

**02 DIRECTOR'S REPORT**

**04 PROGRAM OVERVIEW**

- Victorian Programs
- Commonwealth Programs
- International Programs
- Research and Development Activities
- 2004 Activities
  - Outbreak Investigations
  - Victorian HIV Reference Laboratory
  - Mycobacterium Reference Laboratory
  - MRL Molecular Laboratory
  - Melbourne Sexual Health Centre
  - Viral Gastroenteritis
  - Vaccine Preventable and Related Diseases
  - Respiratory Pathogens
  - Sexually Transmitted Infections
  - Diagnostic Testing
  - New Test Development
  - New Test Validation

**20 RESEARCH AND DEVELOPMENT ACTIVITIES**

- 2004 Activities

**26 COMMONWEALTH PROGRAMS**

- National Poliovirus Reference Laboratory
- Acute Flaccid Paralysis Surveillance
- National Measles Reference Laboratory
- National High Security Quarantine Laboratory (NHSQL)

**30 INTERNATIONAL PROGRAMS/WHO COLLABORATION**

- Collaborating Centre for Virus Reference and Research
- Collaborating Centre for Biosafety in Microbiology
- WHO Regional Measles Reference Laboratory Western Pacific Region
- WHO Regional Poliovirus Reference Laboratory Western Pacific Region
- Collaborations with WHO

**36 OPERATIONS**

**38 WORKS AND INFRASTRUCTURE**

**40 APPENDICES**

- Appendix 1 - Publications
- Appendix 2 - Presentations at meeting
- Appendix 3 - Collaborators
- Appendix 4 - Visitors 2004
- Appendix 5 - Appointments
- Appendix 6 - Staff in 2004
- Appendix 7 - Abbreviations

**TABLE OF CONTENTS**



# DIRECTOR'S REPORT

# DIRECTOR'S REPORT

It seemed during 2004 that the stage was set for the outbreak of what had been anticipated for so long: the emergence of a new pandemic influenza strain. While the year came and went without this threat materialising, there is a new sense of urgency in planning for an event that many now regard as inevitable. This year VIDRL senior staff have made significant contributions to developing these plans at both national and state level. In addition, a collaborative project with the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne saw importation of influenza A H5N1 viruses to VIDRL's physical containment level 4 (PC4) laboratory for preparation of control material and validation of influenza tests used in public health laboratories around Australia.

VIDRL was also represented on a Commonwealth Department of Health and Ageing delegation to Singapore and China led by Ms Mary Murnane, Deputy Secretary for Health. This delegation was to explore cooperation on infectious disease surveillance and responsiveness, especially relating to SARS and highly pathogenic influenza.

In recent years, VIDRL has been developing capability for laboratory services needed in large scale community outbreaks of infectious disease. This activity will continue in 2005 with a significant upgrade of nucleic acid testing facilities. Our successful laboratory support of DHS Victoria in two large scale events in recent years provided valuable experience: 8500 Legionella urinary antigens tests were performed over three weeks during the Melbourne Aquarium outbreak in 2000, peaking at 1663 in one day. Two years later, 13,000 Q-fever complement fixation titrations were performed with the same day turnaround in a month, representing 14 years normal throughput for that test. The three-month project to develop new nucleic acid tests and serology for diagnosis of SARS in 2003 was also an extremely useful learning experience, even though there were ultimately no cases acquired in Australia.

While of great importance, this work was only a small part of what VIDRL achieved in 2004. VIDRL's work this year can be summarised as follows:

## Respiratory Outbreaks

Two sizeable outbreaks of influenza A (H<sub>3</sub>N<sub>2</sub>) occurred at a military base in Victoria this year, some three months apart. As had been the case last year, an outbreak at this base was the first significant cluster of influenza infection detected by VIDRL in Victoria for the respiratory season. Another two outbreaks of influenza-like illness that occurred at aged care facilities turned out to be due to rhinovirus infection.

## Outbreak Investigations

*Mycobacterium ulcerans* was responsible for a cluster of 17 cases of so-called "Bairnsdale ulcer" acquired in the Point Lonsdale/Queenscliff area. This outbreak was the subject of considerable publicity fuelled by media interest in one of the cases, a well-known sporting identity. A DHS-funded collaborative study of the epidemiology of *M. ulcerans* in Victoria is now in progress.

Laboratory support was again provided to DHS investigations of apparent transmission of blood borne viruses this year. The results of nucleic acid sequencing and phylogenetic analysis supported epidemiologic evidence suggesting transmission of hepatitis B virus between two hospital inpatients. Blood glucose monitoring equipment shared between patients on the ward in question was considered the most likely route of transmission. Another investigation focussed on suspected transmission of HIV from a sex worker to two contacts, allegedly unaware of his positive status. Again nucleic acid sequencing and phylogenetic analysis showed a degree of identity between the three infecting viruses, compared to a control group that was consistent with a transmission link between the individuals.

In contrast, an investigation of apparent transmission of hepatitis C on a surgical list found no laboratory evidence consistent with such transmission. Instead, evidence consistent with transmission between the index case and a domestic co-habitant was obtained.

An outbreak of 26 cases of *psittacosis* at a poultry farm and abattoir was investigated this year by DHS and VIDRL. A serosurvey of workers identified another 29 asymptomatic seropositive individuals. A case control study showed that new workers were at significantly increased risk of pneumonia.

Importations of measles continued to cause small clusters of infection, predominantly among family contacts of returned travellers. This year, four such clusters were investigated by serology and molecular epidemiologic analysis.

## Surveillance

VIDRL noted a significant increase in the number of Victorian returned travellers infected with hepatitis E virus during the first half of 2004. This increase was seven fold compared to the six-monthly mean number of infections diagnosed during the last five years.

Notifications of syphilis, a disease until recently rare among Victorians, continue to climb as it has done since 2000. Sixty-seven cases were diagnosed at VIDRL in 2004, including a case of congenital syphilis. This is a 40% increase compared to laboratory-diagnosed cases in 2003.

The percentage of patients newly infected with HIV strains resistant to reverse transcriptase (RT) inhibitor antiviral drugs remained stable at 12.5%. There has been no significant increase since surveillance started in 1996.

## Quality Assurance

The global measles IgM serology proficiency testing program provided by VIDRL's WHO measles reference laboratory distributed its fifth panel this year. A total of 99 WHO national measles laboratories now participate, representing all WHO regions except the Americas. Performance standards continue to be very high.

Three HIV serology proficiency panels were distributed this year by VIDRL's HIV state reference laboratory to 30 approved testing laboratories. An HIV-2 positive specimen was included for the first time this year, and this was detected by all participants.

## New Technology

A number of new PCR tests for pathogen detection were introduced this year including influenza C, mumps, *Pneumocystis carinii* and a coronavirus multiplex of common cold coronaviruses OC43 and 229E together with the newly discovered NL63, which causes paediatric respiratory disease. New improved PCR primers for hepatitis E virus and hepatitis A virus upgraded existing PCR tests for these viruses.

In addition, the sensitivity and dynamic range of hepatitis B virus DNA quantitation in blood was improved with the introduction of the Bayer VERSANT HBV DNA 3.0 (6DNA) assay.

## Consultation and Published Works

VIDRL senior staff continued to participate in a wide range of expert advisory committees and working groups at state, national and international levels. This is an important facet of VIDRL's reference and public health role.

