

Viral Diagnostics Unit

Enterovirus Section

BACKGROUND

The Enterovirus Section of the Viral Diagnostic Unit provides diagnostic enterovirus isolation testing and serves as a WHO Regional Reference Laboratory for poliovirus isolation. In this capacity, the enterovirus laboratory serves seven countries within Africa in AFP surveillance, including South Africa, Botswana, Namibia, Lesotho, Angola, Mozambique and Swaziland. However, the responsibilities of the laboratory go beyond those indicated above to performing confirmatory testing on samples tested in other WHO reference labs, also within Africa. The Enterovirus Section also performs Coxsackie B and poliovirus 1-3 serology. The poliovirus serology testing allows for the immune status of new employees to be determined and effects of post-vaccination.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

SPECIMENS TESTED FOR POLIOVIRUS FROM AFP CASES

Table of frequency by country

Country	Frequency	Comments
Angola	723	Routine
Botswana	22	Routine
Cameroon	2	Confirmation
Chad	50	Confirmation
Ghana	2	Confirmation
Lesotho	28	Routine
Mozambique	208	Routine
Namibia	247	Routine
South Africa	686	Routine
Sudan	2	Confirmation
Swaziland	44	Routine
Togo	2	Confirmation
Zimbabwe	3	Confirmation
Total	2019	

- The lab scored 100% in the annual WHO PT distributed to the African network in September 2008.
- The lab was audited by WHO HQ and AFRO in November 2008 and full accreditation was granted.

JOINT ANNUAL WORKSHOP OF THE MEASLES AND POLIO LAB NETWORKS, ZAMBIA

Objectives were to update lab heads and data managers regarding the current orientation of the polio eradication program; assess the progress in implementing in-house QC procedures in all lab techniques; assess progress in implementation of the revised polio isolation algorithm; and orient the data managers in order to enhance quality outputs.

- Presentation by Portia Ngcobondwana at the above workshop on the challenges in implementing the new virus isolation algorithm.

CAPACITY BUILDING

Experiential technologist students
Microbiology registrars
WHO AFRO Polio staff

Viral Isolation (Respiratory & General) Section

BACKGROUND

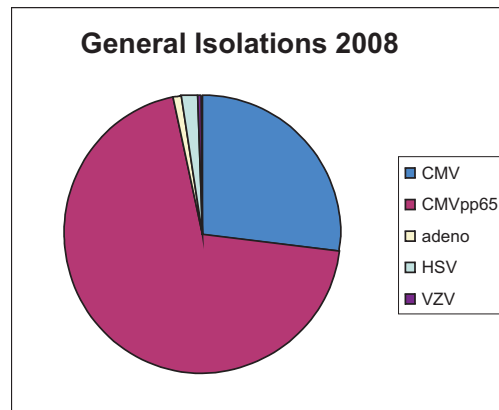
The Respiratory and General Virus Isolation Section has been part of the influenza Viral Watch programme since 1984 and provides data on circulating, seasonal influenza strains. The data is also used by the WHO global influenza programme to assist in the formulation of the annual influenza vaccine. The VIRAL WATCH sentinel surveillance programme which monitors Influenza activity was expanded in 2008 to include all 9 provinces in South Africa. The sentinel sites are further distributed into 3 regions: central plateau, north-eastern sub-tropical and southern coastal belt, which should provide interesting data regarding differences in strains and seasonality, if any, in these areas throughout South Africa.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- For 2008, a total of 3,195 samples were processed and tested, of which 1890 were for respiratory investigations. (258 from routine samples and 1,379 from VIRAL WATCH) and of these, 441 influenza isolates were made (30% overall isolation rate). The

majority of the isolates (374) were Influenza A. The isolates were sub-typed by the Hemagglutination Inhibition test (HAI), for which reagents were supplied by WHO Collaborating Centre (WHO CC) for Reference and Research on Influenza in Melbourne, Australia.

