

## Specialized Molecular Diagnostics Unit

### BACKGROUND

Molecular diagnostics tests are commonly used to diagnose viral diseases, because they are sensitive, can be performed rapidly, with high throughput, and at a moderate cost. The Specialized Molecular Diagnostic Unit (SMDU) offers several clinical molecular assays for preventing, diagnosing, treating and monitoring of viral diseases, such as PCR, qualitative PCR, quantitative PCR, real-time PCR, multiplex PCR and sequencing.

Recent advances in technology, instrumentation and efforts in standardization and validation have been implemented in the unit. The development of internal controls, robotics and bead reagents are providing improved performance and higher-throughput testing of existing tests and new technologies. With any molecular test, assay quality assurance must be performed on an ongoing basis, which includes the use of proficiency panels to measure test performance and competency of staff. There is constant review of test performances, enhancement of operational workflows and ways to improve performance (sensitivity and specificity) in the laboratory.

The objectives of the laboratory are:

- To provide molecular diagnostic tests for the identification for a variety of viral human diseases. To ensure that the laboratory adheres to and performs under the guidelines of GCLP and ISO 17825 and ISO 15189.
- To provide a training service for intern medical technologist, intern scientist and registrars.
- To develop, evaluate and improve current tests with new technologies.

The laboratory's workload has once again increased with the growing demand for tests to detect diseases early and for more targeted therapies. SMDU has continued NAT testing as part of the HVTN 503 and 404 algorithms.

### SPECIALIZED MOLECULAR SECTION

### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

- **HIV-1 DNA diagnosis of HIV-1 infections in infants:** HIV-1 DNA PCR assay using DBS offers a sensitive and specific test appropriate for early

diagnosis of HIV-1 in infants. SMDU is collaborating with the Clinton Foundation and the Center for Disease Control (CDC) by assisting with scaling-up and establishing infant diagnosis of HIV-1 in Lesotho, Swaziland, Liberia and the Caribbean. The LightCycler 480 real-time PCR platform is used for confirming all positive test results.

- **COBAS Ampliprep/COBAS Taqman HIV-1 Qualitative test on DBS samples:** Validation of the COBAS Ampliprep/COBAS Taqman HIV-1 Qualitative test was initiated during 2008. It is a qualitative nucleic acid amplification test for the detection of Human Immunodeficiency Virus Type 1 proviral DNA in dried blood spots (DBS). The system utilizes the COBAS Ampliprep Instrument for automated sample processing, and the COBAS TaqMan 96 analyzer for automated real-time amplification and detection. Sample throughput is improved, due to the automated extraction and real-time amplification on the system. Validation will be completed in the first quarter of 2009.
- **The ANRS 1265 male circumcision trial:** Further human papillomavirus screening and genotyping: 638 swabs processed to screen, detect and genotype low-, intermediate- and high-risk strains of human papillomavirus (HPV).
- **FACSCount and Panleucogating:** Panleucogating (PLG) has been evaluated and validated as a second technology for CD4+ assessment in addition to the FACSCount (BD Biosciences) in SMDU.
- **HIV-1 viral load testing:** The COBAS Ampliprep/COBAS Taqman instrument for real-time viral load tests for HIV (and HBV and HCV) has been evaluated against the NASBA/EasyQ viral load platform during 2008. Offering fully automated sample preparation and analysis, this platform increased laboratory productivity and test result integrity. The NASBA/EasyQ and the COBAS Ampliprep/COBAS Amplicor semi-automated systems are currently utilized for different projects. For future projects the COBAS Ampliprep/COBAS Taqman will be utilized. The unit performed HIV-1 RNA testing for the HVTN 503 trial as confirmation of infection in conjunction with serology testing and in the HVTN 404 trial for monitoring of infected patients.
- **Herpes, Enterovirus PCR:** Only Herpes 1 and 2 and Enterovirus PCR specimens are currently analyzed for aseptic meningitis diagnosis, and a real-time multiplex PCR for the most common aetiological agents responsible for aseptic meningitis is under development, to be able to

## SPECIALIZED MOLECULAR DIAGNOSTICS UNIT

report results within 48 hours to improve treatment schedules and decrease hospitalization of patients.

