis positivity.

**GERMS-SA surveillance activities**

**NICD researchers:** A von Gottberg, C Cohen, L de Gouveia, V Quan, S Madhi

**Collaborators:** C Whitney (CDC); K Klugman (Emory University); GERMS-SA collaborators

**Funding:** GERMS-SA is supported by the NICD/NHLS and in part through a cooperative agreement from the CDC

The CRDM is a partner of the GERMS-SA national active laboratory-based surveillance programme. Key outputs from the centre related to this programme include:

**Trends in invasive pneumococcal disease after 7-valent pneumococcal conjugate vaccine (PCV-7) introduction in South Africa, 2005-2011**

Invasive pneumococcal disease (IPD) was tracked through active, national surveillance; isolates were serotyped by Quellung. IPD rates and the number of cases (January to August each year) during pre-vaccine years (average from 2005-2008) were compared to 2011 figures. IPD incidence (episodes per 100,000 population) in children <2 years decreased from 24 to nine (-61%). Among children <2 years (~70% with known HIV status), the reduction of vaccine type (VT) (77 to 10, -87%) was similar to non-PCV serotype (NVT) (48 to 14, -71%) in HIV-infected children. A decrease of VT (44 to 24, -45%) and increase in NVT (33 to 58, 78%) was observed in HIV-uninfected children; serotype 19A increased from six to 16 cases. IPD reductions among HIV-infected children <2 years were likely mainly due to HIV prevention and treatment.

**Risk factors which predict ceftriaxone non-susceptibility of Streptococcus pneumoniae**

**NICD researchers:** C von Mollendorf, C Cohen, L de Gouveia, V Quan, S Madhi, A von Gottberg

**Collaborators:** K Klugman (Emory University), GERMS-SA collaborators

**Funding:** GERMS-SA is supported by the NICD/NHLS and in part through a cooperative agreement from the CDC

From 36,679 IPD cases (2003-2010), 9,217 random isolates were tested for ceftriaxone susceptibility using broth microdilution: 733 (8.0%) were ceftriaxone non-susceptible, with no proportional change from 2003 (32/451, 7.1%) to 2010 (225/2,859, 7.9%). On multivariable analysis, controlling for province, ceftriaxone non-susceptibility risk factors were age <1 (54/447, 12.1%) and 1-4 years (55/415, 13.3%) compared to 15-44 years (52/1,182, 4.4%), vaccine-serotype (210/1,290 (16.3%) vs 12/1,558 (0.8%) and recent β-lactam use (23/154 (14.9%) vs 129/1,770 (7.3%).

**RESEARCH AND SPECIAL PROJECTS**

**Influenza-related mortality among adults aged 25-54 years with AIDS in South Africa and the USA**

**NICD researchers:** C Cohen, S Madhi

**Collaborators:** L Simonsen, J Sample (George Washington, USA); J-W Kang, M Miller, C Viboud (Fogarty International Centre, National Institutes of Health, USA); M Campsmith (CDC)

**Funding:** NHLS

Monthly all-cause and pneumonia and influenza (P&I) mortality rates were compiled for adults with AIDS aged 25-54 years in South Africa (1998-2005) and the US (pre-highly active antiretroviral therapy [HAART] era: 1987-1994; HAART era: 1997-2005). Influenza-related deaths were estimated as excess mortality above a model baseline during influenza epidemic periods. In the US pre-HAART, influenza-related mortality rates in adults with AIDS were 150 and 208 times greater than in the general population for all-causes and P&I, respectively, and 2.5 times higher than in seniors. Following HAART introduction, influenza-related mortality in adults with AIDS dropped 3-6-fold but remained elevated compared to the general population. Influenza-related mortality in South African adults with AIDS in recent years was similar to that in the US in the pre-HAART era.
Genetic and expression level differences in the non-structural proteins as determinants of respiratory syncytial virus pathogenesis

NICD researchers: M Venter; S van Niekerk (MSc student)
Funding: Poliomyelitis Research Foundation (PRF), MRC

Respiratory syncytial virus (RSV) is unique among paramyxoviruses in having two non-structural (NS) proteins that play a major role in inhibiting the host’s interferon response. Sequence and quantification analysis of these proteins in clinical specimens were performed to determine its role in disease severity. The researchers were unable to attribute specific protein polymorphisms with differences in disease severity but identified genome heterogeneity (quasispecies) in 7/57 NS1 and 9/57 NS2 proteins from patients which may contribute to immune evasion of the innate immune response. When comparing patients with (25) mild and (33) severe disease, significantly higher expression levels of NS2 (mean of five times higher) in the out-patient group relative to the hospitalised group were measured. This may suggest that patients who had low NS2 expression had a higher inflammatory response which resulted in more severe disease.

Effectiveness of 7-valent pneumococcal conjugate vaccine against invasive pneumococcal disease in South Africa: a matched case-control study

NICD researchers: C Cohen, A von Gottberg, N Govender, L de Gouveia, M du Plessis, S Meiring, V Quan, C von Mollendorf, M Fortuin-de Smidt, N Naidoo, B Kgokong, V Nokeri, R Ncha, S Lindani, S Madhi
Collaborators: C Verwey (Chris Hani Baragwanath Hospital); S Varughese (Charlotte Maxeke Johannesburg Academic Hospital and Wits); M Archary, F Naby, K Dawood, R Naidoo (University of KwaZulu-Natal and Pietermaritzburg Metropolitan Hospitals Complex); T Avenant, N du Plessis (Steve Biko Hospital and Kalafong Hospital, Paediatric Infectious Diseases Unit, University of Pretoria); G Elliott, U Hallbauer (Universitas and Pelonomi Hospitals, University of the Free State); B Eley, J Nuttal (Red Cross Children’s Hospital, University of Cape Town); L Cooke, H Finlayson, H Rabie (Tygerberg Hospital, Department of Paediatric Infectious Diseases, University of Stellenbosch); A Whitelaw (NHLS/Division of Medical Microbiology, University of Cape Town); D Perez (Nelson Mandela Academic Hospital, Walter Sisulu University); P Jooste, D Naidoo (Kimberley Hospital); G Reubenson, R Kularatne (Rahima Moosa Maternal and Child Hospital and Wits); B Spies (Rob Ferreira Hospital); L Sono (Rustenburg Hospital); P Maredi, K Hamese (Polokwane & Mankweng Hospitals); M Moshe, M Nchabeleng (Dr George Mukhari Hospital and Medunsa University); N Ngobgo, J van den Heever (national Department of Health); L Conklin, J Verani, C Whitney, E Zell, J Loo (CDC); K Klugman (Emory University); K O’Brien (Johns Hopkins University)

In April 2009, South Africa introduced a 7-valent pneumococcal conjugate vaccine using a three-dose schedule (6 and 14 weeks and 9 months) with no catch-up. Four hospitalised controls, matched for age, HIV status and hospital were selected for each case. One hundred and thirty-three HIV-uninfected cases and 535 controls, and 83 HIV-infected cases and 254 controls were enrolled from March 2010 through September 2011. Effectiveness of ≥2 doses against vaccine serotypes was 68% in HIV-uninfected and -18% in HIV-infected children. Effectiveness against all IPD was 46% in HIV-uninfected and 26% in HIV-infected children. These findings suggest that a schedule including three primary doses, efficacious among HIV-infected children in an earlier South African trial, may be needed for HIV-infected children.