VIDRL senior staff were represented on a range of advisory committees, including the National Influenza Pandemic Action Committee and chairmanship of its surveillance working group, and the Influenza Pandemic Planning Committee of DHS Victoria and chairmanship of its influenza surveillance sub-committee.

This year, VIDRL staff chaired the WHO Workshop on Laboratory Surveillance of Measles in the Western Pacific Region, Manila, and the WHO Meeting on the Standardization of the Nomenclature for Describing the Genetic Characteristics of Wild-Type Rubella Viruses, Geneva. VIDRL was also represented at the WHO Biosafety Advisory Group Special Meeting on Polio Eradication Biosafety Issues, Geneva.

A full listing of VIDRL staff appointments is provided in the appendices.

VIDRL staff also published 48 papers this year and delivered presentations at national and international scientific meetings. A full listing of both is also provided.

## Research

Work initially undertaken in 2003 to investigate the range of cell lines permissive for SARS-CoV replication was confirmed and extended this year. Twenty-one cell lines routinely used for viral culture were studied using indirect immunofluorescence and quantitative polymerase chain reaction (PCR). Fourteen cell lines were found to support SARS-CoV replication. These were mostly kidney cell lines and, with one exception, were of human or primate origin. Most permissive cell lines were not among those commonly used for diagnostic viral culture from respiratory specimens.

A pilot project was started this year in collaboration with the Victorian Infectious Diseases Service (VIDS) and three other Victorian hospitals to collect and store specimens and patient data on encephalitis cases of unknown aetiology. It is hoped that future studies undertaken after diagnostic technology advances or new pathogens are discovered may improve understandings of the causation of this serious condition.

As part of a National Institutes of Health (NIH) USA-funded project to study HIV and hepatitis B virus (HBV) coinfecting patients, nucleic acid sequencing and analysis of the entire genome of HBV strains commenced this year. Virological factors associated with antiviral resistance and their frequency are being studied, with comparison to equivalent markers from a group of HBV mono-infected patients.

Last year, VIDRL reported the first characterization of resistance to adefovir dipivoxil (ADV) in HBV. Studies conducted this year have characterised three clusters of mutations associated with ADV resistance, and further have characterised resistance to the antiviral drug entecavir (ETV) consisting of unique mutations in association with lamivudine resistance mutations.

The antiviral genotype and phenotype of 41 herpes simplex strains collected over the last 20 years from patients clinically resistant to acyclovir were characterised this year. Novel stop codons or amino acid substitutions in the thymidine kinase (tk) were identified in 7% and 15% of isolates respectively. This work contributes to the growing understanding of mutations known to contribute to acyclovir resistance.

A NHMRC-funded collaborative study with the Burnet Institute of social networks and the molecular epidemiology of hepatitis C (HCV) strains infecting injecting drug users continued this year. Several infection clusters were identified by phylogenetic analysis but there was interestingly a low correlation between social network data and molecular epidemiology of the infecting viruses.

## Training

VIDRL continues to host trainees of the Victorian Public Health Training Program, The Royal Australasian College of Physicians, and the Royal College of Pathologists.

Michaela Riddell graduated as Doctor of Philosophy from Melbourne University this year. Her thesis "Simple and practical approaches to the surveillance and diagnosis of measles in the era of measles control" produced five first author peer reviewed publications. Michaela had co-authorship on a further nine peer reviewed publications relating to measles but not directly arising from the thesis produced during these studies. Michaela is currently undertaking post-doctoral studies at John Hopkins University and will return to VIDRL in 2006.

## Operations

The sequence analysis module for HIV in the Medipath laboratory database was refined further this year. This records and reports antiviral resistance mutations detected by the Victorian HIV Reference Laboratory. Further refinement to the HBV sequence analysis software developed by the Molecular Research and Development Division in 2001 was undertaken to accommodate full genome sequence analysis of HBV. This increased analytic power and data handling capacity is required for the HBV and HIV coinfection study described above.

## Summing Up

This was a year of consolidation against a backdrop of preparation for the threat of an influenza pandemic that has yet to materialise. We have made good progress in this area, but considerable work remains to be done. Laboratory incidents with coronavirus SARS-CoV, and the possible threat of Bioterrorism have also contributed to an increasing focus on biosafety and biosecurity. It will be important to ensure that new measures developed to manage risks in these areas contribute meaningfully to community safety without compromising necessary laboratory activity.

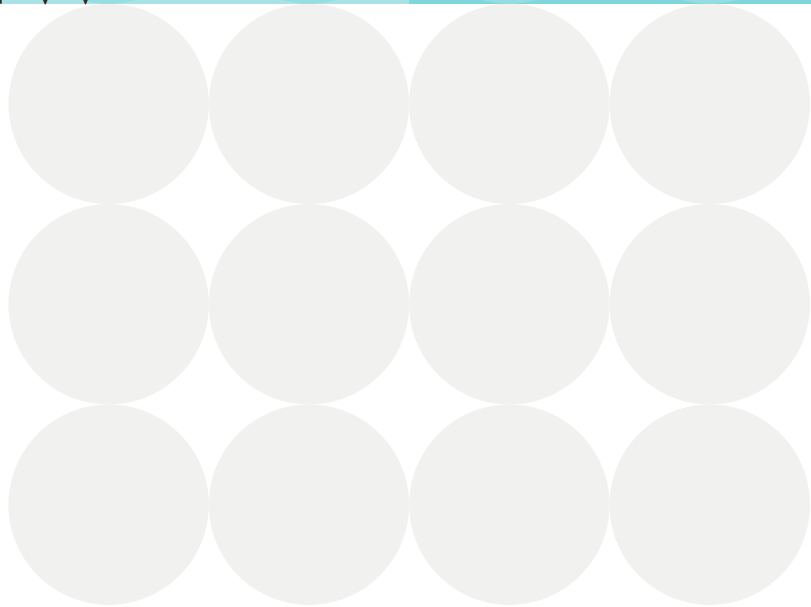
Once again I would like to thank the staff of VIDRL, our partners at the Department of Human Services, Melbourne Health, the Commonwealth Department of Health and Ageing and the World Health Organisation, our collaborators, clients and friends for their continuing support.



**Mike Catton**  
Director



# PROGRAM OVERVIEW



# PROGRAM OVERVIEW

This report summarises the activities of the Victorian Infectious Diseases Reference Laboratory (VIDRL) during the calendar year 2004. The report is organised into sections grouping VIDRL's scientific work at the state, national and international levels, as well as reports of research activities and new developments in laboratory operations. VIDRL's programs may be summarised as follows:

## Victorian Programs

### PUBLIC HEALTH PROGRAMS

VIDRL is Victoria's virology and mycobacterial public health reference laboratory. VIDRL also provides expertise in bacteriology, parasitology, epidemiology and molecular detection technologies. Services provided by VIDRL include:

- laboratory capacity for infectious diseases outbreak investigations by the Department of Human Services, Victoria (DHS)
- infectious disease surveillance
- diagnostic pathology for the Melbourne Sexual Health Centre (MSHC) and other sexual health clinics
- reference laboratory testing
- quality assurance testing and analysis
- applied public health research
- test development
- managed reference collections
- expert advice in major program areas
- advanced teaching and training in microbiology

### DIAGNOSTIC PROGRAMS

An important facet of VIDRL's work is the provision of diagnostic microbiology services to Victorian hospitals and private pathology laboratories, primarily in the areas of viral detection and characterisation, and viral serology. Diagnostic pathology services are also provided to private medical clinics with special expertise in HIV/AIDS, sexual health and travel medicine.

