

Intern Scientist Training Programme – Molecular Biology
National Institute for Communicable Diseases

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1. Introduction

1.1 Background to the National Institute for Communicable Diseases

The National Institute for Communicable Diseases (NICD) provides laboratory based surveillance and diagnostic testing for diseases of public health importance to South Africa and the Southern African region. The NICD also sends outbreak response teams to sites confronted with infectious disease epidemics. The NICD houses national and regional referral laboratories. The NICD comprises ten centers, each of which focuses on different diseases, including HIV, Tuberculosis, malaria, diarrhoeal diseases and meningitis. The NICD serves as an expert authority, providing advice to Department of Health and medical practitioners. The NICD is a resource to all universities and technical colleges in South Africa, with multiple training programs in place and a strong complement of university-affiliated staff. The NICD is a division within the National Health Laboratory Service (NHLS), the national laboratory diagnostic network.

2. Training programme in molecular biology

2.1 Description of Training programme

Each intern medical scientist will complete a 2 year training program, unless special circumstances lead to the HPCSA accepting a shorter training period (such as the candidate already having completed a masters or doctoral degree – see special cases below). The training program will comprise a minimum of 18 months in the host Centre. Options for the host Centre are given in section 5 below. Core competencies are expected to be met by each intern. Each host Centre will additionally train in Centre specific competencies.

There will be two optional 3 month rotations. If the candidate or Centre wishes, these rotations may occur in the host Centre. Alternatively they may occur in a different Centre at the NICD or as part of the established 3 month rotation programme run annually in which all labs at the NICD are visited for 2-3 days each.

We have capacity to train two interns in molecular biology per Centre.

2.2 Summary of training programme

A	18 months	Host Centre at NICD	List of Centres given below
B	3 months	Rotation*	Established annual rotation for doctors and scientists spending 2-3 days in each laboratory at NICD
C	3 months	NICD Centre other	List of Centres given below

		than host Centre*	
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*Note: the 3 month modules are optional and may be chosen to occur in the host Centre if necessary, depending on workload and logistical considerations. Choice will be determined by consultation between NICD staff and candidate intern scientist.

2.3 Outline of training programme during eighteen month program

The following general principles will be covered in the 18 month rotation.

- **Good Laboratory Practice:** Regular training is conducted for all staff. Laboratory divisions conducting patient testing have SANAS accreditation for ISO 15189. This will include exposure to: laboratory management, quality assurance activities of the department, role of standard operating procedures and adherence to these, documentation such as quality manual, safety manual etc. This will involve an orientation program and ongoing bench exposure.
- **Safety Training** – regular training provided for all staff. The safety representative in the laboratory will be responsible for the training.
- **General Laboratory techniques:** centrifugation, pipetting, sample preparation, chain of custody, laboratory information system, sample storage.

The 18 month rotation will ensure that the intern emerges with expert knowledge in molecular biology, be able to troubleshoot as well as use initiative to instigate new work in a particular area. During this time, they will be expected to spend at least 50% of their time on routine work done by the laboratory. Research projects they are doing, including the possibility of a Masters project, should fit within the remaining 50% of time.

Each Centre will offer at least two modules to the intern scientist during the 18 month period. Intern scientists will be expected during this time to become proficient in running the routine assays carried out by their unit. They will become expert in the clinical indications for the assays, other testing available for related conditions, requirements and pitfalls of the assays, instrument maintenance and troubleshooting. They will be expected to attend the academic teaching available in the unit eg tutorials, journal clubs etc. They will also attend the bi-monthly NICD research meeting. Interns will also be given an opportunity to present at university research days and/or national conferences.

There are also a selection of optional courses from which to choose including

- All CEU courses offered by NHLS (booklet available)
- Research related courses offered by collaborating universities

The intern scientist will be expected to compile a portfolio suitable for assessment as determined by the HPCSA. This will include a logbook of all assays witnessed and performed as well as one or more projects. The project(s) may include

- Instrument validation
- method validation
- method optimisation
- research question

with appropriate university or ethics approvals if necessary.

2.4 Outline of training included in the 3 month modules

During the 3 month rotations, the intern will be exposed to the theory and techniques spanning the tests offered by the laboratory. The aim is to give an overview of tests available, equipment and expertise available, an introduction to the pathology tested in the various units, and to stimulate the

interest of the intern. The intern will be expected to understand the principles involved in the techniques. They will NOT however be expected to have performed all the techniques mentioned, nor to be able to run the tests without supervision. Rather the aim is to learn which tests are available and for which patients they would be applicable.

3. Assessment:

Assessment will be performed according to rules and regulations stipulated by the HPCSA. Requirements for internal assessment of the candidate will be the following:

3.1 : Ongoing assessment:

Ongoing assessment will consist of an evaluation report of the intern scientist by their direct supervisor 6 monthly and at the end of each rotation completed (ie 6 monthly during the 18 month rotation as well as at the end of each 3 month rotation). The report will be based on the interim portfolio being collated by the intern (see below) as well as an evaluation of his/her general laboratory demeanour including:

- Attention to good laboratory practice
- Participation in academic activities
- Laboratory expertise acquired
- Personal interaction with other staff members
- Research work
- Presentation skills

The evaluation report will be discussed in full with the scientist during an interview and relevant feedback given. Opportunities for improvement will be discussed and noted. A hard copy of the report will be placed in the intern's portfolio.