Whole genome sequencing of Streptococcus pneumoniae

NICD researchers: Dr A von Gottberg, Dr M du Plessis, Dr N Wolter, KM Ndlangisa
Collaborators: D Everett, (Malawi-Liverpool-Wellcome Clinical Research Programme (MLW); M Antonio (MRC); F Banjul (The Gambia); J-M Collard (Centre de Recherche Medecale et Sanitaire); N Niger, K Klugman, J Vidal (Emory University); L McGee, B Beall (CDC); S Bentley, J Parkhill (Sanger Institute); W Hanage, M Lipsitch (Harvard School of Public Health); D Aanensen, C Fraser (Imperial College)
Funding: Gates funding through Liverpool and Emory Universities

Building on a previous collaboration with the Sanger Institute performing pneumococcal whole-genome sequencing – the first project of over 250 global strains (including from South Africa) of the Pneumococcal Molecular Epidemiology Network clone 1 and which provided insight into both vaccine and antibiotic-mediated evolution of the organism - a follow-up study on pneumococcal serotype 1 whole genomes is planned in collaboration with Malawi, Niger and The Gambia. In addition, a large-scale programme for sequencing of strains related to vaccine introduction in developing countries, in collaboration with Emory University, has been funded by The Gates Foundation and will commence in 2012.
Undiagnosed causes of aseptic meningitis in South Africa: West Nile virus detected in neurological cases in humans in hospitals

**NICD researchers:** M Venter, D Zaayman

A project was undertaken to investigate specific causes of undiagnosed aseptic meningitis cases in hospitals in South Africa. As a pilot study for this project West Nile virus (WNV) was investigated as a cause of neurological disease in hospitalised South Africans in the Pretoria area. From 206 specimens from patients that presented with neurological symptoms that tested negative for common causes, 36 samples (18%) had evidence of WNV exposure as detected by neutralisation assays. Out of 190 specimens that were adequate to perform RT-PCR, IgM and neutralisation assays, seven cases of probable acute WNV infection were identified that were either RT-PCR or IgM and neutralisation-positive on cerebrospinal fluid. This suggests that WNV is being overlooked as an aetiology of neurological disease in South Africa.

**HONOURS**

MM Lassaunière received an award as joint winner in the category ‘Best publication by a young researcher (<35years)’ at the University of Pretoria for her paper entitled ‘A novel multiplex real-time RT-PCR assay with FRET hybridisation probes for the detection and quantitation of 13 respiratory viruses.’

Dr Mignon du Plessis received the Robert Austrian Research Award 2012 in Pneumococcal Vaccinology at the 8th International Symposium on Pneumococci and Pneumococcal Diseases, held in Iguazu, Brazil, March 2012 for a project entitled ‘Molecular epidemiology of Streptococcus pneumoniae serotype 1 in adults and children from South Africa, pre- and post-PCV13 introduction.’

Dr Nicole Wolter was awarded the Discovery Clinical Excellence award for best poster at the Federation of Infectious Diseases Societies of Southern Africa conference, Durban, September 2011 titled ‘Increased risk of pneumococcal pneumonia among HIV and influenza co-infected patients hospitalised with pneumonia in South Africa, 2009-2010’.

Prof Marietjie Venter and Dr Mignon du Plessis were awarded C (established researcher) ratings by the National Research Foundation. Both were previously rated as Y (promising young researcher).

**TEACHING AND TRAINING**

The CRDM assisted with a week-long hands-on training workshop on isolation, identification and anti-microbial susceptibility testing of epidemic-prone organisms in August 2011 for eight laboratory technologists and scientists from various African countries. The workshop was sponsored by WHO AFRO. In conjunction with the CDC, the centre hosted a course on data management and basic epidemiological analysis in African countries in November 2011 at the NICD for scientists from several African countries.

**WHO antimicrobial susceptibility testing workshop**

Centre staff delivered lectures to clinicians on a number of topics at hospitals throughout the country, and lectured to medical students and registrars from the Universities of the Witwatersrand and Pretoria and the South African Society of Travel Medicine.

**PROFESSIONAL DEVELOPMENT**

Postgraduate candidates graduated: 7 (1 PhD [medical virology], 6 MSc [2 in epidemiology, 1 in medical microbiology, and 3 in medical virology])

**RESEARCH OUTPUT**

Publications

A total of 28 papers were published by members of the CRDM. Some key papers are highlighted below.


**Synopsis:** This paper described the evolution of respiratory syncytial virus genotypes since they were first described 10 years ago in South Africa and suggested that experimental vaccines developed against the prototype strains may not be relevant for current circulating subtype B genotypes.

**Synopsis:** This paper has implications for the use of deletion mutants as vaccine candidates in immune-compromised individuals since this method of attenuation may not be sufficient to prevent disease in these patients. It was the first time that mutations lacking the whole attachment protein were detected in humans. This paper was awarded a prize for the best poster at the 7th International RSV Symposium in the Netherlands that was held from 2 to 5 December 2010.


**Synopsis:** The factor H binding protein (fHBP) is currently under investigation as a potential vaccine antigen for protection against meningococcal serogroup B disease. These data highlighted that other serogroups also harbor fHBP and this may increase potential coverage of vaccines initially targeted against only serogroup B.


**Synopsis:** This analysis demonstrated that pneumococcal meningitis among South African children is associated with a high case-fatality ratio and that mortality is increased by HIV co-infection. Increasing access to antiretroviral therapy and a catch-up programme for pneumococcal conjugate vaccine among HIV-infected and malnourished children could reduce this excess mortality.


**Synopsis:** Using surveillance data, it was shown that vaccine failures occurred in both HIV-infected and -uninfected children, and comprised half of the rise in invasive *Haemophilus influenzae* b (Hib) disease detected in South African children 10 years after national introduction of Hib conjugate vaccine. These findings suggest that Hib conjugate vaccine schedules that do not include booster doses may require revision.

**Additional publications**


**Conference presentations**
*International*: 17  
*National*: 12

“West Nile Virus is being overlooked as an aetiology of neurological disease in South Africa”
CENTRE FOR TUBERCULOSIS
Heads

TUBERCULOSIS

The National Tuberculosis Reference Laboratory (NTBRL) was renamed Centre for Tuberculosis.

Strategic functions identified for the Centre for Tuberculosis include provision of specialist diagnostic services, policy development and standardisation of diagnostic methods, and the development and evaluation of novel technologies to advise strategic planning and policy. Further functions concern developing an integrated surveillance system providing epidemiological data for the public, government and scientific community, and utilising surveillance and microbiological data available to design and implement research on a national basis and ensure representation across the country. In addition to these, the centre will continue to support the national Department of Health (DoH) in the development of new TB guidelines and policy, support training programmes and work towards the detection, integration and response to outbreaks and increasing trends in disease prevalence and TB drug resistance.

The Centre for Tuberculosis provides support for the government of South Africa’s objective for its 2012-2016 strategic plan of halving the number of new TB infections and deaths from TB by 2016. The centre, through the Corporate Data Warehouse (CDW), provides weekly notification of new multidrug-/ extensively drug-resistant (MDR/XDR)-TB cases identified by NHLS laboratories to the provincial coordinators of the National TB Control Programme (NTBCP). Surveillance data provided by the centre are available for ongoing evaluation of the impact of TB control measures instituted by the national DoH. The centre plans to also assist the TB control activities of other Southern African Development Community countries.