## Commonwealth Programs

VIDRL holds three Australian national reference laboratory designations:

- National Poliovirus Reference Laboratory
- National Measles Reference Laboratory
- National High Security Quarantine Laboratory (NHSQL)

The main role of the National Poliovirus Reference Laboratory is to detect and characterise viruses from cases of acute flaccid paralysis (AFP). The national coordination of AFP surveillance is conducted by staff of the National Poliovirus Reference Laboratory and the Epidemiology Unit at VIDRL. Funding for these roles is from the DHS and the Commonwealth Department of Health and Ageing (DoHA).

The National Measles Reference Laboratory was first designated in 1999. It is the focus for Australia's contribution to the emerging global effort to control measles infection. A national reference service for genotyping of measles viruses is provided for molecular epidemiological purposes.

The NHSQL is a physical containment level 4 (PC4) facility funded through a service agreement with the DoHA. As such it provides Australia with laboratory testing facilities for the diagnosis of the quarantinable viral haemorrhagic fevers Lassa, Ebola, Marburg and Crimean Congo haemorrhagic fever, as well as infections exotic to Australia, including SARS and avian influenza.

## International Programs

VIDRL holds the following World Health Organization (WHO) designations:

### WHO COLLABORATING CENTRE FOR VIRUS REFERENCE AND RESEARCH

The WHO Collaborating Centre in Virus Reference and Research is a designated centre of excellence in virology and encompasses much of the virological activities of VIDRL.

The Centre is responsible for:

- the detection and characterisation of viruses in clinical material;
- maintaining reference collections;
- training and consultancy in virology;
- the provision of infectious disease surveillance information;
- research and development in areas such as antiviral therapy, mechanisms of antiviral drug resistance and new technology for detection of viruses.

### WHO COLLABORATING CENTRE FOR BIOSAFETY IN MICROBIOLOGY

The WHO Collaborating Centre for Biosafety in Microbiology undertakes training in biosafety, provides biosafety related advice and support to institutions and participates in the work of the WHO Biosafety Advisory Group.

### WHO REGIONAL MEASLES REFERENCE LABORATORY

In 1999 VIDRL was designated the Australian reference laboratory for measles, and in the following year, was designated as the first WHO measles regional reference laboratory for the Western Pacific Region.

The objectives of the measles program at VIDRL are:

- to provide a timely diagnostic service for measles surveillance and outbreak investigation in Victoria;
- to provide a reference service for measles diagnosis for laboratories around Australia;
- to provide laboratory support for the improved understanding of the epidemiology of measles in Victoria and Australia;
- to provide a reference service for measles diagnosis and surveillance for laboratories within the WHO Western Pacific Region;
- to develop and evaluate alternative laboratory methods for measles diagnosis and surveillance that may be appropriate for resource poor countries.

### WHO REGIONAL POLIOVIRUS REFERENCE LABORATORY

The Poliovirus Reference Laboratory was established in 1994 and has played a major role in Australia's commitment to the WHO program for the global eradication of poliomyelitis.

The laboratory is accredited by the WHO and has the following responsibilities as a member of the global poliovirus laboratory network:

- national laboratory for Australia;
- national laboratory for the Pacific Island countries;
- national laboratory for Brunei Darussalam;
- one of two regional reference laboratories for the Western Pacific Region.

### WHO NATIONAL INFLUENZA CENTRE

The influenza surveillance program at VIDRL aims to assist in the early detection of influenza, define the distribution of influenza in the community and provide timely information regarding circulating strains. As a National Influenza Centre VIDRL:

- serves as the key point of contact with the World Health Organization
- collects appropriate patient specimens for influenza virus isolation
- undertakes initial identification of virus type and subtype
- forwards representative and potential variant virus isolates to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne
- alerts the WHO Global Influenza Programme regarding any untypable influenza isolate
- alerts national authorities and the WHO immediately on the emergence of unusual outbreaks of influenza or influenza-like illness

### Research and Development Activities

VIDRL undertakes a diverse range of research projects. These include studies of antiviral drug resistance and the transmissibility of viruses and viral variants. Investigations of the epidemiology of infectious agents of public health significance, and the development of potential new laboratory technologies are also important areas of study. Research is undertaken in all program areas.

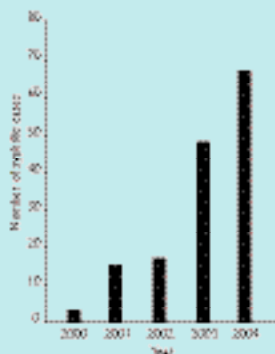
The main areas of research activity in 2004 were:

- further development of an internet-based program for the analysis of hepatitis B virus sequence
- investigation of patients co-infected with HIV/HBV (an international collaborative study funded by the US National Institute of Health)
- hepatitis B virus vaccine escape mutants
- hepatitis B viral antiviral resistance
- social network analysis of hepatitis C virus in a cohort of injecting drug users (in collaboration with the Burnet Institute and funded by NHMRC)
- a pilot project to establish the clinical and laboratory investigation of undiagnosed encephalitis
- development of a real time PCR assay for the diagnosis of mycobacteria in birds (in collaboration with the Melbourne Zoo)
- an observation from serological testing of an increase in hepatitis E virus infection among Victorian travellers
- replication of SARS-CoV in cell-lines used in diagnostic virology laboratories
- molecular analysis of clinical isolates of acyclovir resistant herpes simplex virus.

In addition applied public health research included:

- investigation of possible hepatitis C virus transmission in a surgical setting
- demonstration that norovirus RNA can be excreted before and after acute symptoms (a project funded by a DHS public health research grant)
- estimation of influenza vaccine effectiveness from an outbreak where antiviral medication was used as a control measure
- demonstration of the usefulness of a medical locum service as a sentinel site for the surveillance of influenza-like illness
- a pilot study of influenza in patients from a paediatric hospital Emergency Department
- a review of the changing epidemiology of genital herpesvirus infection

**Figure 1: Number of syphilis IgM positive cases 2000-2004**



## 2004 Activities

### Outbreak Investigations

#### RESPIRATORY VIRUS OUTBREAKS

Several outbreaks involving respiratory virus infections were investigated by VIDRL and DHS during the year. Identification of the causative agent in each of these outbreaks involved use of the multiplex respiratory virus polymerase chain reaction (PCR). Two involved outbreaks of rhinovirus infection presenting as influenza-like illness in aged care facilities. Rhinoviruses appear capable of causing more severe respiratory infection than is generally appreciated, especially among the elderly. Two discrete epidemics of influenza A (subtype H3N2) occurred three months apart in personnel at the same naval base. This is the second year that an influenza outbreak amongst these military personnel has been the first cluster of influenza infection detected in Victoria for the season.

#### SYPHILIS CASES

The number of syphilis cases detected by serology continued to increase. Forty-eight cases were detected in 2003 and 67 in 2004; of these, four were female and 63 male including one baby boy with congenital syphilis. Figure 1 shows the increase in the number of syphilis cases detected by serology from 2000 to 2004.