3.2: Final Portfolio

For registration in the discipline of **Molecular Biology**, the portfolio will consist of:

- logbook of tests performed
- logbook of tests witnessed but not performed
- printout of any oral presentations eg powerpoints given
- copy of any journal articles presented with short explanation of for which forum it was presented eg "presented at haematology journal club, 27 January 2013" and signed by a senior staff member.
- minimum of one project demonstrating capability in the scientific method and computer literacy with relevance to molecular biology. This may have the form of a research paper ie including introduction, methods, results, discussion, conclusion and references or a instrument/test validation report (including background, intra-run precision, inter-run precision, accuracy and references) or a bioinformatic analysis of relevance to patient care.
- Evaluation reports from head of relevant units at the end of each block, indicating strengths and areas for improvement.

- Log of any complaints received or corrective actions undertaken with regards to errors in specimen processing or communication.

4. Special cases

4.1 Six month or one year (shortened) internship period

This rotation will be applicable to those candidates already in possession of a Masters or PhD degree, or other circumstances as dictated by the HPCSA. In the case of a 6 month rotation, the intern will complete 2 X 3-month long modules. One of those modules may include the structured rotation through all labs at NICD.

In the case of a one year rotation, the intern will complete 6 months in the host Centre with 2 X 3 month modules.

The intern will be expected to comply with HPCSA rules and regulations comprising full registration. For internal assessment, the requirement for a research component in their portfolio will be waived. Additionally they will only require two evaluation reports in their final portfolio.

4.2 Concurrent registration in Molecular Biology and another discipline (eg Virology/Microbiology/Immunology)

In the case of an intern wanting to register in two categories, for example Immunology/Virology/Microbiology with Molecular Biology, it will be expected that they meet the assessment criteria for both disciplines. This involves submitting a final portfolio that meets the criteria for a Molecular Biology portfolio as well as for the other discipline. The research project(s) done should be applicable to both disciplines.

5. Competencies

The following are the generic competencies expected from all intern medical scientists on completion of internship:

5.1 Core laboratory Competencies in Molecular Biology

Core laboratory competencies expected from a scientist in molecular biology include the following:

- perform DNA extraction
- perform RNA extraction
- perform quantification and purity assessment of DNA/RNA/protein
- perform a traditional PCR assay
- interpret PCR results via gel electrophoresis
- perform a real-time PCR assay
- interpret results of a real-time PCR assay
- ability to make up solutions using calculations of molar concentration and molecular weights
- sequence sequence assembly and application of general/ advanced tools to analyse data
- genotyping (DNA fingerprinting) of microorganisms (using techniques such as PFGE, MLST, MLVA, etc)

5.2 Core theory for a scientist in Molecular Biology

Core theoretical knowledge for a scientist in molecular biology includes the following:

- principles of cell biology and cell structure

- principles of DNA replication, transcription, translation, cell division
- Understanding of nomenclature for triplet amino acid codes
- principles of epigenetics
- principles of molecular amplification techniques including PCR, isothermal amplification methods.....
- use of bioinformatics tools
- principles of DNA sequencing
- understanding of statistical concepts including sensitivity, specificity, positive and negative predictive values of laboratory assays,
- principles for method evaluation and method comparison

5.3 Optional Host Centres with specific competencies

5.3.1	Centre for Vaccines and Immunology
5.3.2	Centre for HIV and STI
5.3.3	Centre for Respiratory Disease and Meningitis
5.3.4	Centre for Enteric Diseases
5.3.5	Centre for Tuberculosis
5.3.6	Centre for Opportunistic, Tropical and Hospital Infections
5.3.7	Centre for Emerging and Zoonotic Diseases

5.3.1 Centre for Vaccines and Immunology

The Centre for Vaccines and Immunology provides laboratory support to South African and Southern African departments of health for surveillance of vaccine preventable diseases including acute flaccid paralysis (polio) and measles. Specialized molecular diagnostic services are offered to South African stakeholders for Hepatitis B and Hepatitis C. The Centre leadership comprises Dr Melinda Suchard (administrative head and immunology lead), Dr Nicky Gumede (polio lead), Dr Nishi Prabdial Sing (hepatitis lead), Sheilagh Smit (measles lead).

Polio testing

In support of the Global Poliomyelitis Eradication Initiative (GPEI) initiated in 1988 by the World Health Assembly, any new onset of hypotonic weakness (acute flaccid paralysis) in a child aged less than 15 years of age is investigated for polio virus. Acute flaccid paralysis surveillance is a GPEI strategy to detect poliovirus circulation, re-importation of wild poliovirus into polio free-areas or regions and emerging vaccine derived polio viruses (VDPVs).

The Centre for Vaccines and Immunology is a national and World Health Organisation accredited regional referral laboratory for AFP surveillance. The Centre conducts polio virus isolation, identification and molecular analysis for South Africa, Angola, Botswana, Lesotho, Mozambique, Namibia, Swaziland, Angola and the Democratic Republic of Congo. Testing involves three aspects – maintenance of sterile cell lines, isolation of stool samples into cell lines with daily monitoring for cytopathic effects, and molecular typing of strains using polymerase chain reaction (PCR) and sequencing. Reporting of isolates and sequence information is performed weekly according to standardized templates in the format required by the World Health Organisation. Results are also given to clinicians treating the patients.