SURVEILLANCE SERVICES

Prime activities of the Centre for Tuberculosis in support of the NTBCP concern national surveillance of new cases of laboratory-confirmed TB, as well as MDR-TB and XDR-TB and the introduction of enhanced surveillance in line with expanded epidemiologically based strategies of the centre.

A nationwide survey of MDR-TB

MDR-TB remains a significant threat to TB control in South Africa. The first national survey of TB drug resistance in South Africa was performed in 2001/02, producing relatively low estimates of 0.9% - 2.6% of primary MDR-TB. Persisting high prevalence of HIV, as well as the emergence of XDR Mycobacterium tuberculosis strains highlighted the extreme gravity of the TB drug resistance situation in South Africa. The national DoH and the Centre for Tuberculosis are undertaking a nationally representative survey to determine the prevalence and trend of MDR-TB in the nine provinces compared to 2001/02 as well as describe on a population basis the types of MDR M. tuberculosis-complex strains circulating in the country and HIV prevalence in this group.

For the above-mentioned drug resistance survey (DRS), population-proportionate cluster sampling was used to determine the sample size of the survey, the survey sites and to ensure that patients entered into the survey are representative of TB in the country. Patients will be eligible for inclusion in the survey if they are older than 18 years and present as a TB suspect case, according to WHO and International Union Against Tuberculosis and Lung Disease definitions. Sputum specimens will be collected from all consecutive TB suspects attending the facilities of the clusters selected for inclusion. Using the liquid medium-based Mycobacterium Growth Indicator Tube (MGIT) system (Becton-Dickinson, Sparks, Md, USA), cultures will be performed on sputum of all suspect cases in all participating clusters. Cases will be categorised and recorded according to whether TB treatment had been received in the past. Sputum from all survey participants will also be tested for HIV antibodies.

Representative study sites have been chosen across the country and training of clinical and laboratory personnel undertaken to enable standardised processing of specimens. The survey initiation date is May 2012, with one province starting each month and enrolling patients for a maximum of one year. Final results will be available late 2013.
This is the first national DRS being done with cultures and drug sensitivity testing of all TB suspects rather than only smear microscopy positive cases. Prevalence data of drug resistance among new and previously treated cases are key performance indicators of South Africa’s TB control programme and will inform future strategies. Characterisation of M. tuberculosis isolates by molecular typing methods could delineate transmission patterns and clonal expansion and also identify infection control deficiencies.

**Optimising surveillance of TB utilising routinely collected data from the NHLS Corporate Data Warehouse**

All laboratory data from the two laboratory information management systems in use across all public sector laboratories in South Africa are downloaded in real time, creating a sophisticated epidemiological and surveillance resource. Until now, this has not been collated and analysed for TB surveillance. Data for eight provinces are available from 2004, while data from KwaZulu-Natal have been available since 2011. A major project to transform specimen-based data to patient-based data has recently been completed with electronic matching and allocation of unique patient identifiers requiring greatly reduced human intervention. This automated matching will be extended with ‘probabilistic matching’, improving the data matching further.

This project is aimed at improving utilisation of the CDW data for surveillance and feedback to district and municipal level over time, on the rate, nature of TB, laboratory requisition patterns (penetration rates), re-infection rates, treatment outcomes etc. In addition to this, routine drug resistance monitoring will be achieved by matching these data to the Electronic Drug-Resistant TB Register (EDR) which was introduced in 2009 to develop an interface that could on a daily basis transfer data relating to MDR-TB and XDR-TB patients into the EDRWeb of the national DoH.

Envisaged outcomes include good, timely and accurate surveillance data and interpretation to the national programme and provincial health departments on MDR-TB and XDR-TB, identified throughout the country.

**Prospective sentinel surveillance of rifampicin-resistant TB**

In 2011, South Africa began a phased implementation of the Xpert MTB/RIF rapid TB diagnostic testing for TB suspects. This provided an opportunity for the diagnosis of TB and simultaneous assessment of rifampicin resistance to be potentially completed within two hours as it identifies rifampicin resistance on primary testing, albeit at a significantly increased cost. South Africa is the first country to roll out the Xpert MTB/RIF rapid TB diagnostics to scale and this offers the opportunity for a unique surveillance approach in hospitals where it has been rolled out.

In South Africa there is substantial geographical variation in TB rifampicin mono-resistance and true rifampicin plus isoniazid-resistant MDR-TB rates. Little is known about how individuals with rifampicin mono-resistance differ clinically and epidemiologically from those with MDR-TB in the South African setting, including the relationship with clinical outcomes. Enhanced surveillance for rifampicin-resistant TB has recently been incorporated into the already well-established hospital-based enhanced surveillance platform - Group for Enteric Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA) - at sites that have introduced Xpert MTB/RIF rapid TB diagnostics.

The objective will be to deliver accurate, timely and detailed surveillance information on patients with rifampicin-resistant TB to stakeholders in selected sentinel surveillance sites using Xpert MTB/RIF.

With this hospital-based surveillance system, surveillance officers will collect by standardised questionnaire, detailed clinical and epidemiological information on all rifampicin-resistant TB cases diagnosed using the Xpert MTB/RIF platform in sentinel hospital locations and their referring clinics. Information will be collected on previous and current antimicrobial use, HIV status and previous hospital admission data, by patient interview where consent is obtained, or medical record review where patients cannot be located, are discharged, or have died in hospital. A specific addition to current GERMS-SA activities will be an evaluation of the sputum conversion status at six months after treatment initiation using clinical data collected on the EDR.

Surveillance will be initiated with a pilot study at Chris Hani Baragwanath Hospital in June 2012.

**TB in hospitalised patients with severe respiratory infections using an existing surveillance network**

Prospective, hospital-based sentinel surveillance was initiated for SARI in 2009. In this surveillance system, persons hospitalised with acute respiratory illness who meet inclusion criteria, have clinical data and specimens obtained for aetiology testing. During the 2010 influenza season, the severe acute respiratory infection (SARI) surveillance programme was enhanced at selected sentinel sites to expand the case definition for enrolment to include patients with suspected or confirmed TB.
It is important to understand the contribution of TB to severe respiratory illness especially in the context of high TB and high HIV prevalence in South Africa. Including TB in the hospital-based SARI surveillance programme will involve active testing for TB in all patients admitted with respiratory infection at enhanced sentinel sites. The SARI surveillance programme operates at six hospitals in four sentinel sites. These sentinel sites are in the provinces of Gauteng (Chris Hani Baragwanath Hospital), KwaZulu-Natal (Edendale Hospital), Mpumalanga (Mapulaneng and Matikwana Hospitals), and North West Province (Klerksdorp/Tshepong Hospital complex).

All enrolled patients provide respiratory specimens (oropharyngeal [throat] and nasopharyngeal swab in cases ≥5 years old or nasopharyngeal aspirate in cases <5 years of age) for diagnosis of respiratory viruses. Clinical data are collected through a structured interview and hospital record review and include socio-demographic factors, presenting symptoms, onset of symptoms and underlying illnesses including HIV and TB exposure, treatment and immunisation history (children <5 years – routine immunisations and all patients – influenza immunisations). A TB questionnaire on presenting symptoms will be completed for all patients admitted with a respiratory illness.

Surveillance officers at each site will be responsible for identifying cases that fit the current SARI and TB case definitions, as well as taking suitable samples and filling in a SARI case investigation form. Samples collected will be tested for the presence of *M. tuberculosis* by culture or PCR.