- The laboratory participated in a HIV-Vaccine study (107 samples) from Chris Hani Baragwanath hospital.
- The unit assisted in processing specimens from different African countries, including the Seychelles and Zambia.
- The Virus Isolation Section is also part of the WHO measles-supported programme at the NICD. Sera and urines countrywide are sent to the NICD for measles detection and surveillance.
- The section continues to serve the local academic hospitals and some private laboratories/clinicians as a diagnostic facility to facilitate effective and early detection of disease. The commercial kit (CMV TURBO BRITA) from PRO-Gen Diagnostics has been introduced for the detection of pp65. The test has advantages in terms of traceability of reagents and controls, as well as faster turnaround time. 963 tests were performed with 122 positives. Other viruses detected as part of the unit's activities include RSV, PIV, Adenovirus, VZV, HSV and CMV.
- Participated in annual Influenza Symposium aimed at informing, educating and thanking sentinel Viral Watch centers for co-operation and input.
- Virus Isolation laboratory played a role in identifying an influenza outbreak in Marydale, a small village in the Northern-Cape. Influenza A/H1N1 was identified.
- Representative isolates were forwarded to the WHO laboratories in the UK (London) and Australia (Melbourne) for further analysis as part of global Influenza surveillance. This serves as pre-alert or early warning of novel Influenza strains as well as supplying possible vaccine candidates.
- Molecular studies on the H1N1 isolates from the Virus isolation laboratory were shown to be highly resistant (100%) to the anti-viral drug Oseltamivir, unlike the situation in the country in 2007 where all isolates were sensitive to the drug. Early detection was possible and clinicians alerted.



- A total of 121 positive isolates were received from 3 NHLS referral laboratories (Tygerberg, Groote Schuur and Albert Luthuli Hospitals) for strain typing.
- The laboratory passed 2 EQA proficiency panels (UKNEQAS) with 100%.

CAPACITY BUILDING

Training of delegates from other countries included:

- A Tanzanian technologist was trained in seasonal and avian influenza detection during the period 4 - 15 February 2008.
- During February 2008, a group of three registrars were introduced to virus isolation techniques, principles and interpretation.
- 25 - 29 February Mr Logan Reddy from the KZN NHLS laboratory was trained in general Influenza Isolation techniques.
- May 2008 four registrars were trained on Isolation, Shell Vial, HA and HAI techniques.

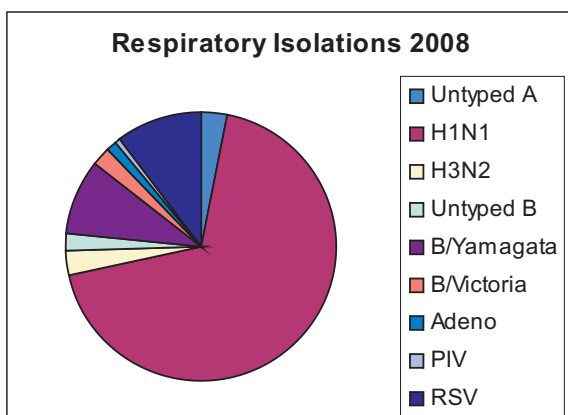
Local staff also attended the following training courses:

- 25 March to 25 April Amelia Buys visited the CDC Atlanta laboratory for training in micro-neutralization assays.
- Internal auditing training in April for Mr Nathi Ndlovu.
- Xolisa Stuurman attended a 2 week course in July and later in October on introduction to epidemiology.

Viral Serology Section

BACKGROUND

The Serology Section serves various functions, including the reference laboratory for measles and rubella serology testing for South Africa, while serving as the regional reference laboratory for the WHO-supported programme for measles and rubella control. The NICD is currently reviewing the testing strategy for measles in the context of a policy of measles eradication and low incidence. The current strategy is to integrate measles serology testing with nucleic acid testing (PCR). The NICD's other major role includes coordinating the laboratory testing for the Annual



Antenatal HIV-1 Prevalence Survey, as well as incidence testing for the survey. A key contribution of the section is to the recent publication, in collaboration with the HSRC, on HIV-1 incidence in South Africa. The findings are relevant with regard to the Department of Health's key strategic objective to reduce HIV incidence by 50% by 2011.

For 2008, the section continued to extend its activities to collaborate in various research and surveillance areas, described below.