#### CHLAMYDIA PSITTACI

An outbreak of *C. psittaci* was identified at a Victorian poultry farm and abattoir. The first case was identified in October 2003. Positive serology was reported on one case in January 2004, three in February, seven in March and others scattered throughout the year as the investigation continued. DHS investigated the outbreak. Overall 26 cases were identified. A serosurvey and case control study were also performed in order to identify any high-risk exposures.

There were 97 workers tested by serology, of whom 53 (55%) were seropositive and, of these positives, 29 (55%) showed no symptoms.

Seropositive workers were older compared to the seronegative group (median age 43 compared to 32.5). Sixty-five percent of the positives worked in the abattoir and 43% were from the farm.

The case control study showed that workers who worked in the abattoir for seven months or less were significantly more likely to have pneumonia.

#### NOROVIRUS GASTROENTERITIS OUTBREAKS

During 2004, specimens were received from 207 gastroenteritis outbreaks for testing for viruses. Norovirus was detected in specimens from 149 (72%). There was a major epidemic of norovirus gastroenteritis for the months August to November. Details can be found in the 'Viral Gastroenteritis' section.

#### INVESTIGATION OF POSSIBLE HEPATITIS C VIRUS TRANSMISSION IN A SURGICAL SETTING

A patient who developed acute hepatitis a few weeks after gastroscopy and colonoscopy was diagnosed with hepatitis C (HCV). Blood samples from other patients on the surgical list and a co-habitant of the patient (known to be anti-hepatitis C positive) were tested for the presence of HCV. One patient who also had surgery on that day and the co-habitant had detectable HCV and further molecular studies were conducted to determine the relatedness of the viruses. Phylogenetic analysis established a strong epidemiological link between the virus from the patient with acute hepatitis C and his co-habitant. No evidence of transmission from the other patient was evident.

### Victorian HIV Reference Laboratory

#### HIV SEROLOGY REFERENCE LABORATORY

HIV confirmatory testing by Western blot was performed on 994 specimens. There were 216 newly diagnosed cases of HIV infection in 2004, compared to 226 in 2003 and 234 in 2002. The number of newly diagnosed cases in females increased to 26 in 2004 compared to 19 in 2003. Fourteen of the newly diagnosed females acquired HIV infection in high prevalence regions of Africa or Southeast Asia.

The serology reference laboratory changed to the Abbott/Murex assay for HIV antibody screening in early 2004, replacing the Abbott HIV gO bead assay. The Triturus automated analyser was installed to run the new assay. The Biorad Genscreen combined antigen/antibody assay was introduced as a supplementary assay to aid in the diagnosis of very recent infection.

Three quality assurance panels each containing 20 specimens were distributed to the 30 approved HIV testing laboratories in Victoria. On the December panel, one laboratory obtained equivocal results for the negative samples. No other discordant results were reported for the year. An HIV-2 positive specimen was included in the December panel, which all laboratories reported as positive.

**Table 1: Number of HIV viral load and genotyping tests performed from 2000-2004**

Test	Year				
	2000	2001	2002	2003	2004
Standard viral load	3690	3896	3845	3610	3303
Ultra sensitive viral load	999	1570	2279	3194	3714
Total VL tests	4689	5466	6124	6804	7017
Genotype	735	277	333	334	296

**Table 2: Mutations detected and likely effects on antiretroviral drug susceptibility in patients whose primary HIV infection was associated with drug resistant virus**

Patient	Resistance mutations detected:	Antiretroviral drugs to which resistance is conferred:	Antiretroviral drugs to which there is possible resistance:
1	M41L, K103N	zidovudine, nevirapine, delavirdine, efavirenz	abacavir, stavudine, tenofovir
2	M184V	lamivudine	didanosine, zalcitabine, abacavir
3	M41L	zidovudine	abacavir, stavudine, tenofovir
4	Y181C, G190A	delavirdine, nevirapine	efavirenz
5	V106V/A	nevirapine	
6	M41L, K103N, M184M/I	zidovudine, nevirapine, delavirdine, efavirenz	abacavir, stavudine, tenofovir, lamivudine
7	T69N, K70R, K219Q	didanosine, zalcitabine, zidovudine	abacavir, stavudine

## HIV CHARACTERISATION LABORATORY

In 2004, the number of HIV RNA quantitative tests (viral loads) performed rose by 4% compared with 2003. There has been an increase of 35% in the number of viral loads performed in the last five years (see Table 1). The number of HIV genotyping tests performed (used to determine antiretroviral drug susceptibility) has remained constant at an average of 310 per year since 2001.

Both Australian-based and international quality control panels were performed for viral load and genotyping. The laboratory also participated in the Immunovirology Research Network quality assurance program established to assess lymphocyte preservation efficiency.

Laboratory staff were involved in several trials co-ordinated by the National Centre in HIV Epidemiology and Clinical Research. These include PHAEDRA (monitoring disease progression in recently infected patients); SMART (a comparison of structured antiretroviral drug treatment interruptions versus no treatment interruption); ESPRIT (the effects of subcutaneous interleukin-2 on disease progression); and INITIO (an international study comparing RT inhibitors versus RT inhibitors plus protease inhibitors for first-line treatment of HIV infection). The laboratory performed the viral load testing for the Australian arm of this study.

As well as assisting treatment decisions in patients with extensive exposure to antiretroviral drugs, genotyping is able to identify the transmission of drug resistant virus in cases of recently acquired infection. As part of the laboratory's reference role, in 2004 a total of 56 genotyping tests were performed to determine whether such transmission had occurred. No evidence of transmitted virus resistant to protease inhibitors was found. However, the viruses of seven patients (12.5%) had evidence of genotypic resistance to one or more RT inhibitors, a percentage which is consistent with observations made between 1996 and 2003. Table 2 shows the mutations detected and an interpretation of which drugs would be affected by them. Interestingly six patients had zidovudine-associated intermediate mutations at codon 215 of the RT. In the absence of drug pressure in these newly infected patients, viruses with resistance mutations at codon 215 mutate to these intermediate forms, which have greater replicative capacity than the resistant viruses. These intermediates, which are susceptible to zidovudine, are likely to be rapidly replaced by resistant viruses if zidovudine therapy is initiated, thereby compromising that therapy.

The availability of sequence data on the RT and protease genes obtained through genotyping enables the HIV subtype to be determined. Of the 56 patients whose HIV strains were genotyped (above), 54 were infected with subtype B strains, one was infected with a subtype C virus and one with a CRF01-AE strain.

A prospective study investigating virological and immunological outcomes in patients who are infected with antiretroviral drug resistant viruses has been commenced. Preliminary results have shown that the viruses of seven of 10 untreated individuals whose infecting virus was drug resistant maintained all their resistance mutations throughout the study period. Patients who were not treated with antiretroviral drugs during follow-up had viral loads and CD4 counts that suggested more rapid disease progression than a control group infected with wildtype virus. Because of the need for long-term follow-up of these patients, this study will continue beyond 2004.

## Mycobacterium Reference Laboratory

The Mycobacterium Reference Laboratory (MRL) at VIDRL provides a statewide facility for referral of all mycobacterial isolates, including those from veterinary and environmental origins. Isolates are identified using a combination of biochemical and molecular techniques, including PCR based assays and DNA sequence analysis. Antibiotic susceptibility testing is performed when indicated for isolates identified as *Mycobacterium tuberculosis*. A total of 7,865 specimens were received in 2004. These included primary specimens for culture and various PCR investigations and culture isolates referred for identification.

## ISOLATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX

*M. tuberculosis* was isolated for the first time from 261 patients and there was also one isolation of *M. bovis*. This is a slight increase from previous years. There were 143 males and 118 females ranging from three to 92 years of age. Two male patients were known to be HIV positive, one had abdominal TB and the other had the organism isolated from sputum, urine and blood. Five children under 15 years of age were diagnosed with tuberculosis.

Figure 2:

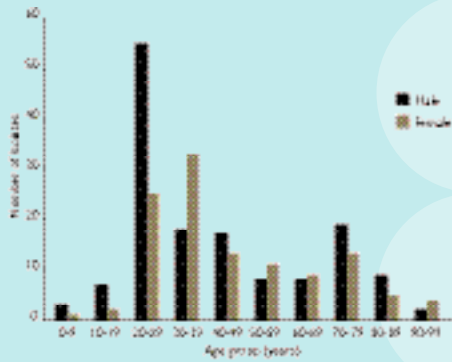


Table 3: Drug resistant *M. tuberculosis* by site of isolation 2004

Site of isolation	INH	INH(I)	INH, STR	INH, RIF*	INH,RIF, STR,PYZ*	INH,EMB, STR,RIF*	INH,RIF, EMB, STR, PYZ*
Pulmonary	4	2	2		3	2	1
Lymph Node	1	1	3				
Pleural			1				
Peritoneal				1			

Key:  
 INH Isoniazid  
 INH (I) Isoniazid intermediate resistance (reported as per NCCLS guidelines)  
 STR Streptomycin  
 RIF Rifampicin  
 EMB Ethambutol  
 PYZ Pyrazinamide

\*Multi drug resistant strains (resistant to INH and RIF +/- other drugs)

The relationship of tuberculosis to age and gender is shown in Figure 2. For males, there was a distinctive rise of cases in the 20 – 29 age group. Female cases peaked in the 30 – 39 age group. Overall, the male: female ratio was 1.6:1 for sputum isolates, but the ratio was reversed for lymph node isolates, 1:1.5.

The predominant specimen types were pulmonary (sputum, bronchoscopy and two gastric aspirates) 47%, lymph node 27%, pleural and abdominal 4.9% each. Thirteen patients had isolations from multiple sites. 'Other sites' included pleural specimens, urine, lymph node, blood and ascites.

Specimen site	No. of isolates
Pulmonary	123
Pulmonary + other sites	12
Lymph node	70
Pleural	10
Abdominal	10
Skin	8
Bone/joint	5
Genito-urinary	3
CSF	2

*M. bovis* BCG was isolated from seven patients. Four were from vaccination sites in infants and three were from urine following intravesical BCG.

### DRUG SUSCEPTIBILITY TESTING

A total of 21 *M. tuberculosis* isolates were resistant to one or more of the first line anti-tuberculosis drugs. Resistance to at least both isoniazid and rifampicin (defined as multi drug resistance) was detected in seven isolates (2.7%), which is a significant increase from previous years (Table 3). The single isolate of *M. bovis* was not included in these results.

### ISOLATION OF MYCOBACTERIUM AVIUM COMPLEX AND OTHER MYCOBACTERIA

The most commonly isolated mycobacterium species other than *M. tuberculosis* is the *M. avium* complex (MAC). The organism was isolated for the first time from 179 individuals. Confirmed concurrent infection with HIV occurred in eight patients. MAC is commonly isolated from lymph nodes in young children and there were seven such isolations from children under five years of age; six males and one female.

As well as being isolated from human specimens, MAC was also recovered from six specimens of veterinary origin, a quokka, a plumed whistling duck, a little pied cormorant and three pigs. Other veterinary specimens included those from a cat, an elephant fish, a dwarf bearded dragon and a separate quokka from that above, yielding a variety of mycobacterial species.

### MRL Molecular Laboratory

The role of the MRL Molecular laboratory is to develop and apply rapid, molecular techniques to the identification of mycobacterial isolates, detection and identification of mycobacteria in clinical specimens and the typing of *M. tuberculosis* isolates for epidemiological purposes. The provision of expertise and resources to a range of collaborative research projects is also central to the role of the laboratory.

### MOLECULAR IDENTIFICATION OF PATIENT ISOLATES

Identification of mycobacteria increasingly relies on molecular techniques, as the range of unusual isolates referred to the reference laboratory is increasing, along with the available sequence information on the databases. Both the 16s rRNA gene and the 16s-23s intergenic spacer region (ITS) are useful targets for the identification of mycobacteria, and in 2004, 179 isolates were referred to the molecular lab. The majority of these isolates were able to be speciated on the basis of sequence matches with characterised strains, however a significant number appeared to be novel species. Among the species commonly identified by sequence analysis included *M. chelonae*, *M. abscessus*, *M. lentiflavum* and *M. shimoidei*. 16s rDNA sequencing was also helpful for the identification of an increasing number of *Nocardia* (including nine different species), *Streptomyces*, *Gordona*, *Actinomadura* and *Tsukamurella* isolates.

## RAPID DIAGNOSIS OF *M. TUBERCULOSIS* COMPLEX IN CLINICAL SPECIMENS

The real-time PCR (TaqMan MGB) assay, developed at VIDRL, for the rapid detection of *M. tuberculosis* complex in clinical specimens, continued to be in demand in 2004. A total of 905 specimens were submitted for TBPCR, compared with 792 during the previous year. Eighty-two positive test results were obtained, (including four from formalin-fixed, paraffin-embedded specimens), corresponding to 76 patients.

## PCR DETECTION OF *M. ULCERANS* IN CLINICAL SPECIMENS

The TaqMan real-time PCR assay, introduced in 2003, continued to perform well in 2004. Compared to the previous year, the demand for *M. ulcerans* diagnosis and the number of new cases was significantly increased, with 361 tests performed, identifying 27 new cases which were later confirmed by culture. Two of the new cases were patients living in the Northern Territory. Seventeen of the Victorian cases either lived or had stayed in the Point Lonsdale/Queenscliff area, which represented a significant outbreak. An additional five cases had links to *M. ulcerans* endemic regions including Phillip Island, East Gippsland and the Mornington Peninsula.

## PCR DETECTION AND IDENTIFICATION OF ATYPICAL MYCOBACTERIA IN CLINICAL SPECIMENS

This test is a nested PCR capable of detecting any mycobacterial species, present in sufficient numbers in a range of clinical specimens, including paraffin-embedded formalin fixed tissue sections. Subsequent identification of the species is then possible by restriction enzyme digestion and/or sequence analysis of the PCR product (ITS region). In 2004, 119 specimens (both human and veterinary) were referred. Mycobacterial DNA was detected in 20 of these. The species identified were *M. avium* (5), *M. intracellulare* (3), *M. chelonae* (4), *M. kansasii* (3), *M. marinum* (2), *M. haemophilum*, *M. leprae* and a member of the *M. avium* complex was identified from a tree kangaroo.

## GENOTYPING OF *M. TUBERCULOSIS* ISOLATES

The MRL Molecular laboratory routinely performs molecular typing on all new *M. tuberculosis* isolates for epidemiological purposes. PCR-based typing based on estimation of the numbers of tandem DNA repeats at various loci (variable number of tandem repeats and mycobacterial interspersed repeating units [MIRU] typing) are used as first-line typing methods. Where further discrimination is required, selected strains are then fingerprinted using the restriction fragment length polymorphisms method. The MIRU profiles obtained for all the 2004 Victorian isolates were submitted for inclusion in a national *M. tuberculosis* genotype database.