Measles testing

As one of the most contagious infectious disease, measles virus causes significant morbidity and mortality in children, and especially those who are malnourished and/or immune-compromised. Since the aim of Millenium Development Goal 4 is to reduce the overall number of deaths among children under 5 years of age by two-thirds from 1990 to 2015, routine measles vaccination coverage was selected as an indicator of progress towards this goal. Aggressive efforts to improve measles vaccination coverage resulted in an estimated 86% reduction in measles-related mortality globally between 1990-2008, representing a 23% reduction in all-cause mortality in the under-5 age group in this period.

The Centre for Vaccines and Immunology is the national and WHO regional referral laboratory for measles surveillance. Serology, specifically the detection of measles-specific IgM antibodies, is the most commonly used method of laboratory diagnosis of acute measles infection. The Centre provides a diagnostic service to referring clinicians. Additionally the Centre provides reference testing for the external quality assurance program run by the World Health Organisation for measles. The Centre also uses PCR and sequencing to genotype measles strains and analyse the phylogenetic trees for any measles outbreak. Molecular epidemiology provides information to authorities regarding geographic distribution and evolution of measles strains.

Since rubella presents with similar clinical symptoms, laboratories often test for IgM against both viruses in suspected measles cases. Rubella surveillance projects have been run by the Centre and future rubella surveillance is planned.

Hepatitis B testing

Hepatitis B vaccination was introduced into the South African Expanded Programme of Immunization in 1995. The HIV epidemic has increased the burden of disease in South Africa from Hepatitis B. There is no national surveillance program for hepatitis B. The Centre for Vaccines and Immunology provides a diagnostic service for hepatitis B, releasing results to referring clinicians. Additionally the Centre performs genotyping to identify circulating strains of hepatitis B. Any positive sera from the South African National Blood Service are also genotyped by the Centre. Genotyping results are interpreted together with serological testing results. Genotyping gives a snapshot of the burden of disease caused by each strain.

Hepatitis C testing

There is currently no vaccine against hepatitis C and no national surveillance program. The burden of disease of hepatitis C in South Africa is not well defined. The Centre for Vaccines and Immunology provides diagnostic testing for hepatitis C, reporting results to referring clinicians. The Centre also performs genotyping to identify the strain. Strain information provides prognostic information to the clinician. Additionally the Centre performs genotyping for any positive hepatitis sera from the South African National Blood Service.

Immuno-regulation laboratory

The immuno-regulation laboratory in the Centre aims to identify correlates of protection to Tuberculosis and other infectious diseases by characterising the immune response and factors that limit the effectiveness of the immune response. Factors such as regulatory T cells, cytokines, Human leucocyte antigens and antibodies to Human leucocyte antigens are studied by flow cytometry and molecular methodologies.

Specialized Facilities and equipment in Centre for Vaccines and Immunology

- Thermal Cycler
- 7500 Real Time PCR System
- Horizontal Gel Electrophoresis Apparatus
- Flash Gel Dock
- UV Transilluminator
- Nanodrop Spectrophotometer
- Life Technologies 3130XL Genetic Analyzer

Specific competencies from Centre for Vaccines and Immunology

- Neutralization assay for assessment of polio immunity
- Exposure to Intratypic differentiation (ITD) and Vaccine Derived Poliovirus (VDPV) screening of the Poliovirus using Real Time PCR
- Sequencing and Phylogenetic analysis of the Poliovirus
- PCR for measles virus
- PCR for rubella virus
- Genotyping measles virus
- Genotyping rubella virus
- Luminex based HLA typing

5.3.2 Centre for HIV and Sexually Transmitted Infections

The Centre for HIV & Sexually Transmitted Infections (STI) is a resource of knowledge and expertise in HIV and other regionally relevant STIs to the South African Government, to SADC countries and to the African continent at large, in order to assist with the planning of policies and programmes related to the control and effective management of HIV/STIs. The Centre also aims to be a place of academic excellence in terms of both research and teaching/training. 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 PhD and MSc students. The Centre for HIV & STIs leadership team consists of Professor David Lewis (Administrative Centre Head, STI section lead), Professor Lynn Morris (HIV Research section lead), Professor Adrian Puren (HIV Sero-Molecular Diagnostics section lead) and Professor Caroline Tiemessen (Cell Biology section lead) amongst others.

HIV prevalence and incidence surveillance

The Centre supports the National Department of Health's (NDoH) Annual Antenatal HIV-1 Prevalence and HIV Incidence survey and the South African Prevention of Mother to Child Transmission (PMTCT) Effectiveness study at 4-8 weeks post-partum. The latter survey is critical to inform on the continued success or otherwise of the decline in HIV transmission in the PMTCT setting. To improve surveillance of HIV incidence, methods are being applied to various surveys and since this methodology is new to the field in South Africa, optimum methods for analysis are being assessed.

HIV drug resistance surveillance

The Centre's HIV drug resistance laboratory is the designated Centre for national surveillance activities and also serves as a WHO regional HIV drug resistance laboratory. The laboratory has recently extended the scope of testing to include genotyping of dried blood spot specimens in addition to plasma, allowing for surveillance of resistance in paediatric patients. On-going surveys of transmitted resistance make use

of specimens collected from young women in their first pregnancy who participate in the annual antenatal clinic survey. The laboratory also offers training to visiting scientists from local and sub-Saharan Africa.