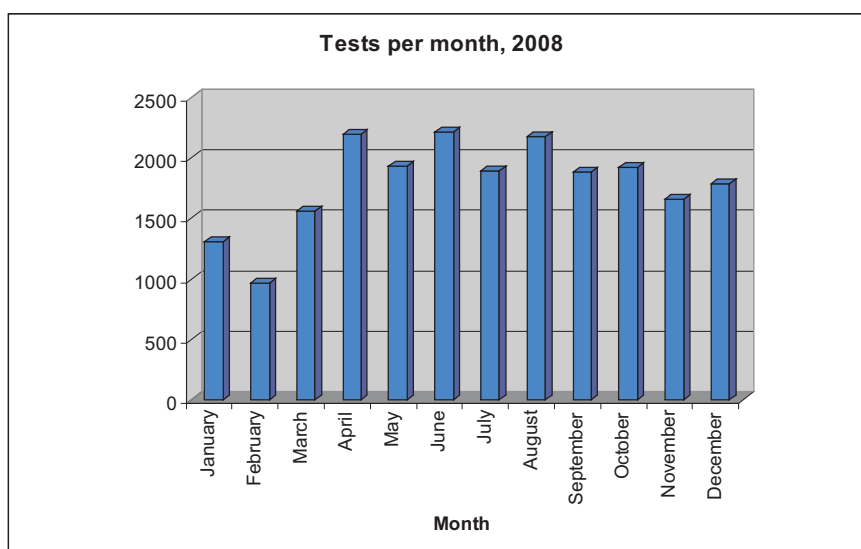
- **JC PCR:** The unit is currently developing a real-time PCR method for the detection of the JC virus to improve turnaround times and the sensitivity of this assay.
- **Multiplex Respiratory Virus PCR:** A Respiratory virus multiplex PCR has been developed. Viruses for the multiplex PCR include: Parainfluenza 1, 2, 3, Respiratory Syncytial Virus, Influenza A, and B, Enterovirus, human Metapneumovirus, Adenovirus, Rhinovirus. The primers and probes for all 10 viruses have been tested and cross reactivity within these virus groups and non-specific amplification have been ruled out. There will be ongoing quality control and validation in the form of further QCMD and Grace Panels as well as culture conformation on specimens chosen on a spot check bases.

SMDU collaborates with other laboratories like the Viral Isolation Unit, Respiratory Virus Unit, Epidemiology department, the CDC and WHO for respiratory virus testing.

- **EQA:** During 2008, the laboratory successfully completed proficiency panels referred by NEQAS, REQAS, QCMD (Quality Control for Molecular Diagnostics), the Centers for Disease Control (DBS HIV-1 DNA PCR) and the Virology Quality Assessment Program (VQA) for the various test methods offered within SMDU. For the VQA program, additional EQA programs have been subscribed to for HIV-1 DNA testing on both whole blood and dried blood spots (DBS). Other new programs include respiratory virus and aseptic meningitis EQA from QCMD for planned new diagnostics tests to be offered in the near future.