## RESEARCH PROJECTS WITHIN THE MRL

In 2004, the MRL Molecular group hosted a part-time MSc student based at the Melbourne Zoo who has now completed her project developing a molecular assay for the detection of *M. genavense* infection in birds. The TaqMan assay developed from this project is now offered by the MRL for both human and veterinary specimens and is highly sensitive and specific. In addition, the laboratory is part of a collaborative group, along with the Austin Research Foundation and Department of Primary Industry, which has gained funding from the DHS to identify possible modes of transmission of *M. ulcerans* infection, particularly associated with the Point Lonsdale outbreak.

## Melbourne Sexual Health Centre

Thirty-three newly diagnosed cases of HIV infection were detected in 6061 samples tested. Of the 1,088 specimens tested, there were 81 cases of HCV infection diagnosed. Of the 4537 specimens tested for hepatitis B, 24 were positive for surface antigen, although there were no cases of acute infection diagnosed.

## Viral Gastroenteritis

Noroviruses are a major cause of both sporadic and epidemic gastroenteritis in humans but the mechanisms by which norovirus circulates within the community are poorly understood. Two studies were completed in this area in 2004.

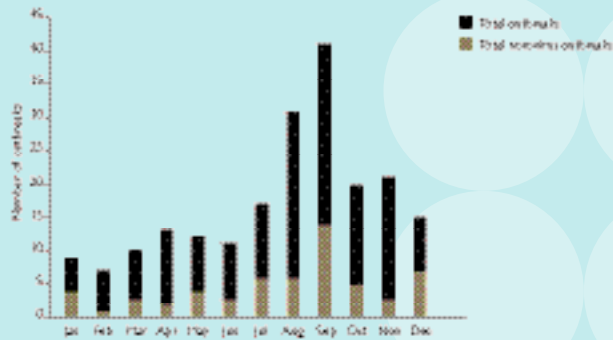
## FAILURE TO DETECT NOROVIRUS IN A LARGE GROUP OF ASYMPTOMATIC INDIVIDUALS

The hypothesis that asymptomatic people act as a reservoir for norovirus was examined. Faecal specimens from 399 asymptomatic individuals, ascertained as part of a larger cohort study of gastrointestinal disease following exposure to filtered or unfiltered water, were tested for norovirus by reverse transcriptase polymerase chain reaction (RT-PCR), and no norovirus was detected. The findings suggest that, if norovirus is carried by asymptomatic people, the carriage rate is very low; the upper limit of the 95% confidence interval for carriage was only 0.8%. It appears unlikely that asymptomatic people are an important reservoir for norovirus.

## LONG-TERM FEATURES OF NOROVIRUS GASTROENTERITIS IN THE ELDERLY

The relationship between clinical symptoms and norovirus excretion and the possible role of asymptomatic carriage of norovirus in the elderly are poorly understood. This study examined symptoms and norovirus excretion in elderly individuals associated with a norovirus outbreak in an aged care facility. Ten individuals aged 79-94 years were recruited for the study. One or more sequential faecal samples were collected from all participants over the same period and tested by RT-PCR. Norovirus was detected in faecal samples from all 10 study participants and was commonly detected in formed stools. In the nine symptomatic participants, acute symptoms such as diarrhoea and vomiting had largely resolved by the third or fourth day of illness, but non-specific symptoms such as headache, thirst and vertigo could persist for as long as 19 days. Both acute and non-specific symptoms appeared to resolve and recur in some participants. The median excretion time for norovirus was 8.6 days (range 2-15 days) in symptomatic participants (n = 5). There was no general relationship between the duration of norovirus excretion and the duration of either acute or non-specific symptoms. A faecal sample collected from an asymptomatic contact the day before gastroenteritis symptoms began was positive for norovirus, demonstrating prodromal excretion of norovirus. The relationship between norovirus shedding and duration of clinical symptoms (acute and non-specific) for each symptomatic participant is given in Figure 3.

**Figure 3: Norovirus outbreaks**



**Table 4: Outbreak settings**

Setting	Total outbreaks	Outbreaks with norovirus	Norovirus outbreaks as a percentage of total (%)
Aged care facility	131	104	79
Child care and Schools	11	3	27
Disabled care	2	2	100
Restaurants/ social functions	23	10	43
Hospital	40	31	78

### NOROVIRUS GASTROENTERITIS OUTBREAKS

During 2004, specimens were received from 207 gastroenteritis outbreaks for testing for viruses. Norovirus was detected in specimens from 149 (72%). The seasonal periodicity of all outbreaks received for testing, and those positive for norovirus, are given in Table 4. It can be seen that there was a major epidemic of norovirus gastroenteritis for the months August to November.

The settings of all outbreaks tested, including those where norovirus was detected, are given in Table 5.

Most outbreaks occurred in aged care facilities, hospitals and in gatherings; in all cases norovirus was a major cause of illness.

### Vaccine Preventable and Related Diseases

#### ENHANCED MEASLES SURVEILLANCE

The enhanced measles surveillance program continued for its eighth year. Eighty-nine notifications were tested by serology with 15 (17%) confirmed cases of measles. There were a number of individual cases and four small clusters identified throughout the year. Where an index case was identified, all were returned travellers.

The first cluster for the year began with the index case returning from Bali in December 2003 and transmitting measles to other household members. Nucleic acid sequencing of the nucleoprotein gene identified this virus as genotype G3. In March 2004, a traveller recently returned from Indonesia developed measles and infected relatives and household members. This virus typed as genotype D9. In July 2004, another two cases were linked with the index case, having just returned from a trip to Queensland. In November 2004, a traveller recently returned from Thailand infected a sibling and another relative. This was a genotype D5 virus.

In addition, an isolated case of genotype G2 measles infection was linked to travel to Singapore, and a case of genotype D3 was detected where the source could not be identified. Two of the notifications for measles were found to be parvovirus and two that were provisionally rubella IgM positive by serology were found to be false positive reactions when retested in an alternative assay.

#### ENHANCED MUMPS AND RUBELLA SURVEILLANCE

Surveillance for mumps and rubella also continued throughout 2004. Two notifications of mumps were tested for mumps IgG and IgM and Epstein Barr Virus IgG and IgM for differential diagnosis. The samples were also tested for measles and rubella IgG to monitor the seroprevalence of these vaccine preventable diseases. No cases were confirmed.

There were no notifications of rubella in 2004.

#### Q FEVER VACCINATION PROGRAM

The Q fever vaccination program remained active throughout Victoria in 2004.

VIDRL performed 1881 pre-vaccination serology tests, an increased number of tests compared to the years prior to the introduction of the Q fever management program by the Federal Government.