STI clinical syndrome, aetiological and gonococcal antimicrobial resistance surveillance

The Gauteng STI surveillance project, run by the Centre in collaboration with the Gauteng Provincial Department of Health (DoH), collects STI syndrome data from public clinics. In collaboration with the NDoH, provincial DoHs, Alexandra Health Centre and NHLS laboratories, the Centre undertakes aetiological surveillance of three major STI syndromes (male urethritis syndrome, MUS; vaginal discharge syndrome, VDS; genital ulceration syndrome, GUS), as well as surveillance of gonococcal antimicrobial resistance, in Gauteng (Johannesburg), Mpumalanga (Nelspruit) and the Northern Cape (Kimberley) provinces. Molecular, serological and bacteriological methods are employed to test for a variety of STI pathogens

HIV-1 rapid testing quality assurance and post-marketing surveillance of HIV rapid test devices

The NDoH has expanded HIV testing in South Africa in the past three years with well over 15 million individuals tested. A critical component is the quality assurance of testing. PEPFAR-funded quality assurance coordinators conducted 235 on-site monitoring and evaluation visits to assess progress with HIV rapid testing and specifically the introduction of the use of internal quality assurance specimens as part of quality assurance monitoring. Three HIV rapid test kits were awarded the government tender in 2011. A key follow-on activity undertaken by the Centre was the post-marketing surveillance of the lots/batches of devices prior to release in testing sites.

HIV external quality assurance schemes

Centre staff coordinate the HIV EQA program for NHLS-participating laboratories. Serology panels are distributed to 181 laboratories and HIV RNA panels to 18 participating laboratories. Participation in the schemes is mandatory and reporting of both the serology and molecular scheme results as part of the quality improvement processes.

Support for HIV vaccine trials

The Centre provides results from validated end-point humoral antibody and molecular HIV assays for the HIV Vaccine Trial Network (HVTN).

Correlates of Protection against HIV-1

The Centre is involved with multiple projects to characterize innate and adaptive aspects of protection against HIV disease. These include the role of CCR5 and its ligands, the role of natural killer cells and the role of host genetics, particularly at the human leucocyte antigen (HLA) loci.

Equipment for Centre for HIV and STI

Areas for molecular work include a nucleic acid extraction laboratory (18 m²) with a MagNa Pure Extraction System, or NUcliSens easyMAG 2 pre-PCR areas (2 x 10m²) with a UV light to decontaminate surface areas and a post-PCR laboratory (80 m²) with 10 PCR thermocyclers, a Gel-doc system, a Nanodrop spectrophotometer, a transilluminator, 2 Biocap extraction hoods and 3 electrophoresis power units. A separate laboratory (24 m²) is available for bacterial work and contains a Biohazard class II safety cabinet. The sequencing laboratory (10 m²) contains 2 Genetic Analyzers, a 3130xl and a 24 capillary 3500xl and 2 ultra-deep sequencing platforms, a 454 Junior and MiSeq. Currently these areas are utilized in the single genome amplification, sequencing and cloning of HIV-1 envelope genes.

Protein purification facilities include an AKTA Prime Pump, Chemi-doc gel system, SDS-PAGE tanks, Western-Blot equipment, a microplate washer and a microplate spectrophotometer.

Specific competencies from Centre for HIV and STI

HIV drug resistance genotyping

- Sample handling.
- Extraction of RNA from plasma and dried blood spots.
- PCR for HIV-1 *pol* gene.
- Sequencing of the pcr products.
- Sequence analysis using 2 softwares, Sequencher Version 5 and ReCall.
- Interpretation of HIV-1 drug resistance report from the Stanford database.

Bioinformatics for HIV drug resistance

- Align and edit sequences using Bioedit and Mega 5.
- Calculate genetic distances between patient viruses.
- Generate neighbour-joining tree and maximum-likelihood tree using Mega 5, PhyML and PAUP.
- Interpretation of phylogenetic trees and contamination identification.

5.3.3. Centre for Respiratory Diseases and Meningitis

Background

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 Centre is also a source of capacity building and formal training within South Africa and the African region. CRDM includes bacteriology and virology laboratories, and a team of epidemiologists and surveillance field staff.

Specific competencies

- Molecular detection and serotyping/grouping of *S. pneumoniae*, *N. meningitidis* and *H. influenzae*
- Strain characterization of organisms using multilocus sequence typing or whole genome sequencing
- Molecular detection of atypical pneumonia-causing pathogens (*B. pertussis*, *M. pneumoniae*, *C. pneumoniae*, *Legionella* spp.)
- Taqman Array Card technology for simultaneous detection of multiple respiratory pathogens (viruses and bacteria)
- Influenza and other respiratory virus isolation
- Hemagglutination inhibition assays to determine sensitivity of circulating influenza viruses to vaccine induced antibodies or to evaluate exposure to novel or zoonotic influenza A viruses and

other respiratory pathogens and lastly this assay can be used to evaluate the immune responses induced in vaccine recipients.