The primary objective is to determine the proportion of patients with laboratory-confirmed TB among patients admitted with severe respiratory infection at enhanced surveillance sites by age group, HIV status, duration of symptoms and hospital site.

**DIAGNOSTIC SERVICES**

The Centre for Tuberculosis continues to evaluate new technologies for diagnosis and treatment monitoring of TB. Some of these molecular-based methods evaluated by the centre produce rapid results and promise to revolutionise management of drug-susceptible and especially drug-resistant TB. Recent examples of this are the evaluation and rollout of the GenoTypeMTBDRplus assay (Hain Lifescience, Nehren, Germany) and the GeneXpert (Cepheid, Sunnyvale, CA, USA) technologies in South Africa, in which the NTBRL and subsequently the centre played a prominent role. Future evaluation and possible roll out of other novel technologies will keep South Africa on the cutting edge of TB management.

**RESEARCH**

**Epidemiology of drug-resistant TB among children and adolescents in KwaZulu Natal, Eastern Cape, Limpopo and Gauteng**

**NICD researchers:** Dr G Coetzee, Dr N Ismail, Dr L Erasmus

**Collaborators:** Dr H Menzies, Dr S Bamrah (CDC); Dr M van der Walt (MRC); Dr D Mameja, N Ndjeke, Dr S Dlamini (DoH)

The Centre for Tuberculosis is collaborating with the CDC, the MRC and the national DoH in a study to describe the epidemiology of and access to treatment for drug-resistant TB among children and adolescents in Eastern Cape, Gauteng, KwaZulu-Natal and Limpopo. In addition, the study will evaluate current recording and reporting systems by comparing data collected at the facility, provincial and national levels and the CDW. Information will be provided to the NTBCP on current epidemiology and care practices to guide interventions and plan for future research.

**Automated computer-aided smear microscopy**

**NICD researchers:** Dr G Coetzee, N Ramdin

**Collaborators:** S Kennedy, T Sonoh, Dr J Paganini (Guardian Technologies); Dr D Clark, Prof G Churchyard (Aurum Institute for Health Research)

This study is a joint venture of the Aurum Institute for Health Research, Guardian Technologies International, USA and the NTBRL/Centre for Tuberculosis to develop and evaluate the performance of an automated computer-aided smear microscopy system involving digital image scanning of *Mycobacterium tuberculosis* cells in sputum smears stained with the fluorescent auramine O stain. The system has an automated slide loader and image-capturing hardware which captures fields from stained slides. Images which have been captured are then interpreted using TBDx software. A fully functional system has been developed and assessment of the performance of the system has reached the stage of clinical evaluation under routine laboratory testing conditions.
Host and pathogen contributions in the emergence of XDR-TB: cross-sectional and prospective observational studies

**NICD researchers:** Dr G Coetzee, Prof H Koornhof

**Collaborators:** Prof G Kaplan, Prof D Fallows, Prof B Kreiswirth (University of Medicine and Dentistry of New Jersey, USA); Prof C Gray (University of Cape Town); Prof K Klipstein-Grobusch (University of the Witwatersrand)

The baseline cross-sectional study to determine drug-resistance-related mutations of the MDR/XDR-TB epidemic in Gauteng has been completed and ~300 *Mycobacterium tuberculosis* isolates have been collected and shipped to New Jersey to Dr Kaplan’s laboratory for molecular typing and drug resistance profiling. In the prospective study, cultures from sputum specimens are collected at monthly intervals from MDR/XDR-TB patients at Sizwe Hospital. Of the 200 (100 HIV-positive and 100 HIV-negative) patients earmarked for the study, 109 have to date (April 2012) been entered for investigation of their isolates for acquisition of drug-resistance-determining mutations over time. Genes encoding resistance to isoniazid (*katG* and *inhA*), rifampicin (*rpoB*, core region), quinolones (*gyrA* and *gyrB*), amikacin, kanamycin and capreomycin (*rrs* and *tlyA*), streptomycin (*rpsL*), pyrazinamide (*pncA*) and ethambutol (*embB*) are being studied and sequenced when relevant.

Evaluation of Xpert MTB/RIF assay in mine setting – accessory to the Thibela study

**NICD researchers:** Dr L Erasmus, Dr G Coetzee, M Ramdin

**Collaborators:** Prof G Churchyard, H van der Meulen (Aurum Institute for Health Research)

The centre collaborated with Dr Churchyard (Aurum Institute) on the laboratory work for the Thibela study which initially evaluated the effect of community-wide preventive therapy with isoniazid among mine employees on the incidence of TB in a clustered randomised study performed at the Centre for Tuberculosis. Accessory to this study, was the evaluation of the performance of the GenoTypeMDRTBplus line probe assay in 2009 and subsequently the Xpert MTB/RIF assay in gold miners and the impact of their implementation, compared with conventional smear microscopy, culture and drug susceptibility testing in the MGIT system. The latter study was initiated in 2010 and was completed in April 2011. Approximately 6,900 specimens from this study were processed during this period.

Laboratory support for Médecines Sans Frontieres TB research in Swaziland

**NICD researchers:** Dr G Coetzee, N Ramdin, Z Bhyat

Since 2010, the NTBRL/Centre for Tuberculosis has been providing laboratory support for a project on TB management in Swaziland conducted by the Médecines Sans Frontieres. Part of this project is the evaluation of the performance of the GenoTypeMTBDRplus line probe assay (LPA) at Mbabane and Manzini sites in Swaziland compared with smear microscopy, culture and drug susceptibility testing (DST). The LPA together with conventional smear microscopy, culture and DST are performed at the Centre for Tuberculosis on specimens as they arrive and results issued within acceptable turnaround times.

Transmission of HIV-associated XDR-TB in South Africa (TRAX Study)

**NICD researchers:** Dr G Coetzee, Z Bhyat, M Ramdin

**Collaborators:** Prof B Kreiswirth (University of Medicine and Dentistry of New Jersey)

Following the disastrous Tugela Ferry outbreak of XDR-TB in KwaZulu-Natal in 2006, the present study was designed to prospectively investigate the transmission of XDR-TB in KwaZulu-Natal. The study aimed to determine the proportion of new XDR-TB cases with primary drug resistance, identify risk factors associated with such transmission through epidemiological and social network analysis, and using molecular genotyping, to demonstrate transmission patterns involving persons and locations associated with XDR-TB transmission. For this study, the Centre for Tuberculosis collects from the CDW laboratory-based XDR-TB data from KwaZulu-Natal, and mails these on a weekly basis to the Inkosi Albert Luthuli Hospital laboratory which is responsible for all DST testing in KwaZulu-Natal. XDR-TB isolates identified this way are shipped to the Centre for Tuberculosis for confirmatory testing and assurance of culture purity. These cultures are stored in the centre’s culture repository and an aliquot of each is shipped to Professor B Kreiswirth at the PHRI TB Center, New Jersey for molecular characterisation.

HONOURS

Professor Hendrik Koornhof received the NHLS Lifetime Achievement Award at the Laboratory Medicine Congress in September 2011.
TEACHING AND TRAINING

Teaching and training of medical technologists associated with culture-based routine TB testing, together with medical scientists and technologists involved with molecular technology-based research projects initiated or supported by the Centre for Tuberculosis are being offered in structured courses, as well as in-house training by the African Centre for Integrated Laboratory Training (ACILT), in close collaboration with the Centre for Tuberculosis. During the past year, ACILT assisted with teaching and training of eight technicians or scientists in TB culture, DST and identification of mycobacteria, nine in LPA technology and 15 in both culture- and molecular technology-based courses. More than 600 healthcare staff, both clinical and laboratory-based were trained in Xpert MTB/RIF technology as part of the implementation of Xpert MTB/RIF testing throughout the country.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates enrolled:
1 MSc (Microbiology)

RESEARCH OUTPUT

Publications
Staff authored/co-authored seven peer-reviewed publications during 2011/12.