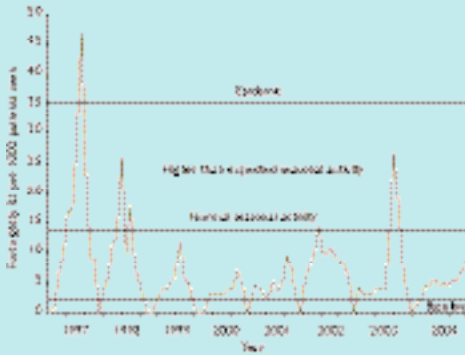
### Respiratory Pathogens

#### VICTORIAN SENTINEL INFLUENZA SURVEILLANCE PROGRAM

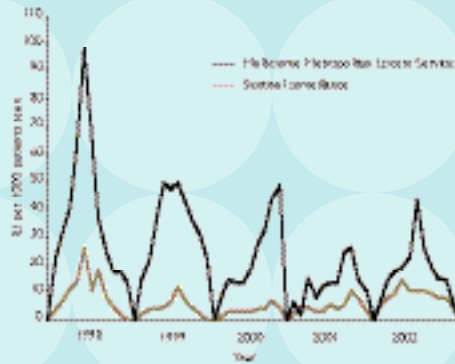
Sentinel surveillance at VIDRL for influenza-like illness (ILI) entered its ninth year in 2004. Coordination of influenza surveillance for Victoria continues to be based on the combination of general practitioners (GPs), ILI notification with laboratory confirmation, locum service ILI notification and laboratory reporting of influenza detections.

In 2004, influenza surveillance was performed over a 23-week period from May to October. Thirty-eight sentinel general practices participated, 15 from metropolitan Melbourne and 23 from rural Victoria. GPs reported weekly on ILI and overall consultation rates as well as collecting nose and throat swabs from patients who fulfilled the case definition of fever, cough and fatigue. ILI data was also once again collected from the Melbourne Medical Locum Service (MMLS) and laboratory confirmed influenza figures were obtained from The Royal Children's Hospital, Monash Medical Centre, VIDRL and the Alfred Hospital.

**Figure 4: Fortnightly consultation rates for influenza-like illness in Victoria 1997-2004**



**Figure 5: Retrospective comparison of locum service data and sentinel surveillance data, 1998-2002**



The season was characterised by low influenza activity with no clearly defined peak. There was an average weekly rate of ILI from sentinel surveillance of 5.4 per 1000 consultations (Figure 4). The data received from MMLS reflected the low activity and absence of seasonal peak.

Specimens were collected from 238 patients for testing using an in-house polymerase chain reaction (PCR). Influenza A was detected in 37 (13.9%) specimens and influenza B in 11 (4.1%) specimens. Further strain analysis was carried out on all positive samples at the WHO Collaborating Centre for Influenza. Of the influenza A samples recovered, 88% were A/Fujian/411/2002-like and 12% were A/Wellington/1/2004. All influenza B samples were B/Shanghai/361/2002-like. The composition of the influenza vaccine was A/New Caledonia/20/99(H1N1)-like virus, A/Fujian/411/2002(H3N2)-like virus, and B/HongKong/330/2001-like virus.

Excel spreadsheets are currently being used to store all influenza surveillance data. Ongoing efforts will see the completion of the development of a database designed specifically for influenza surveillance in early 2005.

## SEVERE INFLUENZA INFECTIONS IN CHILDREN, 2003

Influenza has an annual attack rate of up to 20% of children in developed countries. Although most cases are self-limiting, severe complications can occur. Neurological manifestations are infrequently reported outside Japan. During the influenza season in 2003, four cases of Victorian children with severe influenza A infection with neurological or cardiac complications occurred. Two children died. All cases occurred in late August or early September 2003, when the influenza season in Victoria was well established. For all cases, samples positive for influenza A were confirmed as influenza A/Fujian/411/2002-like by the WHO Collaborating Centre for Influenza Reference and Research.

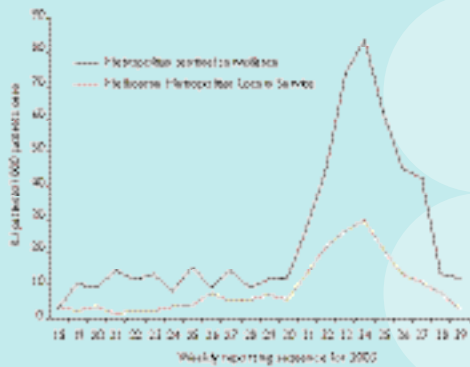
Victoria's influenza season in 2003 was characterised by a late peak and higher than normal seasonal activity. Almost all viruses detected from both diagnostic and surveillance specimens were influenza A/Fujian/411/2002-like. This same strain was found in each of these four cases: cases one and four had encephalitis due to influenza A, case three had influenza A infection with widespread cortical damage on MRI, and case two had influenza A infection complicated by myocarditis. It was not clear whether the apparent increase in severity of infection was due to increased influenza virus circulation or an increased virulence of the Fujian strain. Paediatric deaths due to the Fujian strain were also reported in the Northern Hemisphere influenza season of 2003-4.

Although influenza in infancy and childhood is usually associated with a high attack rate, the clinical course of the illness is generally benign. Nonetheless, authorities in the USA have recently recommended annual vaccination for children aged six to 23 months. A recent European review does not recommend universal annual influenza childhood vaccination and, at the time of writing, neither had Australian authorities recommended routine influenza vaccination for children.

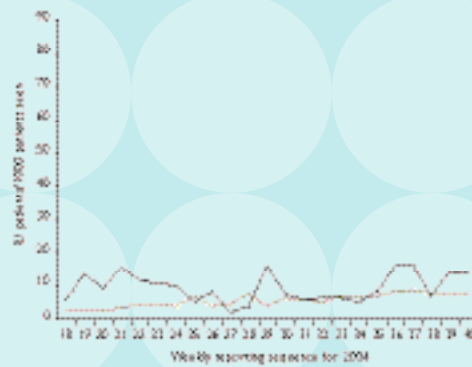
## INFLUENZA PANDEMIC PLANNING IN VICTORIA

DHS has been coordinating influenza pandemic planning for the last two years. VIDRL staff have been involved in the development of the plan, providing the Chair and a member of the surveillance sub-committee and contribution to the desktop review of the plan, held in December 2004. In addition to continued coordination of influenza surveillance in Victoria, VIDRL continues to refine its diagnostic algorithm for the diagnosis of influenza. From the use of immunofluorescence and viral isolation, in 1998-9 the diagnosis of influenza moved to an influenza multiplex PCR that identified influenza haemagglutinin I (HI) and H3 sub-types. Further evolution of the multiplex PCR technology allowed the diagnosis of a number of other respiratory viruses known to cause an influenza-like illness, in addition to the diagnosis of the HI and H3 sub-types. In 2004, the multiplex PCR was again modified to identify any of the known haemagglutinin subtypes. Further evolution of the approach to the diagnosis and surveillance of influenza will allow the rapid identification of haemagglutinin subtypes such as H5, H7 and H9 that might have the potential for pandemic spread.

**Figure 6a:** is there a 6b? Comparison of general practice surveillance and MMLS surveillance for influenza-like illness



**Figure 6b:**



### ESTIMATING INFLUENZA VACCINE EFFECTIVENESS FROM AN OUTBREAK

Previously two outbreaks of influenza in aged care facilities have been reported and attention drawn to the low levels of staff vaccination coverage and the emerging use of antiviral medication to control the outbreaks. Influenza vaccine effectiveness (VE) was estimated in one of these outbreaks, which occurred between 25 December 2001 and 21 January 2002. Neuraminidase inhibitors were used to control the outbreak. Residents and staff with respiratory symptoms were tested for influenza using reverse transcriptase polymerase chain reaction (RT-PCR) and/or serology. Influenza VE was estimated for the prevention of laboratory confirmed influenza.