- Conventional live virus based microneutralization assays.
- Pseudovirion-based microneutralization assays for BSL3 pathogens performed under BSL2 conditions
- Conventional PCR and sequencing of respiratory virus gene fragments
- Allelic discrimination real time RT-PCR assay to identify influenza B lineages and to identify known drug resistant mutation in the M and NA genes of influenza
- Phenotypic assay to determine sensitivity of influenza virus neuraminidases to antiviral drugs
- Multiplex real time RT-PCR for human respiratory viruses including influenza viruses
- Determination of cytokine levels in respiratory samples and/or other biological samples
- Investigation of zoonotic respiratory viruses that cause respiratory / neurological symptoms
- Virus discovery for unknown causes of respiratory disease
- Genome sequencing using both Sanger and next generation sequencing methods

Specialized Facilities and equipment in Centre for Respiratory Diseases and Meningitis

- Thermal Cyclers
- 7500 Real Time PCR Systems and Roche 480
- ViiA7 real time PCR machine with block and centrifuge for TLDA cards
- Horizontal Gel Electrophoresis Apparatus
- Western blot apparatus with semi-dry transblot
- UV Transilluminator
- Nanodrop Spectrophotometer
- Bio-Rad Bio Plex 200
- Multilabel plate reader with stacker
- Glomax Luminometer
- GeneGnome imaging system for luminescence
- Bio-Rad Gel documentation system
- Magpure 96 RNA extraction system
- Nanophotometer

5.3.4 Centre for Enteric Diseases

The Centre for Enteric Diseases (CED) of the NICD was established in 2012 through the amalgamation of the Enteric Diseases Reference Unit and the Viral Gastroenteritis Unit of the NICD. 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, we actively motivate all diagnostic laboratories throughout the country to voluntarily submit limited demographic details and isolates to us centrally. In exchange, we offer 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, E-tests are used to determine the minimum inhibitory concentration (MIC) of each isolate to antimicrobial agents, according to CLSI guidelines. The bacterial division also performs genotypic characterization 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 *Salmonella* Typhi, *Shigella dysenteriae* type 1 and *Vibrio cholerae*. A multiplex polymerase chain reaction is used to elucidate the presence of virulence (toxin) genes in diarrhoeagenic *E. coli*. The division is developing its molecular research laboratory involved with characterizing the molecular basis for antimicrobial resistance in these pathogens and has plans to further characterize the mechanism of disease due to these pathogens at a molecular and cellular level.

The introduction of the rotavirus vaccine into the national expanded program of immunization (EPI) in August 2009 was a positive step in combating diarrhoeal disease burden in children < 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 program 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 in the South African population. Expansion of the current diarrhoeal surveillance program to include more sentinel sites and offer a wider range of screening options will increase the quality and representativeness of the data generated.

Specific competencies in Centre for Enteric Diseases

- *Salmonella* species: identification, serotyping and antimicrobial susceptibility testing
- *Shigella* species: identification, serotyping and antimicrobial susceptibility testing
- *V. cholerae* (O1 and non-O1): identification, serotyping and antimicrobial susceptibility testing
- Diarrhoeagenic *Escherichia coli*: identification (via conventional PCR to detect for virulence genes), serotyping and antimicrobial susceptibility testing
- Stool processing to extract DNA and RNA
- *Campylobacter* species: real-time PCR detection and identification
- *V. cholerae* and cholera toxin: real-time PCR detection and identification
- *Salmonella* species and *Salmonella* Typhi: real-time PCR detection and identification
- Genotyping: pulsed-field gel electrophoresis (PFGE) analysis of enteric pathogens
- Genotyping: multiple-locus variable-number tandem-repeats analysis (MLVA) of enteric pathogens
- Genotyping: multi-locus sequence typing (MLST) of enteric pathogens
- DNA sequencing

- Rotavirus: detection and genotyping via ELISA and RT-PCR
- Adenovirus: detection via ELISA and real-time PCR
- Norovirus: detection via real-time PCR
- Astrovirus: detection via real-time PCR

5.3.5 Centre for Tuberculosis (CTB)

Functions of the CTB include provision of specialist diagnostic services, policy development and standardization 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 to design and implement research on a national basis. In addition to these, the Centre will continue to support the National Department of Health (NDoH) in the development of new TB guidelines and policy, support training programmes and work towards the detection, integration and response to outbreaks. The CTB 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 also plans to assist with TB control activities of other Southern African Development Community Countries.

Trends in multidrug-resistant TB and emergence of extremely drug-resistant TB

There was an estimated prevalence of ~650000 cases of multidrug-resistant TB (MDR-TB) in the world in 2010, while in 2008 there were ~1500 MDR-TB-related deaths. The World Health Organization (WHO) calculates that only 16% of the estimated number of MDR-TB patients that needed treatment in 2010 received treatment directed against MDR-TB.

As part of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance of the WHO/International Union against Tuberculosis and Lung Disease (IUATLD), a country-wide drug resistance survey was conducted in South Africa during 2000-2001. The survey recorded MDR-TB rates in the provinces which varied from 0.9% to 2.6 % in new cases and from 3.9% to 13.7 % in retreatment cases. A second national drug resistance survey, details of which feature in this report, will be conducted in South Africa during 2012-2013.

The National Health Laboratory Service (NHLS) of South Africa provides facilities for mycobacterial culture and drug susceptibility testing (DST) covering rifampicin and isoniazid for the diagnosis of MDR-TB as well kanamycin/amikacin and the fluoroquinolones ofloxacin/moxifloxacin for the detection of extremely drug-resistant TB (XDR-TB) on a regional basis throughout the country.

Based on information extracted from the CDW, the numbers of laboratory-confirmed MDR-TB patients in eight provinces (figures for KwaZulu-Natal were not available on CDW) were 5348 in 2007 and 6198 in 2010. The proportion of MDR-TB cases compared with newly diagnosed culture-confirmed TB cases during this period was 5.0%. During the same period the numbers of XDR-TB patients diagnosed per year in the eight provinces increased from 260 to 454, constituting on average 6.2% of the annually diagnosed MDR-TB cases.