### SMDU TEST STATISTICS FOR 2008

2008	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
CDLS	46	17	41	230	204	126	274	156	234	75	73	14	1490
HBVL	0	14	16	16	20	19	12	23	34	48	34	34	270
HCGEN	4	2	5	12	6	156	13	7	5	3	5	2	220
HCVL	5	5	5	15	16	169	18	13	16	39	20	4	325
HIV1R	0	25	74	49	35	13	0	0	0	0	0	0	196
HIVVL	0	0	0	1	0	0	0	0	0	0	17	1	19
PCREV	0	19	0	1	1	1	0	0	0	0	2	1	25
PCRHB	23	22	12	16	22	11	12	15	15	24	11	14	197
PCRHC	19	37	39	37	33	26	18	33	28	36	27	27	360
PCRHP	0	0	0	0	0	0	0	0	0	0	0	638	638
PCRHQ	1151	789	1039	1123	1372	1546	1247	1747	1301	1630	1419	1001	15365
PCRHS	3	5	2	3	5	12	5	5	1	4	11	3	59
PCRHV	44	18	321	677	206	127	281	164	238	49	38	26	2189
PCRJC	9	9	5	16	14	14	11	11	15	19	0	18	141
<b>Total</b>	<b>1304</b>	<b>962</b>	<b>1559</b>	<b>2196</b>	<b>1934</b>	<b>2220</b>	<b>1891</b>	<b>2174</b>	<b>1887</b>	<b>1927</b>	<b>1657</b>	<b>1783</b>	<b>21494</b>



## COLLABORATIONS

Centers for Disease Control and Prevention (CDC):  
HIV-1 diagnosis in infants  
Clinton Foundation: HIV-1 diagnosis in infants  
HVTN 503 and 404 HIV-1 Vaccine Trials: HIV-1 RNA viral load testing on participants  
STIRC, NICD: CD4 testing for Women at High risk (WAHR) study  
CDC and WHO: Respiratory Virus testing

## CAPACITY BUILDING

**Training:** The PRF centre at the NICD hosted two successful training sessions for Early Infant Diagnostics (EID) using the Roche HIV-1 DNA PCR during 2008. The first training session took place from 1-6 September 2008, and this was the first official training hosted in the Polio Research Foundation's Training Laboratories but the NICD in conjunction with ACILT (African Centre for Integrated Laboratory Training), the CDC (Centers for Disease Control, USA) and PEPFAR (United States President's Emergency Plan for AIDS Relief). Attendees from Tanzania, Zimbabwe and Ethiopia were amongst the first, and trainers from NICD, Roche and the CDC were on hand to assist. The second training took place from 1-6 December, with attendees from Zimbabwe and Mozambique, and trainers once again from NICD, Roche and the CDC.

Two registrar training sessions with a total of 3 registrars trained, was held in 2008. Registrars received introductory lectures on the entire molecular diagnostic test performed at SMDU. Three medical technology students were introduced and trained on the full range of molecular tests performed in the unit. Medical technology students are trained to a level of competency and ability to perform all molecular diagnostic tests. New staff and medical scientist interns in the unit are trained to be competent in all diagnostics assays performed in the unit. The unit has 4 intern students.

**Registered for Degrees:** Ewaldé Cutler, Msc

## HBV AND HCV SECTION

### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

Diagnostics and research studies in the hepatitis unit continue to be confined to the hepatitis B and C viruses.

#### THE PREVALENCE OF HEPATITIS C VIRUS (HCV) GENOTYPES IN JOHANNESBURG

Last year HCV subtypes were determined in isolates from plasma from two patient groups (one from the Haemophilic Clinic and the other from the Liver Clinic both at the Johannesburg General Hospital) collected

over an eight-year period. Sentinel surveillance was continued this year, in collaboration with the South African National Blood Services (SANBS), and the prevalence of genotypes in the volunteer blood donor population in the period 2006-2008 was determined.

Two hundred and one specimens were tested for the presence of HCV RNA. From the demographic data collected, there were more males than females in this group with an average age of 39 years. Caucasians made up 44% of the infected blood donors for which ethnic data was disclosed followed by the Black population (21%), Asians (4%) and people of Mixed race (0.75%). There was no demographic data for a large percentage (30.25%) of the specimens.