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- The Serology Section supports numerous projects related to HIV-1 and HIV-2, HSV 2 IgG, measles and rubella testing. For 2008, the laboratory participated in the following activities:
- **HIV-1 Prevalence and Incidence Surveillance:** The annual Antenatal Survey is conducted each year in October across the nine provinces of South Africa in collaboration with nine NHLS laboratories. Testing is conducted for HIV Serology and RPR on pregnant women attending the antenatal clinics in the month of October, sometimes extending into November. Antenatal Survey incidence testing was performed on serum samples using the HIV-1 BED ELISA for 2005 and 2006 samples. Incidence testing was carried out using the HIV-1 BED ELISA. Incidence testing was also completed in an HSV-2 Episodic treatment study. The objectives of such studies are to define likely false-positive rates of the BED assay and the validity of incidence estimates against predetermined/calculated incidence, as well as apply correction factors. Incidence is also being conducted on positive samples from various sites within South Africa where rapid testing is being conducted. This is being done in collaboration with Internal Partnerships for Micobicides (IPM). Incidence testing for the HSRC SABSMM 111 study is being done on DBS samples.
- **Orange Farm** : In preparation for a large-scale male circumcision trial in Orange Farm, a household HIV prevalence survey and questionnaire was conducted in collaboration with the ANRS and STIRC Unit, NICD.
- **CERG (Cancer Epidemiology Research Group):** survey samples are received from the Cancer Research Unit for HIV serology testing.
- **PlasmAcute Study:** A WAHR (Women at High Risk) study was facilitated through the STIRC unit at NICD. This study involved testing sex workers from Carletonville for HIV serology, including Western Blots, depending on the algorithm as part of a study to identify acute HIV infection.
- **HSV 2 IgG Comparative Study:** This study was conducted on samples tested previously for HSV 2 IgG from the HSV-2 Episodic Therapy Study. The initial test used for HSV2 IgG serology was the Kalon. Samples were retrieved from 2005 to 2007 and re-tested on the Kalon, FOCUS, Trinity, Biokit (Rapid Test) and Novagnost HSV 2 IgG ELISA Tests. A panel of 120 blinded samples was also tested on each of these test kits, except for the Biokit. Additionally, the HSV 2 Rapid BLOKIT was assessed by two different operators. Additional studies to evaluate HSV-2 testing included the Male Circumcision Study for HSV 2 IgG detection.
- **In collaboration with the HSRC, DBS (dried blood spot) and Oral Fluid (OF) testing for HIV was performed on children from 0 to 14 years.** The testing was done on two different sample types for the suitability of OF for the forthcoming general population survey on HIV prevalence and incidence. Further evaluations included, for example, the AWARE OMT test (Calypte). OF samples were tested and compared to the Determine and Unigold HIV Rapid tests at the Chris Hani Baragwanath PHRU VCT clinic, and ELISA testing algorithm at the NICD. All results were compared to produce one final result. Where there were discordant results, Western Blots and HIV Viral Load were carried out on the samples. The objective of the study was to determine the sensitivity and specificity of the Aware™ OMT HIV-1/2 Rapid Test in a diverse population. The test performed within the defined specifications and is now placed on the USAID waiver list of HIV rapid tests.
- **HSRC (JEMS Study):** a study was conducted for HIV serology on DBS samples from males having sex with males.
- **Medical Research Council:** study for HIV serology conducted on males only.
- **Hepatitis B Survey:** testing performed on the Axsym for Hepatitis B Surface Antigen and Surface Antibody for the Age Specific prevalence of Hepatitis B virus immunity and carriage in the Eastern Cape Province 11 years after the introduction of universal infant vaccination.
- **Inverness Study** - this study is conducted on HIV Determine Rapid HIV Ag/Ab Combo Kit. A retrospective evaluation will be done on previously tested positive and negative samples and reported to Inverness for final evaluation. A prospective study will also be conducted together with NEWSTART where samples will be drawn from clients visiting VCT centres and then these samples are forwarded to NICD for testing as per algorithm.
- **NICD support for HIV quality management systems included support for NEWSTART (Society for Family Health) Quality Assurance Program:** a DBS re-testing programme for HIV serology for 15 sites (Gauteng(Johannesburg, Khutsong and Orange Farm), Western Cape, Musina, Bloemfontein, Mafikeng, Umtata, Middledrift, East London, Durban, Pietermaritzburg, Polokwane, Welkom and Nelspruit) is in place. Initially the programme started in 5 sites, and in late 2007, early 2008 this was extended to 10 sites and in the latter part of 2008, 5 more sites were opened. The NICD retested approximately 5% - 10% (site dependant) of the total numbers of clients tested by New Start. There was approximately 1.4% discordance in results.