Koornhof HJ, Coetzee GJ. Molecular diagnostics for TB: Here to stay. South Afr J Epidemiol Infect 2011; 26: 47-49

Koornhof HJ, Coetzee GJ. Times have changed! The molecular revolution in TB diagnostics and detection of drug resistance. South Afr J Epidemiol Infect 2011; 26: 229-234

Klugman KP, Hayden Smith SW, Koornhof HJ. Evidence that prevention of carriage by pneumococcal capsular vaccines may be the mechanism of protection from pneumococcal pneumonia. South Afr J Epidemiol Infect 2011; 26: 221-224


“The Centre for Tuberculosis supports the government’s objective of halving the number of new TB infections and deaths by 2016”
CENTRE FOR VACCINES AND IMMUNOLOGY
The Centre for Vaccines and Immunology (CVI) consists of three working groups addressing the vaccine-preventable diseases of polio, measles and rubella and hepatitis B (the latter working group also manages the other viral causes of infectious hepatitis, in particular hepatitis C). The CVI houses the WHO Regional laboratories for polio, measles and rubella to provide international support for diagnostics, special testing services, provision of reagents especially accredited cell lines, external quality assessment and training to African countries through WHO AFRO. In addition, laboratories for specialised molecular diagnostic testing for a spectrum of viral pathogens are housed in the centre.

Polio working group

The polio laboratory supports seven countries within southern Africa for isolation of poliovirus in addition to performing confirmatory testing on samples tested from other WHO AFRO national laboratories, parallel testing with countries that do not meet accreditation criteria to determine accuracy of results, as well providing assistance with routine testing to laboratories when requested. The laboratory also performs poliovirus serology against all three poliovirus serotypes to monitor immune status of new employees as an occupational requirement to be followed with subsequent vaccination if necessary.

During the course of 2011/12, the molecular laboratory performed intratypic differentiation tests on polio isolates and identified 238 wild-type 1 and 43 wild-type 3 infections from acute flaccid paralysis patients. The countries classified as having re-establishment transmission in Africa (DRC, Chad and Angola) had continuous wild-type 1 transmission that accounted for 95% of identified wild-type 1 cases in Africa; however, only three cases of wild-type 3 poliovirus were identified. Twenty-four circulating vaccine-derived polioviruses (cVDPV) were identified in four countries, i.e. three countries had cVDPV 2 and one had cVDPV 1. In Mozambique, two cases were identified as cVDPV 1. Regarding cVDPV 2, 14 cases were identified in the DRC, seven in Somalia and one in Niger.

A new protocol for transporting poliovirus was established using FTA Elute cards. FTA Elute Cards provide a safer and cost-effective method for transporting and processing poliovirus isolates. Laboratories shipping FTA cards no longer have to be concerned with maintaining the cold chain as they may be transported at ambient temperature. The principal behind these cards is that they are impregnated with a chemical that lyases cell membranes and denatures proteins on contact. Nucleic acids are physically entrapped, immobilised and stabilised for storage at room temperature. The cards protect nucleic acids from microbial and fungal attack, UV damage, oxidation and nucleases. Poliovirus cell culture specimens are applied to FTA cards, allowed to air dry and then packaged for shipping purposes. Once received, the cards are punched and RNA is retrieved for downstream processes through precipitation.

Measles and rubella working group

Technology platform assessment: Alternative sample methods are a useful consideration as part of surveillance and in particular if there are difficulties in obtaining the required specimens and logistics challenges while maintaining specimen integrity. The serology laboratory assessed two methods, namely dried serum spots (DSS) and oral fluid testing. The serology laboratory validated the use of DSS for measles virus detection. The validation was performed as a proxy for the dried blood spot (DBS) filter cards which are in wider use with expansion of the PMTCT programme as it is easier to obtain the blood heel prick rather than venipuncture which is an invasive technique and not ideal for infants, and also since the volume of blood collected is not always adequate. A standardised WHO protocol for DBS preparation and elution of proteins was used and elution was performed for 1.5 hours or overnight intervals. There was no difference in terms of the sensitivity and specificity using either time period with a sensitivity of 90.7% and a specificity of 100% and a sensitivity of 91.3% and a specificity of 100% for the 1.5 hour and overnight elutions, respectively. Alternate sampling technique for measles and rubella IgM testing as well as genotyping using oral fluid is a consideration as this too is a convenient specimen to collect. The serology laboratory tested an oral fluid panel prepared by the HPA and achieved 100% concordance.
The WHO has an EQA programme in place where the national laboratories from WHO AFRO send, on a quarterly basis, a selection of the serum specimens to their regional reference laboratory for retesting. These EQA serum samples are often the only specimens that are available for genotypic analysis of the measles virus strains circulating in those countries, and therefore all EQA sera which had either a positive or indeterminate result for measles-IgM were tested by RT-PCR and hemi-nested PCR for the presence of measles virus RNA. The amplicons of PCR-positive specimens were sequenced and the genotypes determined by phylogenetic analysis. Of the 58% (55/95) of sera that were found to be PCR-positive, genotype B3 (identical to the South African outbreak strain) was detected in specimens from Angola, Botswana, Mozambique, Namibia, Zambia and Zimbabwe. In addition, a different strain of genotype B3 was detected in specimens from Angola, Botswana and Mozambique. It was also found that a single strain of genotype B2 was co-circulating with the genotype B3 strain in Namibia whereas two lineages of genotype B2 were co-circulating with the genotype B3 strain in Angola. Of the specimens received from other African countries, 75% (191/255) were PCR-positive. Genotype B3 comprising several lineages were detected in specimens from Benin, Cote d’Ivoire, Kenya, Malawi, Mauritania, Niger, Nigeria, Rwanda, Sierra Leone, Somalia and Tanzania.

**Hepatitis working group**

The NICD, in collaboration with the CDW, collates the demographic and laboratory data on serology, PCR and genotypes of hepatitis B and C, respectively, from all national NHLS laboratories. This database surveillance provides numbers of positive hepatitis B and C virus cases in the country, to indicate the national burden of disease. Over the past year, quantitative PCR tests for hepatitis B and C viruses were validated due to the discontinuation of qualitative assays for hepatitis C and the poor sensitivity of the qualitative PCR for hepatitis B. The new tests are real-time assays with higher sensitivities (with lower limit of detection of 15 and 20 international units/ml), for hepatitis C virus and hepatitis B virus, respectively, are SANAS-accredited and use a reduced sample input volume of 650 µL of either serum or plasma specimen as compared to previous versions. Genotyping for hepatitis B and hepatitis C viruses is performed using the LPAs, which have been found to be more sensitive than sequencing, with better turnaround times.

**Specialised diagnostic tests**

During 2011, the CVI validated and implemented a number of new real-time PCR quantitative tests for the following viruses: JC and BK viruses, varicella zoster virus, Epstein-Barr virus, cytomegalovirus and herpes simplex virus.