Monitoring of the genetic basis of resistance to anti-TB drugs

Not only is surveillance of the extent of resistance to anti-TB drugs important but monitoring of the genetic basis for such resistance is also a priority for the development and upgrading of rapid molecular methods for resistance detection, as well as formulating strategies for prevention and containment, and serving as a basis for new drug development. South Africa with its high prevalence of TB and suboptimal performance of directly observed therapy, short course (DOTS) and DOTS Plus for management of known or suspected drug-resistant TB, coupled with good laboratory facilities and expertise, offers ideal circumstances for the study of emerging and ongoing drug resistance. Unsurprisingly, study of the problem of drug resistance is receiving a very high priority in the 5-year business plan of the CTB and features in a large proportion of the projects envisaged in the plan.

Epidemiological research and need for enhanced surveillance

The realization of the importance of epidemiological research in the field of TB has led directly to the expansion of the National Tuberculosis Reference Laboratory (NTBRL) to include as an equal partner a well-staffed Epidemiology division in the newly established CTB. The Epidemiology division is headed by a highly experienced epidemiologist from the Health Protection Agency in the UK who was specifically seconded to South Africa for three years to promote and expand epidemiological expertise and activities in this country.

Epidemiological research planned for the next five years will focus on public health surveillance including the large drug resistance survey designed to be representative of TB drug resistance in the country. Projects include optimising surveillance of TB utilising routinely collected data from the CDW, an enhanced surveillance project comprising sentinel surveillance of rifampicin-resistant TB and studying the emergence of rifampicin mono-resistance in the country, and surveillance of TB in hospitalised patients with severe respiratory illness, using an existing GERMS surveillance network in South Africa.

Specialized Facilities and equipment in Centre for Tuberculosis

The Centre for Tuberculosis laboratory facilities include a P3 laboratory for handling of viable infectious agents and a P2 laboratory for downstream molecular and typing applications. Our Laboratories have a range of conventional and molecular diagnostics used for the detection of tuberculosis.

P3 Facility: 356m²

The P3 facility is separated into three sections which include dedicated Culture laboratory, Microscopy and Molecular Laboratory and Drug Susceptibility Facility.

P2 Facility: 331m²

The Facility consists of four fully equipped molecular biology laboratories for sample preparation, extraction, Conventional and Real-time PCR assay generation, Post amplification analysis, Sequencing and Typing analysis and storage. The Facility is dedicated to providing diagnostic services, Sequencing and Strain typing for Mycobacteria.

Specialized equipment

- Nucleasens Easy Mag extraction system
- BSC Class II
- PCR workstation
- ABI 3130 sequencer
- StepOne Plus Real-time PCR instrument
- Conventional PCR thermocyclers
- Riboprinter
- Gel-Doc system
- X-Ray developer
- BioTek plate reader
- Gel Electrophoresis system
- NanoDrop (DNA quantification)
- Twincubators (Hain Lifescience PCR detection)
- GT Blot (Hain Lifesciences PCR detection)

- Qubit analyser
- MiSeq sequencer

Specific competencies

- Performing Xpert MTB/Rif assay and interpreting results
- Perform MTBDRplus/CM/SLT assays and interpret results
- Perform Spoligotyping an MIRU-VNTR typing and analyse results including phylogenetic analysis
- Performing sequence run with library preparation on the MiSeq and interpreting sequence data with data quality assessment

5.3.6 Centre for Opportunistic, Tropical and Hospital Infections

The Centre for Opportunistic, Tropical and Hospital Infections aims to prevent and control opportunistic, tropical and hospital infections in South Africa by providing:

- Strategic information obtained through surveillance and research to the Department of Health and other major stakeholders
- Technical support for public health programmes such as the malaria control programme and the cryptococcal screening programme
- Reference laboratory services in the fields of parasitology, mycology, entomology and bacteriology
- Laboratory support for outbreak response
- Training for clinical, laboratory and public health personnel to ensure optimal diagnosis and control of diseases

The Centre focuses its efforts on opportunistic infections, particularly those that are HIV-related; tropical infections, especially malaria and its vectors; and nosocomial infections, concentrating on antimicrobial resistance in the hospital setting.

Parasitology Working Group

The group provides a specialised parasitology reference service for routine diagnostic medical laboratories. In addition, some important opportunistic pathogens form the focus of its diagnostic, research, teaching, and surveillance activities. *Pneumocystis jirovecii* is an unconventional opportunistic pathogen that causes the important AIDS-defining infection, Pneumocystis pneumonia (PcP). The estimation of the burden of PCP in HIV-positive patients at sentinel sites in South Africa is a current project. Other opportunistic diseases being studied are microsporidiosis, toxoplasmosis, and free-living amoeba infections. Malaria and echinococcosis are other areas of interest for the group.

Specific competencies for Parasitology

- PCR for *P. jirovecii*
- RFLP/ Sequencing for *P. jirovecii* DHPS genotypes
- Multiplex PCR for malaria
- PCR for microsporidia

Mycology Working Group

The mycology working group focuses on laboratory-based surveillance for two fungal diseases of public health importance in South Africa: cryptococcal meningitis and candidaemia. Research activities in mycology are focused on developing and validating new diagnostic assays and defining risk factors for

fungal disease and antifungal drug resistance. The reference laboratory also holds a large collection of pathogenic fungi.