- **HVTN 503 (HIV Vaccines Trial Network):** This study involves the use of the Merck Study Vaccine “Merck Adenovirus serotype 5 HIV-1 *gag/pol/nef* in participants enrolled in the study. The sites involved are Soweto, Cape Town, CAPRISA, KOSH and Medunsa. The Serology Unit performs the In-Study HIV Testing and Recent Exposure/Acute Infection Testing, using the *env*-based Biorad Multispot Rapid HIV1/2 test kit and the Western Blot assay as per algorithm.
- **Measles and Rubella Surveillance:** This forms part of the routine testing. No outbreaks were reported for 2008, however there were many suspected cases of measles in KZN, Eastern Cape, Mpumalanga and Limpopo, and were positive for rubella. The lab reported 2 171 rubella IgM positives for 2008 and 40 measles IgM cases. The laboratory acquired and automated instrument (BEP 2000) in September 2008. The objective is to automate the measles and rubella testing. Evaluation of the instrument to take place first quarter 2009.
- **RT-41 Tender (HIV Rapid Kit Evaluation):** The DoH issued a tender for HIV Rapid Test kits. The NICD Serology laboratory is the nominated laboratory for the testing and issuing of reports for each test kit that is submitted for the tender. Kits were submitted since November 2008. The laboratory also evaluated HIV Rapid kit batches for First Response, SD Biotec and Wondo before they were distributed to the field.
- **EQA Distribution:** laboratory is involved in the preparation and distribution of EQA panels for HIV Serology to the NHLS labs in South Africa and to the WHO labs in Africa for each year. Three EQA distributions were issued to 318 NHLS labs and 2 distributions were issued to 69 WHO labs in 2008.
- **EQA participation:** The laboratory participates in 13 different EQA schemes. For 2008, the laboratory achieved 100% satisfactory results for all distributions received and tested.
- **External Audits:** The laboratory was audited by three external bodies (SANAS, WHO for Measles and Rubella and PPD for the HVTN 503 Trial) in 2008. No major findings were reported.
- NEWSTART (Society for Family Health) regarding Quality Assurance at the various testing sites by providing HIV-1/2 results for every tenth client, including training on HIV-1 Rapid Testing and provision of Internal Quality Control samples for HIV Rapid Testing.
- NHLS Quality Assurance Unit regarding EQA distribution and reports submitted to NHLS laboratories for HIV Testing.
- WHO (Dr Gershy Damet) for WHO EQA distributions for HIV-1 testing to the WHO African laboratories.
- Project Accept (part of the HSRC) for the distribution of Internal Quality Assurance Samples for HIV Rapid Testing and quality assurance testing on routine samples.
- Contract Lab Service (CLS): HIV validation panels and Western Blot testing.
- STIRC Unit: Various projects PlasmAcute, HSV-2 testing HIV prevalence and incidence studies.
- HIV Vaccines Trial Network frequent liaison with Laboratory Operations division in Seattle with regard to the HVTN 503 Trial.

CAPACITY BUILDING

- Experiential training for Witwatersrand Technikon student for one week in November 2008. Student trained in the principles and practices of ELISA testing, including Western Blot techniques and HIV Rapid Testing. Department testing algorithms for HIV, measles and rubella testing were covered during training, including the use and care of equipment, specimen audit trail, document control, SOP management, assay validations and interpretation of serology results.
- Intern Students (Medical Technology) Two students trained, one student from May June 2008 and one from August October 2008. Students trained in the principles and practices of ELISA testing, including Western Blot techniques and HIV Rapid Testing. Department testing algorithms for HIV, measles and rubella testing were covered during training, including the use and care of equipment, specimen audit trail, document control, SOP management, assay validations, interpretation of serology results and hands on practice using blind samples and thereafter testing of routine samples.
- Registrar training was conducted on the 6th February, 7th May and 14th August. Areas covered: principles of ELISA testing and Western Blot techniques, Total Quality surrounding serology tests, testing algorithms for HIV, measles and rubella, SOP management, Dried Blood Spot Technology for HIV ELISA testing, and a general overview of lab activities.
- Mirriam Mashele attended the WHO Polio, Measles and Yellow Fever Laboratory Directors Annual Meeting in Lusaka, Zambia in September 2008 and the WHO Measles and Rubella Diagnosis Training

COLLABORATIONS

- The EPI Division at NICD and WHO AFRO with regard to measles and rubella surveillance on a national and regional level.
- The CDC in Atlanta with regard to BED database management and with the CDC in Pretoria with regard to HIV-1 Rapid Testing QMS for the DOH.
- Medical Research Council (MRC) for HIV-1/2 and HSV 2 IgG testing.
- The Human Sciences Research Council (HSRC) for HIV-1/2 prevalence and incidence testing
- International Partnerships for Microbicides (IPM) for Incidence Testing.
- MRC/NHLS/Wits Cancer Epidemiology Research Group at Braamfontein NHLS for HIV-1/2 associations with cancer.

for the Eastern and Southern African countries in November 2008 as a facilitator.

- Sarah Hloma assisted as a Facilitator in various HIV Rapid QMS training workshops throughout 2008.
- Robert Moalosi attended the HVTN global meeting in Washington DC in May 2008 and in Seattle in November 2008.
- Beverley Singh attended the HVTN global meeting in Seattle in November 2008.
- Mahlatse Maleka assisted as a facilitator in the training of nurses and health care workers in the collection and transport of Dried Blood Spot samples for the HSRC in Pretoria and Durban.
- Beverley Singh assisted as a facilitator in the HIV-1 BED Incidence training workshop together with the CDC at the NICD in December 2008