These tests are now SANAS-accredited and being offered for routine diagnostic testing. In addition, the CVI is currently developing a multiplex real-time PCR assay for the detection of viral agents responsible for aseptic meningitis/encephalitis, as well as a real-time PCR assay for the genotypic differentiation of enteroviruses. These assays will be available for routine diagnostic testing in 2012.

**RESEARCH AND SPECIAL PROJECTS**

**POLIO WORKING GROUP**

**The assessment of the combined oral/ inactivated polio immunisation schedule in an African setting**

**NICD researchers:** Dr N Gumede-Moeletsi, G Ntshoe, Prof S Madhi, Prof B Schoub, Prof A Puren

Sera archived at Chris Hani Baragwanath Hospital prior to the introduction of inactivated polio vaccine (IPV) will be compared to sera collected after the introduction of IPV (two years ago) for polio neutralising antibodies. Samples from infants at 6 weeks, 18 weeks, 18 months and 3 years will be tested for polio neutralising antibodies using the standard polio neutralisation assay against all three polio serotypes. Post-IPV introduction data will be obtained from the project below.

**The influence of HIV on the immune response to polio vaccination**

**NICD researchers:** Dr N Gumede-Moeletsi, G Ntshoe, Prof S Madhi, Prof B Schoub, Prof A Puren

Sera archived at Chris Hani Baragwanath Hospital from infants will be tested for polio neutralising antibodies using the standard neutralisation test. Samples will be examined from infants at 6 weeks (for assessment of serotype-specific response to birth oral polio vaccine dose), 18 weeks (after primary vaccine administration), 18 months (after booster dose) and 3 years (for durability of immunity). Neutralising antibody responses will be compared between non-exposed, exposed and HIV-infected infants with or without antiretroviral treatment.
**Polio sero-surveillance to detect serotype-specific immunity gaps in adults**

**NICD researchers:** Dr N Gumede-Moeletsi, G Ntshoe, Prof S Madhi, Prof B Schoub, Prof A Puren

A cross-sectional sero-surveillance study of a convenience sample of adult sera – adult women attending the public sector antenatal clinics in Gauteng – is being conducted. Residual samples will be obtained from the 2011 HIV antenatal study in Gauteng and will be tested for neutralising antibodies to each of the three polio serotypes using the standard polio neutralisation test to assess the extent of gaps in immunity to any of the three polio serotypes.

**HEPATITIS WORKING GROUP**

**Investigation of pre-six-week HBV infection in HIV-exposed infants**

**NICD researchers:** Dr N Prabdial-Sing, Prof AJ Puren, Prof S Madhi, Prof B Schoub, G Ntshoe

This study investigates the extent of intrauterine and peri-natal exposure to hepatitis B virus infection in HIV-exposed and HIV non-exposed infants so as to assess whether a neonatal dose of the hepatitis B virus vaccine is needed in South Africa.

**The prevalence of hepatitis C viral mutations and single nucleotide polymorphisms on the interleukin-28 in patients on pegylated interferon and ribavirin therapy in Johannesburg hospitals**

**NICD researchers:** R Williams, Dr N Prabdial-Sing, Prof A Puren, G Ntshoe

**Collaborators:** Dr A Mohamed, Dr T Burger (Charlotte Maxeke Johannesburg Academic Hospital); Prof R Ally, Dr W Abuelhassan (Chris Hani Baragwanath Hospital).

The objective of this study is to determine whether viral and/or host genetic variations have an influence on the individual’s responses to combination therapy. This study can provide an algorithm to predict therapeutic outcomes, taking into account hepatitis C virus genotype, viral mutations and single nucleotide polymorphisms.

**TEACHING AND TRAINING**

**Polio working group**

In an effort to increase the skills of polio laboratory staff in Africa as well as to optimise laboratory workflow, a consultation workshop with personnel from the polio working group was held in March 2012 to train laboratory personnel from Africa. Training included an overview of the theoretical and practical aspects of real-time PCR, its applicability to poliovirus diagnostics, as well as an overview of result interpretation. The current real-time PCR methodology contains a considerable advantage over conventional PCR diagnostics in that it allows for the identification of vaccine-derived polioviruses as well as viruses which have undergone recombination in either the VP1 or 3D regions of the viral genome without the requirement for the generation of sequence data. Extensive practical training was done, with real-time PCR being used to discriminate between known wild-type and vaccine serotypes, mixtures, as well as known and suspected vaccine-derived poliovirus isolates. Training indicated that reactions may either be performed on cell culture material or extracted viral RNA.

**Measles and rubella working group**

Six registrars were trained in July and two in December 2011. In February 2012, the measles and rubella serology section trained two laboratory personnel from the WHO-supported laboratory in Maputo in Mozambique. The training was aimed specifically towards successfully performing the measles IgM and rubella IgM Siemens EIA assays. Other areas covered were QA and quality control.

**Hepatitis working group**

Lectures on hepatitis were given to five intern medical scientists, one medical technologist, one medical registrar and two new staff members. All were trained on diagnostic laboratory techniques, phylogenetics theory and practical. Intern medical scientists and staff had hands-on practical training according to current standard operating procedures.

**RESEARCH OUTPUT**

**Publications**

Highlights include:


**Synopsis:** Combining global molecular data has proven useful for tracking global measles transmission patterns and for documentation of interruption of indigenous transmission in some countries.


**Synopsis:** The suspected measles case definition also captures rubella cases. Therefore measles surveillance will be improved by the control (and eventual elimination) of rubella transmission. Molecular surveillance is an integral part of any control programme. The dynamics of rubella virus circulation are currently unclear because of limited virological surveillance.


**Synopsis:** The aim of the study was to determine the secondary structure of the 5’ untranslated region (5’UTR) of a predominant HCV genotype in SA, genotype 5a. The 5’UTR is a common target for commercial diagnostic assays, drug therapies and reverse genetics and information on the structural data in this region can be used to better understand and design targets.


**Synopsis:** The 2009 measles epidemic underlined the need for an acceptable vaccine coverage survey to detect vaccination-deficient districts so that national vaccine coverage reaches guideline levels to prevent the cyclical measles epidemics in the country.

“FTA Elute Cards provide a safer and cost-effective method for transporting and processing poliovirus isolates”
DIVISION OF PUBLIC HEALTH, SURVEILLANCE AND RESPONSE
The Public Health Surveillance and Response Division was established to incorporate the Group for Enteric, Respiratory and Meningeal diseases Surveillance in South Africa (GERMS-SA), Outbreak Response, the South African Field Epidemiology and Laboratory Training Programme (SA-FELTP) and Travel Health.

The division facilitates communication and data sharing between the national and provincial health departments and the NICD and provides epidemiological input to other NICD units through collaborative projects and support of surveillance and epidemiological activities and outbreak responses.

**GERMS-SA**

**Section head:** Dr V Quan

**SURVEILLANCE/DIAGNOSTIC SERVICES**

GERMS-SA is a laboratory-based surveillance programme for invasive bacterial and fungal causes of pneumonia, meningitis and diarrhoeal diseases. It is coordinated by the National Microbiology Surveillance Unit (NMSU) and spans many of the centres at the NICD including the Centre for Enteric Diseases, Centre for Respiratory Diseases and Meningitis, and the Centre for Opportunistic, Tropical and Hospital Infections.

This year, a new pathogen, *Candida spp.*, was added to the organisms under surveillance, bringing the total to nine, including: *Salmonella enterica*, *Shigella spp.*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Cryptococcus* spp.

