NICD 2007

Virology Division

Viral Diagnostic Unit

BACKGROUND

The Enterovirus Section provides diagnostic enterovirus isolation testing as well as Coxsackie B and poliovirus serology. It 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 unit served as the endpoint laboratory for the HVNT 503 trial. Adenovirus type 5 was used as the vector in the MRKAd5 HIV-1 vaccine. Pre-existing anti-Ad5 neutralizing antibodies may have an impact on the measurements of immunogenicity elicited by the vaccine and vaccine efficacy. It was therefore essential that this be determined for each participant.

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 programme includes sites from the Eastern Cape and referral laboratories from KwaZulu-Natal and Western Cape (Tygerberg and Groote Schuur Laboratories).

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). 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 2007, the section has extended its activities to collaborate in various research areas, including HIV surveillance and incidence testing for various projects, HIV rapid kit evaluations, HSV-2 association with HIV infection in male circumcision, as well as under conditions of episodic therapy for HSV-2 infection and HSV-2 kit evaluations. A major area of support is for the HVTN 503 trial. The section provides endpoint testing for HIV/Vaccine exposure. An area of great interest is the identification of acute HIV infection. The section therefore supports two initiatives for this purpose, namely serological testing for the PlasmAcute study (described elsewhere in this report), as well as the HIV incidence studies serological testing in combination with nucleic acid testing of acutely infected individuals in the HSV-2 episodic treatment study.

Enterovirus Section

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

In 2006, a new poliovirus isolation algorithm was introduced to the Global Polio Lab Network. This algorithm was successfully implemented in the Enterovirus Lab in 2007.

The lab scored 100% in the annual WHO PT distributed to the African network in September 2007.

The lab was audited by WHO HQ and AFRO in November 2007 and full accreditation was granted.

Two staff members were trained on the Ad5NAB assay in February and April 2007, one at the Merck lab in Philadelphia and the other at the HVTN lab in Seattle. Competency was based on the performance of three successful independent runs performed by each trainee.

The Ad5NAB laboratory was audited by PPD in June 2007 with no major findings

The HVTN503 trial was halted in September 2007, with all Ad5NAB testing for the 801 participants completed by October 2007

Thirteenth Informal Consultation on the Global Polio Laboratory Network, Geneva: The objectives of this consultation were to review the performance of the lab network in all regions; evaluate the progress with implementation and impact on reporting timelines of the new test procedures; establish new accreditation and proficiency test performance targets for labs using the new algorithm; and review the progress in developing new diagnostic reagents for VDPV detection, real-time PCR, and IgM antibody detection.

Joint Annual Workshop of the Measles and Polio Lab Networks, Ghana: 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 new polio isolation algorithm; and orient the data managers in order to enhance quality outputs.

COLLABORATIONS

HVTN Lab, Seattle: Assisted with the set-up of the laboratory at NICD, provided training on the assay, and assisted with the testing of enrollees.

Merck Laboratories, Philadelphia: Provided training on the Ad5NAB assay and supplied reagents (Controls and Ad5 virus) for performance of the assay.

CAPACITY BUILDING

Experiential technologist students

Microbiology registrars

Viral Isolation Section

(Respiratory & General Section)

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

For 2007, a total of 1,624 respiratory specimens were tested (185 from routine samples and 1,439 from VIRAL WATCH) and of these, 533 influenza isolates were made (32.8% isolation rate) (See Table for summary). The majority of the isolates (390) 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 unit assisted in processing specimens from different African countries, including the Ivory Coast, 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. A new 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. Other viruses detected as part of the unit's activities include RSV, Adenovirus and CMV (see Table One for summary of isolates).

CAPACITY BUILDING

Training of delegates from other countries in support of the WHO programme for measles control included:

WHO Measles workshop, 1620 April: One delegate from Zambia, one from Zimbabwe and one from South Africa were trained in measles isolation techniques.

During November 2007, two delegates from Zambia received influenza isolation training.

Cell Culture Section

ACTIVITIES

The major function of the cell culture laboratory is the production and banking of various cell lines used by the Enterovirus and General Isolation laboratories for routine testing. With the increased influenza surveillance activity (Viral Watch) there was an increase in supply of the MDCK cells for virus isolation as well as the distribution of viral transport medium to NHLS laboratories and participating Drs. during the influenza season. The Vero Slam cell line has been introduced as the preferred cell line for the isolation of measles and rubella viruses. The A549 cell line is also a new introduction for the isolation of RSV, Herpes, Varicella and Adenovirus. The laboratory has kept up with the increased demand for L20B and RD cell lines with the change in algorithm used by the Enterovirus virus Laboratory. The laboratory also provided LB20 and RD cell lines to 15 WHO-supported polio laboratories in Africa as well as the Vero Slam cell line for measles and rubella isolation and MDCK cell line for influenza isolation.