Sixty six percent (133/201) were positive by HCV PCR and these specimens were subsequently genotyped by sequencing and a commercial line probe assay, LiPA. Five (3%) discordant results were detected between the two genotyping technologies. Genotype 5a was predominant in this study group (36%) followed by genotypes 1(34%), 3(19%) and 4(7.5%). The commercial genotyping assay, LiPA, was able to detect mixed genotype infections (3%) and this study is the first to report the incidence of mixed infection in South Africa. The mixed genotypes identified were 1b+4 (0.75%), 1+5a (1.5%) and 1b+5a (0.75%). The new LiPA methodology was validated against the gold standard of sequencing and phylogenetic analysis and found to compare well. In addition, it is quick and easy to use resulting in better turn-around times which is a big advantage in a high throughput laboratory. This collaboration with SANBS is ongoing and will form part of our continued HCV genotype surveillance in South Africa.

#### THE DEVELOPMENT AND VALIDATION OF A FAST-THROUGHPUT SEQUENCING PROTOCOL CAPABLE OF EXAMINING THE DISTRIBUTION AND DYNAMICS OF HBV VARIATION WITHIN AND BETWEEN HOSTS IN SOUTH AFRICA

A project has been initiated to establish a multiplex, fast throughput assay with which to monitor change in the hepatitis B virus genome within carriers (before and after treatment). In order to study change, it is necessary to recognize representative sequences and to be able to calculate the percentage similarity among them. Our first step therefore was to establish a database of all known full genome HBV sequences.

- **Database construction:** We obtained 1469 HBV aligned genomic sequences including all known genotypes A to H from all over the world from the International Nucleotide Sequence Database Collaboration (INSDC) database and placed them into an Excel spreadsheet. We then created further fields and filled these with patient data such as country of origin and phenotype of HBV disease. This database will be updated regularly using an in-house Perl Script and will have many other uses in our surveillance of HBV.

- **Finding conserved regions:** The next step in this project was to find conserved regions of the HBV genome to use to target adjacent variable regions of interest. With the help of WebLogo, which provides a graphical consensus of an alignment file, five representative consensus sequences for all genotypes A through H were chosen from the database. These were then submitted to MEME. MEME is a software program which seeks for the best motifs within the sequences it is given. Between 11-20 motifs were found (20 were requested) for each of the genotypes. From these the 28 best strings of conserved sites within HBV were identified.
- **Standard Primer Design in region of interest:** We chose to design our first assay in the region of the polymerase gene containing the YMDD motif which is most susceptible to mutations which induce drug resistance during nucleoside analogue treatment of HBV. Six of the 28 conserved sites were within the Polymerase region from nucleotide 640-1471 (all nucleotide numbering is taken from the unique Eco R1 site of HBV) which overlaps the surface antigen and includes the YMDD motif. We then extracted a smaller stretch of sequence (from nucleotide 603 to 790) from the full genome database and placed it into its own Excel file. This was used to optimize our first primer set. Every column of the conserved region was checked for its percentage consensus and primer candidates were chosen which required minimal degeneracies and which had the required 5°C difference in melting temperature required by the CIPer multiplex technology. We have successfully designed PCR primers which amplify a 76bp region between two amino acids known to be important in causing drug resistance. Included is the wild type methionine of the YMDD motif which can mutate to V, I or L (valine, isoleucine or lysine) during treatment with nucleoside analogues
- **PCR Optimisation:** This PCR has been found to be specific for genotypes A-F (not tested on genotype H yet as no commercially available source of the latter has been found and we have not identified this genotype locally). It has been found to be as effective on the last two QCMD panels after a single round of PCR as the existing accredited nested diagnostic PCR. If successfully validated it will thus save significantly on turn around times (TATs) and because of its careful construction be without genotype bias. It is also a much shorter product.
- **Steps toward the Multiplex PCR:** Several multiplexing technologies have been developed. We are presently applying CIPer technology to our polymerase PCR. We have designed the equivalent CIPer probe for the optimized PCR. CIPer probes (Figure 1) have specific probes at their ends (Sn and Asn) which are connected by universal probes (SnU1 and AsnU2), a barcode region or regions (BC) and a restriction enzyme site (RE). They also differ from standard PCR probes in that both Sn and Asn specific portions target the same strand of DNA and are called the extension and anchor sites, respectively (Figure 2). CIPer probes are reported to

not only allow higher orders of multiplexing than standard PCR but their reaction conditions are also easier to optimize.