Cryptococcal surveillance

In collaboration with the Department of Health and other partners, the group is also leading efforts to implement and monitor a laboratory-driven cryptococcal screening programme at ± 500 clinics in Gauteng and the Free State (phase 1) - this programme aims to prevent deaths associated with cryptococcal meningitis. Development of clinical guidelines for management of fungal infections is an important activity – members of the group continue to participate in development of South African and WHO guidelines for HIV-associated cryptococcal meningitis. As part of surveillance the Centre also has projects focusing on molecular epidemiology of incident cryptococcal disease in South Africa using recently published, consensus guidelines for Multilocus sequence typing (MLST), to determine the genotype and mating type of selected, clinical isolates of *Cryptococcus neoformans* obtained through national, population-based surveillance, 2005-2009.

Candidaemia surveillance

As part of Candidaemia surveillance, the Centre also focuses on detection of undiagnosed outbreaks of *Candida parapsilosis* using microsatellite genotyping assay and genotypic resistance testing for antifungal agents (Echinocandins).

Identification of unknown or difficult fungi

A specialised mycology reference service is provided to routine diagnostic medical laboratories – including phenotypic and sequence-based identification of unusual or difficult-to-identify fungi and antifungal susceptibility testing.

Specialized Facilities and equipment in the Centre for Opportunistic, Tropical and Hospital Infections

- Thermal Cycler
- Horizontal Gel Electrophoresis Apparatus
- G-Box Gel Documentation System
- UV Transilluminator
- Nanodrop Spectrophotometer

Specific competencies from Centre for Opportunistic, Tropical and Hospital infection

- Sequencing and Phylogenetic analysis of unknown / difficult fungi
- *Candida* species antifungal resistance genotyping (D- glucan synthase)
- Genotyping of *Candida parapsilosis*
- Genotyping of *Cryptococcus neoformans*

5.3.7 Centre for Emerging and Zoonotic Diseases

The CEZD aims to establish itself as a national and international centre of excellence for emerging and re-emerging zoonotic diseases. CEZD aim to function as a resource for knowledge and expertise to the South African government, the SADC countries and the African continent, in order 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.

Specialized facilities and equipment in the Centre for Emerging and Zoonotic diseases

The CEZD operates multiple biosafety level 3 laboratories and the only biosafety level 4 laboratory in South Africa. These facilities are geared for bacteriological, virological and animal work. In addition the Centre operates several biosafety level 2 laboratories including PCR facilities and general molecular laboratories.

Equipment for molecular biology applications includes:

Lightcycler® v1.5 real time PCR machine (Roche)

Lightcycler® 480 real time PCR machine (Roche)

Smartcycler II real time PCR machine (Cepheid)

GeneAmp 2720 automated thermocyclers (Applied Biosystems)

Real time turbidimeter for loop mediated isothermal amplification (LAMP) (Teramecs)

Agarose gel electrophoresis equipment

Ultraviolet transilluminator with gel documentation system

PAGE gel electrophoresis equipment

Western blot equipment (i.e. semi dry and wet blotting equipment)

Incubators, waterbaths, centrifuges, laminar flow cabinets, biosafety cabinets etc

Specific competencies for Centre for Emerging and Zoonotic Diseases

Performing and analyzing molecular detection protocols for diagnosis of viral haemorrhagic fevers, rabies and arboviral infection including the use of real time, conventional and isothermal amplification protocols

Additional competencies:

- Molecular sequencing of PCR products and basic analysis of sequence data
- Basic molecular cloning techniques (PCR product clean up, TA or other cloning vector cloning, blue/white and antibiotic selection techniques)
- Basic protein expression techniques in bacterial, baculo- and mammalian expression systems

6.1 Clinical Laboratory Competencies

- ability to provide interpretation of molecular data and a diagnostic opinion, including any further action to be taken by the individual directly responsible for the care of the patient
- understanding of the wider clinical situation relevant to the patients presenting
- ability to develop/devise an investigation strategy taking into account the complete clinical picture
- understanding of the clinical applications molecular biology and the consequences of decisions made upon his/her actions/advice
- awareness of the evidence base that underpins the use of the procedures employed by the molecular biology laboratory
 - must understand the underlying mechanisms of the pathology of diseases tested for by molecular biology
 - must be able to advise on choice of molecular technique
 - must be able to interpret data and recommend further course of action within the wider context of the clinical situation.
 - must be able to relate data from other disciplines to the overall clinical situation.

- must be aware of the strengths and weaknesses of the evidence base for commonly used procedures in molecular biology.
- must be able to contribute to monitoring of patients as appropriate within the diagnostic service.
- Awareness of importance of turn-around times and audit trail to quality of results

6.2 Technical Competencies

- understanding of the principles associated with a range of molecular biological techniques employed
- knowledge of the standards of practice expected from molecular biological techniques used
- experience of performing techniques for clinical diagnosis.
- the ability to solve problems that might arise during the routine application of techniques (troubleshooting)
- understanding of the principles of quality control and quality assurance
- experience of the use of quality control and quality assurance techniques including restorative action when performance deteriorates.
- ability to perform common technical procedures in molecular biology as detailed in the local Standard Operating Procedures.
- a critical ability to review the results and determine the significance of quality control and assessment information for relevant analytical procedures in molecular biology
- a detailed understanding of analytical principles utilised in molecular biology to facilitate method troubleshooting and the development of adequate procedures of preventative maintenance.
- an understanding of the hazards (environmental, biological, chemical, radioisotopic) associated with the practice of molecular biology and the appropriate controlling legislation and appropriate procedures of risk assessment.
- Adequate numbers of samples run involving various techniques relevant to the discipline (eg involving use of a log book recording practical experience in the relevant molecular biology unit)