CAPACITY BUILDING

Three trainee students: Lebogang Mokoko; Nicolas Cumbane; Kearabilwe Mahlabane were trained on all aspects of cell culture.

Viral Serology Section

ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

ACTIVITIES

The Serology Section supports numerous projects related to HIV-1 and HIV-2, HSV 2 IgG, measles and rubella testing. For 2007, 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. 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 NARS and STIRC Unit. CERG (Cancer Epidemiology Research Group) survey samples are received from the Cancer Research Unit for HIV serology testing. Antenatal Survey incidence testing was performed on serum samples using the HIV-1 BED ELISA for 2005 and 2006 samples. In collaboration with the Africa Centre Study, incidence testing was performed on DBS samples from specimens collected from a population survey in KwaZulu-Natal. Incidence testing was carried out using the HIV-1 BED ELISA. Incidence testing was also completed in the 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.

PlasmAcute Study: A WAHR (Women at High Risk) study was facilitated through STIRC 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 ELISA. 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 BIOKIT was assessed by two different operators. Additional studies to evaluate HSV-2 testing included the Male Circumcision Study for HSV 2 IgG detection. Samples that were previously tested on the Kalon HSV 2 IgG EIA were re-tested on the FOCUS HSV2 IgG EIA assay. Moreover, for the Medical Research Council Stepping Stones study HSV 2 IgG testing was conducted on serum/plasma and DBS samples to determine the association between HIV incidence and HSV infection. Samples were tested on both the Kalon HSV 2 IgG EIA and the FOCUS HSV2 IgG EIA.

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.

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 five sites (Gauteng, Western Cape, Musina, KwaZulu-Natal and Bloemfontein) is in place. The NICD retested approximately 5% of the total numbers of clients tested by New Start. There was approximately 1% discordance.

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 2007.

ACHIEVEMENTS

Mahlatse Maleka attended the SANAS ISO 17025 Lab systems course from 7 to 8 May 2007, the SANAS Internal Auditors Course from 9 to 11 May 2007, the Laboratory Safety Course for Health and Safety Reps on 9 October 2007, and a course in Basic Fire Training at NICD on 4 November 2007. Mahlatse Maleka successfully completed her Biomedical Technology Degree in 2007. She passed all subjects (Molecular Biology, Laboratory Management, Research Methodology and Integrated Pathophysiology) and will be graduating in March 2008. Mahlatse started studying for this degree in 2003.

Elias Kekana attended the WHO Measles Workshop as a participant from 16 to 20 April 2007 and the course in Basic Fire Training at NICD on 4 November 2007. Elias Kekana registered for the Biomedical Technology Degree in 2007. He has successfully completed three of the four subjects, i.e. Laboratory Management, Research Methodology and Integrated Pathophysiology. Elias has registered for Molecular Biology in 2008.

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

Chris-Hani Baragwanath Perinatal HIV Research Unit: Project Accept: HIV-1 Rapid Test Training

MRC/NHLS/Wits Cancer Epidemiology Research Group at Braamfontein NHLS for HIV-1/2 associations with cancer

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

Contract Lab Service (CLS): HIV validation panels

STIRC: 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 students for one week in October 2007. Three students were 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.

One registrar was trained on 20 August and one on 17 September. 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.

Beverley Singh and Sarah Hloma facilitated the CDC workshops for Quality Management Systems for HIV-1 Rapid Testing from 29 May to 1 June 2007, from 8 to 10 October 2007, and from 27 to 29 November 2007.

Serology staff trained 50 nurses and healthcare workers from Bara PHRU on the Pareekshak HIV Rapid Test Kit from 5 to 9 March 2007.

Beverley Singh and Sarah Hloma trained two newly employed healthcare workers at Bara PHRU on how to perform HIV Rapid Tests using the First Response and Pareekshak Test Kits, on 30 July 2007.

Martin Masango facilitated a workshop hosted by NEWSTART (Society for Family Health) on HIV Rapid testing and Quality Assurance management systems in KwaZulu-Natal on 9 July 2007 and in Gauteng on 19 October 2007.

Beverley Singh attended the HIV Vaccines Trial Network meeting in Washington, DC, from 1 to 3 May 2007.

Martin Masango attended the Lab Directors' meeting in Ghana for Measles and Yellow Fever from 24 to 26 July 2007.

Martin Masango and Beverley Singh facilitated the WHO Measles workshop on Laboratory Diagnosis of Measles Infection, hosted by NICD from 16 to 20 April 2007.

Martin Masango, Robert Moalosi and Sipo Boltina attended the GCLP (Good Clinical Practice) course hosted by CLS at the NICD from 17 to 19 July 2007.

Martin attended the EQA Workshop hosted by the NICD from 26 February to 2 March 2007.