GERMS-SA is an active surveillance programme and relies not only on participating laboratories to submit isolates but also makes use of the CDW to ensure that all cases meeting the case definition are included in the database.

In this reporting period, approximately 200 laboratories (public and private), from all nine provinces reported 17,967 cases according to specific GERMS case definitions. Almost one quarter of the cases (4,074 (23%)) came from 25 sentinel sites across the country where 28 surveillance officers collected clinical information on 3,590 (88%) of patients relating to these specific pathogens.

The aim of GERMS-SA is to use the data to inform and guide public health policy makers in their decisions. The objectives include estimating the burden of both community- and hospital-acquired infectious diseases under surveillance, monitoring antimicrobial susceptibility trends, monitoring the impact of the HIV/AIDS Comprehensive Care, Management and Treatment Programme in SA on HIV-associated opportunistic infections, and evaluating the impact of vaccines included in the EPI.

The work carried out by the GERMS-SA team has significantly contributed to the development of clinical guidelines for pneumonia, meningococcal disease, cholera, cryptococcosis, typhoid fever, etc., and the introduction of pneumococcal conjugate vaccine into the EPI.

More recently, data emanating from the GERMS-SA activities have contributed to the DoH acting decisively in responding to a proposed cryptococcal antigen screening programme which will facilitate the early diagnosis of cryptococcal meningitis.

GERMS-SA work is funded through a CDC cooperative agreement and through the Global Diseases Detection Center. Supplemental funds to improve overall surveillance and particularly surveillance for IPD was obtained from Pfizer.
TEACHING AND TRAINING

NMSU assists in the training of registrars and SA-FELTP residents on surveillance. The broader GERMS-SA team performed 36 site visits to laboratories and hospitals participating in the programme and provided feedback on surveillance data, and training in clinical and microbiological diagnostics.

PROFESSIONAL DEVELOPMENT

Postgraduate candidates graduated: 1 MSc
Postgraduate candidates enrolled: 3 (2 MPH, 1 MSc)

OUTBREAK RESPONSE UNIT

Section head: Dr J Thomas

The Outbreak Response Unit (ORU) provides technical support for all aspects of communicable disease outbreaks and control in South Africa, with special emphasis on optimising the role of laboratory services during these events. The ORU is a source of intelligence during outbreaks, and through working in close collaboration with the provincial and national health departments and other stakeholders ensures a comprehensive outbreak response, as well as development of systems for early detection and improved reporting of epidemic-prone communicable diseases. In addition, close partnerships with the NHLS diagnostic laboratories and NICD centres provide appropriate laboratory diagnostic services during outbreaks and specialised diagnostic tests as required.

PUBLIC HEALTH SERVICES

The ORU’s role in outbreaks may include, but is not limited to, the following: outbreak detection and reporting, field investigation, development of clinical and laboratory guidelines, management of laboratory data and interpretation of results, and recommendations for prevention and control. During 2011, the ORU assisted with a wide spectrum of outbreaks, including:

- Rift Valley fever
- Pertussis
- Enteroviral meningitis
- Avian influenza H5N2 in ostriches
- Meningococcal disease
- Nosocomial outbreaks
- Odyssean malaria
- Travel-associated Legionnaires’ disease
- Rabies

During 2011, the ORU established a network to improve reporting and investigation of foodborne illness, including DoH stakeholders and all NHLS public health laboratories. A foodborne illness registry was also established, with 81 outbreaks reported to the ORU, all of which were followed up by the unit.

As part of the avian influenza H5N2 outbreak in ostriches (Western Cape Province), the ORU conducted a serosurvey among exposed humans to explore the risk and extent of transmission during the outbreak.

The ORU is a member of the Multisectoral National Outbreak Response Team and assists with the development of provincial and national guidelines for priority communicable diseases.

The CDW Alert system, managed by the ORU, facilitates timely notification of laboratory-confirmed cases of priority communicable diseases detected by NHLS laboratories throughout the country (Salmonella Typhi, Vibrio cholerae, and Neisseria meningitidis) to healthcare and public health workers.

The OutNet programme is an NHLS laboratory-based outbreak network with nine provincial laboratory OutNet representatives who act as the key points of contact for provincial public health staff and facilitate laboratory functions in outbreak detection and response.

The ORU publishes a monthly Communicable Diseases Communiqué, which reports recent outbreak and communicable disease cases/issues of relevance. This is distributed to a wide audience including: general practitioners, specialists, infectious diseases and travel medicine societies, and national and provincial public health personnel. In addition, the unit publishes special urgent advisories and communiqués in response to acute events requiring immediate dissemination of information.

TEACHING AND TRAINING

The unit assisted national and provincial health departments in training healthcare workers and public health personnel in epidemic preparedness and response, with an emphasis on case management and appropriate laboratory diagnostic tests for a number of epidemic-prone diseases.

ORU supported the training of future epidemiologists and public health experts through the SA-FELTP. The unit provides supervision to residents during outbreak investigations, and also gives lectures during both short and long courses offered by the programme.
The ORU supported the training of public health specialists at the University of the Witwatersrand by hosting six-month placements for registrars to gain experience in both outbreak response activities and communicable diseases-related public health. Public health registrars from the HPA, UK were hosted for three-month placements as part of the NICD-HPA exchange programme.

SA-FELTP

Section head: Dr Bernice Harris

The SA-FELTP was formed to build epidemiological capacity in the South African health services through applied epidemiology short courses and a two-year Masters in Public Health (MPH)-accredited residency programme. It is funded by PEPFAR with contributions from the DoH and the NICD.

Applied epidemiology short course
SA-FELTP conducted two short courses attended by 50 public health workers from provincial and district health departments to improve their capacity in applied methods of disease surveillance and outbreak investigation. The courses were sponsored by the Mpumalanga provincial and Ekhuruleni District health departments.

MPH accredited two-year residency programme
Nine first year residents were enrolled in 2011 with four seconded and funded by their health departments (one national DoH, two Limpopo and one Northern Cape). The first year residents attended 16 didactic modules at the University of Pretoria and the NICD. The two laboratory track residents attended two laboratory modules with the second year residents. The residents spent 20 weeks in their appointed field sites where they undertook protocol development for research projects, analysed large data sets, investigated outbreaks and undertook surveillance system evaluations, while supporting the national and provincial health departments, NICD and NHLS in various activities.

Ten second year residents completed their field competencies and supported the national and provincial health departments, NICD and NHLS in various activities. They attended a scientific writing and communication workshop, two laboratory, and one epidemiology track modules.

TRAVEL HEALTH

Section head: Prof Lucille Blumberg

This unit provides a consultative service for health practitioners regarding pre-travel advice for travellers and clinical consultations for returning travellers with suspected infectious diseases; develops guidelines for a number of travel-related diseases and neglected diseases; serves as a point of contact and liaison internationally for infectious diseases acquired in southern Africa, and assists with training of travel health practitioners and those studying tropical diseases. There is a focus on zoonotic diseases and emerging pathogens through the One Health approach brought about by the interactions between animal and human health and the environment.

Tropical and travel-related diseases and international health
The unit was involved in formulating the national guidelines for the elimination of malaria in South Africa. A number of consultations took place both locally and internationally to provide support for the diagnosis and management of travellers with travel-related diseases, including East and West African trypanosomiasis and leishmaniasis.