- **Future challenges of the project:** Optimisation of the ligation chain reaction (LCR) which is performed using the CIPer probe and two thermostable enzymes, a DNA polymerase and a DNA ligase, and consists of an amplification step which closes the region between the extension site and the anchor site followed by ligation of the amplicon to the anchor site. The end product thus consists of specific circles in a soup of unspecific DNA, including the primers themselves, all of which are removed using Exonuclease I and III. Specific circles can then be linearised using the RE site and amplified using the universal primers (on their own or in conjunction with the barcode region which is specific to this PCR). Product detection is done by using a specific primer made up of the barcode plus universal primer as a sequencing primer. We have designed the CIPer primer, sequenced it and are in the process of optimizing the LCR reaction. After which the process will be repeated for each of the reasons of interest.

### DIAGNOSTIC SERVICE

Last year the COBAS AMPLICOR/AMPLIPREP TAQMAN for determining viral load (both HBV and HCV) was introduced and this year it was validated as an accredited diagnostic assay for viral load determination of both viruses.

The commercial LiPA assay was also validated as an alternative for HCV genotyping, previously done only by sequence analysis.

In collaboration with Roche Pharmaceuticals we are currently using these assays to determine HCV genotype and baseline viral load in patients prior to therapy. After commencement of PEG-interferon therapy viral load is then further monitored until the virus is cleared or treatment is withdrawn due to resistance. This study will yield valuable information on the effect of genotype on the HCV carrier response to PEG-interferon therapy in South Africa where genotypes I (known to require longer therapy and to be more prone to resistance) and Va (no formal reports on the response of this subtype to therapy are presently available) are the predominant genotypes.

We are validating our unbiased standard PCR primers developed for the first step of the multiplex PCR for use as standard HBV diagnostic primers.

### QCMD

**HCV:** There were two panels (each of eight plasma specimens) for the QCMD HCV RNA proficiency programme for 2008. These were tested by both the qualitative PCR method (Roche Amplicor Manual) as well as the newer COBAS AMPLICOR/AMPLIPREP TAQMAN. In the first panel using qualitative PCR we

initially scored 75% (as both weak positives were missed). However, since the QCMD final report found that 75% of the participants missed these two specimens our score was revised to 92% which correlates with the limitations of the technology used. The same two specimens were recorded as "less than 15 IU/ml" by the viral load test and the final report accepted these readings and scored the laboratory 100%. The second panel scored 100% by both methods.

There was one panel of eight plasma specimens for the QCMD HCV Genotyping proficiency programme for 2007. We used two methods for genotyping: LiPA (Bayer-Siemens) and sequencing (in-house). The LiPA scored one specimen which was a 1a as 1b although this specimen was successfully genotyped as 1a by the sequencing method. We scored 100% for the panel. Mixed infections in the panel were easily detected by LiPA and sequencing confirmed the LiPA results.

**HBV:** There were two panels (each of eight plasma specimens) for the QCMD HBV DNA proficiency programme for 2008. The first panel was done using the in-house HBV PCR and scored 100%. The second panel was done using both the HBV PCR and the COBAS AMPLICOR/AMPLIPREP TAQMAN which was introduced and validated during 2008. The latter scored 100% but the PCR missed one genotype D specimen. As the PCR has been demonstrated to detect 30 copies per ml in this panel a reason for its inability to detect 923 copies in the missed sample was sought. It was found that this PCR was not reliably picking up genotype D at copies below 1000. For this reason a new PCR (using our new research project primers) is being validated for future use for standard diagnostic HBV PCR.