6.3 Scientific Competencies

- understanding the science that underpins molecular biology and the broader aspects of medicine and clinical practice.
- demonstrating a strong base of knowledge appropriate to the molecular biology specialty and to the investigations and therapeutic options available.
- experience of searching for knowledge, critical appraisal of information and integration into the knowledge base of molecular biology
- ability to apply knowledge to problems associated with the routine provision, and development, of the laboratory service
- ability to identify the clinical decision which the test/intervention will inform
- ability to make judgements on the effectiveness of procedures performed
- application of the knowledge base to the discipline and to the range of procedures/investigations available
- a critical understanding of the application of investigative protocols and diagnostic tests
- critical understanding of the integration and interpretation of molecular biological parameters with other relevant diagnostic parameters in the overall clinical assessment of the patient
- understand the principles of the techniques and methods employed in molecular biology
- able to advise on appropriate choice of investigation and sample preparation
- must be familiar with the evidence for, and limitations of, common molecular procedures used in

the diagnosis and management of patients

- must have a basic knowledge of related disciplines in order to be able to integrate relevant diagnostic results into an interpretation
- must be familiar with information on developments and needs in molecular biology
- a critical understanding of scientific method and the tools required to successfully evaluate, develop and/or modify both current and emerging technologies as routine diagnostic tools
- a critical understanding of classification criteria for disease entities of relevance to tests performed
- a critical understanding of diagnostic criteria in determining disease prognosis and outcome in immunology.
- An understanding of sensitivity, specificity, positive and negative predictive values of an assay and how these are affected by prevalence of a disease.

6.4 Research and Development Competencies

- ability to read and critically appraise the literature
- ability to develop the aims and objectives associated with a project
- ability to develop an experimental protocol to meet the aims and objectives in a way that provides reliable and robust data.
- ability to perform the required experimental work ability to produce and present the results (including statistical analysis)
- ability to critically appraise results in the light of existing knowledge and the hypothesis developed and to formulate further research questions
- ability to present data and provide a critical appraisal to an audience of peers – both spoken and written
- developed research skills and expertise sufficient to support supervised and collaborative research initiative in haematology
- an awareness of the current extent of knowledge in molecular biology an ability to employ appropriate information tools to search for, consolidate and critically examine information
- participation in local research meetings and supervised and collaborative research initiatives, leading to in-house reports (eg validation reports), publications or a research Masters degree
- self-endeavour (eg literature awareness) under the tutelage of an appropriate specialist.

6.5 Communication Competencies

- ability to assess a situation and act accordingly when representing the specialty
- ability to respond to enquiries regarding the service provided when dealing with clinical colleagues
- ability to communicate with patients, carers and relatives, the public and other healthcare professionals as appropriate
- ability to communicate the outcome of problem solving and research and development activities
- evidence of presentation of scientific material at meetings and in the literature
 - must be able to communicate effectively with professional colleagues within the discipline and in the wider scientific and clinical community
 - must be able to present findings effectively in a variety of written and spoken media must be able to educate and train professional colleagues within and without the department
 - must understand the requirements and responsibilities associated with the supervision of junior colleagues

- must be able to use modern communication devices
- must understand basic management techniques and be aware of topical management issues

6.6 Problem Solving Competencies

- ability to assess a situation which may pose a problem
- ability to determine the nature and severity of the problem
- ability call upon the required knowledge and experience to deal with the problem
- initiate resolution of the problem
- demonstrate personal initiative
- must be able to interpret internal quality control and external quality assurance data
- must be able to recognise when a test or procedure is not within adequate performance limits
- must be able to recognise the consequences of inadequate performance of individual tests or procedures
- must be able to identify potential causes of problems and to investigate these appropriately
- must be able to identify and appropriate solution to the problem and propose an effective and timely solution, including any requirement for clinical follow-up

6.7 Management Competencies

- understanding of the legal and ethical boundaries of the molecular biology specialty, and the ethical aspects of scientific research.
- ability to recognise the limits of personal practice and when to seek advice.
- ability to manage personal workload and prioritize tasks appropriately.
- understanding of the principles of clinical governance including importance of confidentiality, informed consent and data security clinical audit, accreditation requirements relevant to the haematology specialty.
- The ability to contribute effectively to work undertaken as part of a multi-disciplinary team
- ability to supervise others as appropriate to area of practice.
- understanding of the role of appraisal in staff management and development.
- understanding of the need for career-long self-directed learning and the importance of continuing professional development.
- understanding of the need for, and ability to establish and maintain, a safe practice environment.
- understanding of the structure and organization of the department

6.8 Ethics and Values Competencies

- apply and maintain appropriate professional ethics, values attitudes and behaviour.
- use science and technology effectively and critically, showing responsibility towards the environment and health of others
- understand and apply ethics in both human and animal research
- understand and comply with the laws of copyright protection, confidentiality and ownership of intellectual property
- take responsibility within own limits of competence and recognise the need for lifelong learning with an awareness of personal and knowledge limitations
- demonstrate an ability to work as a team and to show respect for colleagues and other health care professionals and the ability to foster a positive collaborative relationship with others.
- recognise the ethical and legal aspects in the field including record keeping and documentation
- flexibility to adapt to uncertainty and change