The International Health Regulations (IHR) are due to be implemented by member countries and Prof Blumberg is a member of the IHR working group in South Africa with particular responsibility for the laboratory component of the implementation plan.

TEACHING AND TRAINING

Under- and postgraduate teaching on travel and tropical diseases was provided for undergraduates and postgraduates at the Universities of Stellenbosch and the Witwatersrand as well as participants at the travel medicine course and the Diploma in Tropical Diseases.

RESEARCH OUTPUT

Publications
Staff authored/co-authored the following publications:


**Book chapters**


**Conference presentations**

National: 10
International: 22

“*The aim of GERMS-SA is to use the data to inform and guide public health policy makers in their decisions*”
THE SOUTH AFRICAN REGIONAL GLOBAL DISEASE DETECTION CENTRE
The Global Disease Detection Programme of the CDC is aimed at strengthening the global capacity to rapidly detect, accurately identify and contain infectious disease threats that occur internationally. In July 2010 South Africa was selected as the eighth Global Disease Detection Regional Centre.

The South African Regional Global Disease Detection Centre (SARGDDC) is the only centre in the world with a co-director leadership model and the two co-directors were appointed in the latter half of 2011.

The six core capacities of the Global Disease Detection Programme include:
- emerging infectious disease detection and response;
- field epidemiology and laboratory training;
- pandemic influenza preparedness and response;
- strengthening laboratory capacity;
- zoonotic disease investigation and control; and
- risk communication and emergency response.

The SARGDDC’s strategy relevant to national priorities for the country is being formulated with its partners at the national Department of Health and the NHLS. There are, however, a number of programmes already underway that include the implementation of a cooperative agreement with NHLS to the value of $4.1 million. This agreement supports 21 projects, providing job opportunities to 55 staff members and includes strengthening surveillance of severe acute respiratory disease, avian influenza and TB, GERMS, and various projects for other specific zoonotic pathogens. The agreement also supports the national Department of Health with the malaria elimination programme.

In February 2012, SARGDDC issued a funding announcement as a sole source research cooperative agreement with NHLS to support public health research of national, regional, and global importance. Funding will be determined in the second half of 2013. Three polio projects have been submitted to the CDC Director’s Discretionary Fund for consideration.

SARGDDC has been working with the national Department of Health to strengthen capacities around IHR implementation and supported provincial IHR training of 20 provincial communicable disease coordinators in February 2012.

The SA-FELTP was reviewed by a national and international review team in January 2012 and the recommendations are in the process of being tabled with various stakeholders. The SA-FELTP cohort continues to support outbreak responses nationally and also recently supported the typhoid outbreak in Zimbabwe.

The existing influenza programme activities have been strengthened by the recent appointment of a Deputy Director: Influenza. The programme is aimed at building strong regional partnerships for the detection, surveillance, and response to seasonal, pandemic, and zoonotic influenza in South Africa and selected countries of the Southern Africa Development Community.

The national DoH intends establishing a national health emergency operations centre that will enhance emergency preparedness and improve coordination of emergency responses. The SARGDDC provided funding to the department for the establishment of this centre.

There have been a number of high level visits to South Africa from the US Department of Defense and the Defense Threat Reduction Agency, all aimed at exploring opportunities for collaboration in strengthening early detection and response to emerging and re-emerging health threats and improving biosecurity and biosafety in South Africa and the region.

Co-directors

THE SOUTH AFRICAN REGIONAL GLOBAL DISEASE DETECTION CENTRE

Dr Natalie Mayet

Not present: Dr Rachel Eidex
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>BSL3/4</td>
<td>biosafety level 3/4</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CDW</td>
<td>Corporate Data Warehouse</td>
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<td>CED</td>
<td>Centre for Enteric Diseases</td>
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<td>CEZD</td>
<td>Centre for Emerging Zoonotic Diseases</td>
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<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
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<td>Centre for Opportunistic, Tropical and Hospital Infections</td>
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<td>CRDM</td>
<td>Centre for Respiratory Diseases and Meningitis</td>
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<tr>
<td>CT</td>
<td>cholera toxin</td>
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<tr>
<td>DEC</td>
<td>diarrhoeagenic <em>Escherichia coli</em></td>
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<tr>
<td>DoH</td>
<td>Department of Health</td>
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<tr>
<td>DRC</td>
<td>Democratic Republic of the Congo</td>
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<tr>
<td>DST</td>
<td>drug susceptibility testing</td>
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<tr>
<td>EDR</td>
<td>Electronic Drug-Resistant TB Register</td>
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<tr>
<td>EPI</td>
<td>expanded programme of immunisation</td>
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<tr>
<td>ESBL</td>
<td>extended-spectrum beta-lactamase</td>
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<tr>
<td>EQA</td>
<td>external quality assurance</td>
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<tr>
<td>GERMS-SA</td>
<td>Group for Enteric, Respiratory and Meningeal diseases Surveillance in South Africa</td>
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<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
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<tr>
<td>GUS</td>
<td>genital ulceration</td>
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<tr>
<td>HAART</td>
<td>highly active antiretroviral therapy</td>
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<td>Health Protection Agency</td>
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<td>Health Professions Council of South Africa</td>
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<td>HPV</td>
<td>human papillomavirus</td>
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<tr>
<td>IHR</td>
<td>International Health Regulations</td>
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<tr>
<td>IPD</td>
<td>invasive pneumococcal disease</td>
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<tr>
<td>LPA</td>
<td>line probe assay</td>
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<td>MARV</td>
<td>Marburg virus</td>
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<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
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<tr>
<td>MGIT</td>
<td><em>Mycobacterium</em> Growth Indicator Tube</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<tr>
<td>MRSA</td>
<td>methicillin-resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>MUS</td>
<td>male urethritis</td>
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<tr>
<td>NRF</td>
<td>National Research Foundation</td>
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<tr>
<td>PATH</td>
<td>Program for Appropriate Technologies in Health</td>
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<tr>
<td>Pcp</td>
<td><em>Pneumocystis</em> pneumonia</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PFGE</td>
<td>pulsed-field gel electrophoresis</td>
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<tr>
<td>PMTCT</td>
<td>prevention of mother-to-child transmission (of HIV)</td>
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<tr>
<td>PRF</td>
<td>Poliomyelitis Research Foundation</td>
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<tr>
<td>QA</td>
<td>quality assurance/assessment</td>
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<tr>
<td>RSV</td>
<td>respiratory syncytial virus</td>
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<tr>
<td>RT-PCR</td>
<td>reverse transcription polymerase chain reaction</td>
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<tr>
<td>RVF</td>
<td>Rift Valley fever</td>
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<tr>
<td>SA-FELTP</td>
<td>South African Field Epidemiology Training Programme</td>
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<td>SANAS</td>
<td>South African National Accreditation System</td>
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<td>SARGDDC</td>
<td>South African Regional Global Disease Detection Centre</td>
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<td>SBPRL</td>
<td>Special Bacterial Pathogens Reference Laboratory</td>
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<tr>
<td>STIs</td>
<td>sexually transmitted infections</td>
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<tr>
<td>SVPRL</td>
<td>Special Viral Pathogens Reference Laboratory</td>
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<tr>
<td>TB</td>
<td>tuberculosis</td>
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<td>UCT</td>
<td>University of Cape Town</td>
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<td>VDS</td>
<td>vaginal discharge</td>
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<tr>
<td>VHF</td>
<td>viral haemorrhagic fever</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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