## COLLABORATIONS

Dr Adam Mohamed, Liver Clinic, Johannesburg General

Dr Johnny Mahlangu, Head of both the Haemophiliac Clinic, Johannesburg General, and Medical and Scientific Advisory Council of the National Haemophilia Foundation (MASAC)

Marion Vermeulen and Andrew Saville, South African National Blood Services (SANBS)

## CAPACITY BUILDING

Ongoing training and supervision of student Nishi Prabdial-Sing who was registered for her second year as a PhD student at the University of the Witwatersrand Health Sciences in 2007.

Training and supervision of intern scientist Shirley Muvhulawa who is hoping to develop her intern project on multiplex PCR into an MSc project once initial steps are optimized.

Registrars were trained on hepatitis diagnostics and phylogenetic methods used in the laboratory.

New staff were trained in hepatitis diagnostic methods

**Registered for Degrees:** Nishi- Prabdial-Sing, PhD

## POLIO SECTION - WHO REGIONAL REFERENCE LABORATORY FOR POLIO

### BACKGROUND

Since 1995, the NICD has been a WHO Regional Reference laboratory for the Polio Eradication Initiative. The molecular polio unit serves as a reference laboratory outside the southern African region for many of the countries that fall under the WHO African Regional Office. Major activities within the section using molecular sequence-based analysis have answered several epidemiological questions regarding the likely location of endemic poliovirus reservoirs and patterns of virus transmission. They also determine if a virus is similar to endemic strains or has been introduced (imported), i.e. closely related to viruses circulating in another country or region. The unit contributes to training scientists and technologists from other African countries through WHO-based workshops. Unit members are involved in the following Polio Network activities:

- Global Polio LabNet
- Regional Polio LabNet
- National Polio Expert Committee (NPEC)
- National Task Force for Polio (NTF)
- Global LabNet Working Group
- Data Management for Polio LabNet

### ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

During 2008, the unit received 1,516 poliovirus isolates, which were characterized as vaccine or wild type using two intratypic differentiation methods, PCR and ELISA. These isolates were sent to the NICD from national and regional laboratories throughout Africa. Original specimens from AFP cases were received from several southern African countries and any polio isolates were treated as described above.

PV1 wild-type isolates belonging to India (SOAS), genotype consists of viruses from Namibia, DRC, Central African Republic (CAR) and Angola (Figure 1). Of the identified PV1 wild types in 2008, 3 were from DRC, 4 from Angola and two from CAR. No wild type has been identified in Namibia since the outbreak in 2006.

The only wild PV3 SOAS genotype circulating Africa is for viruses from Angola. The most recent case had onset of paralysis on 21 September 2008 WPV1 from

Cuanza Sul district). NIDs were held on 14 November with mOPV1. Further campaigns took place on the 10<sup>th</sup> December (with trivalent OPV) and in January 2009, as part of 'accelerated routine immunization activities', during which a range of vaccines are offered to communities using a 'fixed site' approach.

## COLLABORATIONS

Centers for Disease Control and Prevention, Atlanta, Georgia, USA: Polio Eradication Initiative (PEI)  
 WHO/HQ, Geneva, Switzerland: Polio Eradication Initiative (PEI)  
 WHO/AFRO, Harare, Switzerland: Polio Eradication Initiative (PEI)  
 African Polio LabNet: Polio Eradication Initiative (PEI)

## CAPACITY BUILDING

**Registered for Degrees:** Nicky Gumede-Moeletsi, PhD

## MOLECULAR MEASLES/RUBELLA SECTION

### BACKGROUND

Characterizing circulating strains of measles virus to determine whether they are of endemic or imported origin is important because this information can focus implementation of control measures. The data is shared with the Global Measles Laboratory Network of the World Health Organization (WHO). The molecular measles laboratory functions as a national laboratory to identify strains circulating in South Africa, as well as a regional reference laboratory to identify strains circulating in southern Africa. The laboratory also provides a service to other African countries that do not have access to nucleic acid sequencing technology.

When the incidence of measles is low, it is known that the positive predictive value of the anti-measles IgM-antibody test is relatively low. Since South Africa is experiencing low measles incidence, molecular methods are being used in a diagnostic capacity, in conjunction with standard epidemiological information, to try and confirm whether measles IgM-reactive specimens are compatible with a diagnosis of measles.

Although rubella vaccination is not part of the EPI programs in South Africa or Africa, it is important to get a baseline measure of the strains circulating on the continent. It will also help to define the global molecular epidemiology of rubella virus which is currently poorly described in Africa.

## ACTIVITIES, HIGHLIGHTS AND ACHIEVEMENTS

Although 40 measles IgM-positive sera were detected by the rash-surveillance program in South Africa in 2008, only 1 specimen contained measles virus nucleic acid. Sequencing of the PCR product, followed by phylogenetic analysis, identified the virus as genotype D8, an imported genotype. Unfortunately, this case could not be linked to other IgM-positive cases in the district. There were also 57 sera with indeterminate measles IgM serology results, but none of these contained measles virus RNA. Urine specimens, more suitable for molecular studies, were available for just over half of all cases (53/97, 55%).

It is possible that some of these 97 serology results could represent false measles reactivity, given that the positive predictive value of the test is not high in settings of low measles incidence. Since a significant proportion of these specimens also had positive/indeterminate rubella IgM results, they were tested for Rubella Virus nucleic acid. The hypothesis that these specimens represent rubella cases was evaluated by RT-PCR followed by nested PCR, and the results are shown in the table below.

**Table: Rubella PCR results for specimens with measles IgM reactivity**

Serology IgM result	Number of sera	Number of urines	Number sera/urines tested for rubella RNA	Number Rubella PCR+ (%)
(M+)(R+)	9	8	9	7 (78)
(M+)(Rindet)	2	1	1	1
(M+)(R-)	29	13	23	3 (13)
(Mindet)(R+)	23	14	23	8 (35)
(Mindet)(Rindet)	1	1	1	0
(Mindet)(R-)	33	16	30	8 (27)

It is clear that specimens that were measles IgM-positive and that had rubella IgM-positive/indeterminate serology represent false-positive measles reactivity because 80% (8/10) were PCR-positive for Rubella Virus. A smaller proportion of specimens that were measles IgM-indeterminate and that had rubella IgM-positive/indeterminate serology were PCR-positive for Rubella Virus (8/24, 33%) and therefore also represent false measles reactivity. It is interesting to note that 20% of the specimens with measles-IgM reactivity that were rubella IgM-negative were rubella PCR-positive: this is not an unexpected result because rubella IgM responses take a few days longer to appear relative to measles IgM responses, thus the rubella antibody level might still be below the test cutoff in the first few days after onset of rash.

With regard to the NICD RRL function, 79 specimens (clinical material/viral isolates/PCR products) were received from 3 countries: Angola, Democratic Republic of the Congo and Côte d'Ivoire (who forwarded

specimens from Benin, Niger, Cameroon and Togo) for molecular analysis. It was possible to obtain sequences for 44 (56%) of these specimens. With the exception of the DRC which had circulation of genotype B2 viruses, all the other countries had transmission of genotype B3.1 viruses.

It was only possible to amplify a portion of the measles virus genome from 3 specimens from the quarterly batches of sera sent by the national labs of the southern African countries for WHO-EQA purposes (the sera were subjected to numerous prolonged freeze thaw cycles as a result of frequent power failures). The amplicons were sequenced and genotype B2 (Zambia, Angola) was identified; this was the first time that this genotype was shown to be circulating in Zambia.

## CAPACITY BUILDING

**Registered for Degrees:** Sheilagh Smit: MSc