Nineteen of 42 (45%) residents and four of 29 (14%) staff were laboratory confirmed as cases. The outbreak was caused by an A (H3N2) strain of influenza, antigenically matched to that season's vaccine. The VE for preventing laboratory confirmed influenza infection was 61% (95% confidence interval (CI), 41-73) among residents and 100% (95% CI, 61-100) among staff. The VE estimates from this outbreak were consistent with other published results for both staff and residents. Outbreaks of influenza in institutions provide a good opportunity to review influenza VE, but use of antiviral medications as control measures may affect the interpretation of findings.

### A MEDICAL LOCUM SERVICE AS A SITE FOR SENTINEL INFLUENZA SURVEILLANCE

In Victoria, surveillance of influenza, with influenza-like illness (ILI) defined by fever, cough and fatigue and influenza seasons described by thresholds is conducted through sentinel general practices. However the coordination of sentinel general practices can be time-consuming and expensive. For the last two influenza seasons a medical locum service has been used as a sentinel site for influenza surveillance. We are not aware of locum service being used as a sentinel site anywhere else in the world.

Although different in magnitude, the pattern of ILI among patients seen by the locum service was similar to the pattern from sentinel general practice surveillance between 1998-2002 (Figure 5). Prospective surveillance during the 2003 and 2004 influenza seasons confirmed similar ILI surveillance patterns. In 2003 an increase in ILI was evident by week 31 with a peak at week 34 for both surveillance systems (Figure 6a) and both systems demonstrated a similar picture in 2004, despite a season of low activity and no clearly defined peak (Figure 6b). The proportion of patients with ILI seen by the locum service continued to be higher than in sentinel general practice surveillance, 2.5% (661/25630) compared to 1.0% (764/75455) in 2003 and 1.0% (216/23470) compared to 0.6% (465/80712) in 2004.

Because of its timeliness, flexibility, patient mix and geographic spread, locum service surveillance may be able to supplement, or even replace, sentinel ILI surveillance. Locum service surveillance may also have a role in the recognition of emerging diseases.

### EVALUATION OF INFLUENZA SURVEILLANCE

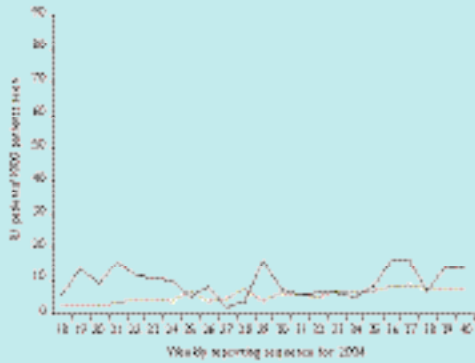
An evaluation of influenza surveillance in Victoria, using the *Updated Guidelines for Evaluating Public Health Surveillance Systems (MMWR 2001; 50:RR-13)*, was conducted between July and December 2004. The evaluation sought to describe the operation and attributes of the system; identify the objectives of influenza surveillance in Victoria and determine how well these objectives were being met; and to assess the usefulness of influenza surveillance in Victoria. Data from both VIDRL and DHS were analysed from 2002 to 2004 for data quality, timeliness of data management and reporting and to determine geographic and age representativeness. Stakeholder interviews and a survey of participating sentinel practitioners were conducted.

The evaluation determined that sentinel practice surveillance provided influenza strain information, important for vaccine formulation. It also captured information on laboratory-confirmed influenza in older children and adults, whereas hospital laboratories notified DHS predominantly of influenza in infants and the elderly. Although this collection of data from several sources was necessary to obtain information across all age groups and geographic areas, it also provided challenges for efficient system coordination with data being reported separately rather than collated and DHS and VIDRL duplicating data collection of hospital laboratory confirmed cases.

The continuity of surveillance over several years has enabled thresholds for influenza activity to be established. Ability of influenza surveillance to contribute to pandemic preparedness through early detection of influenza epidemics and the recognition of new influenza viruses in a timely fashion appears feasible but has yet to be tested.

While influenza surveillance was generally well run and met the stated objectives, some areas for improvement were identified. These included improving coordination of data collecting and reporting and conformity to standard definitions for cases reported from hospital laboratories. Further research to determine the applicability of the current ILI case definition for children and ways to monitor the impact of influenza such as using mortality data were recommended.

**Figure 7: The distribution of respiratory viruses detected in the pilot study samples compared to specimens collected as part of general practice surveillance**



## PILOT STUDY OF INFLUENZA AMONG PATIENTS IN A PAEDIATRIC EMERGENCY DEPARTMENT

In response to the USA 2003 revised recommendation for annual influenza vaccination to include children aged six to 23 months, a pilot study was commenced looking at influenza among children attending the Royal Children's Hospital emergency department (ED). The study aimed to determine the extent of, and risk factors for, influenza in children in order to assess the need for, and potential impact of, the introduction of a similar annual influenza vaccination program in Australia.

Patients were eligible to participate if they presented at the ED with a respiratory illness, were assigned triage category one to four (one is the most serious illness) and aged between six months and 16 years. Provision of informed consent was necessary to comply with requirements of the ethics committee. A one-page clinical history and presentation questionnaire were completed and a nose and throat swab collected and sent to VIDRL. Swabs were tested by multiplex respiratory PCR, and for human coronavirus OC43, 229E and metapneumovirus by separate PCR assays.

Between 26 July and 30 September, 21 specimens were collected from eligible children aged 0 to 12 years. Influenza was detected from 14% (3/21) of specimens; one influenza A and two influenza B. An alternative respiratory virus was detected in an additional 48% (10/21) specimens, giving a total virus detection rate of 62%. No human coronaviruses or metapneumoviruses were detected. The pilot study detection rate was higher than that found in general practice surveillance (48%), however the distribution of viruses detected was similar with picornavirus the most frequent (Figure 7). After consideration of the pilot study results and limitations, the study will be continued in 2005.

## Sexually Transmitted Infections

### THE CHANGING EPIDEMIOLOGY OF GENITAL HERPESVIRUS INFECTION

In last year's Annual Report, we reported an increasing proportion of herpesvirus (HSV)-1 compared to HSV-2 in genital infections known to be caused by herpesviruses. As reported between 1980 and 2003, the overall proportion of VIDRL laboratory-diagnosed genital herpes due to HSV-1 rose from 16% to 35%. In 2004, the proportion of HSV-1 rose further to 41%. In patients under 20 years of age, more than 90% of laboratory-diagnosed genital herpes was due to HSV-1 in 2004, up from 77% in 2003.

## Diagnostic Testing

### BACTERIOLOGY

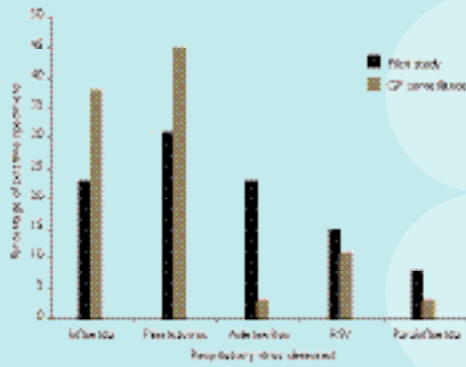
The Bacteriology Laboratory provides a diagnostic service for a targeted group of medical clinics with special expertise in HIV/AIDS, sexually transmitted infections and travel medicine. It is envisaged that in the future these clinics will be considered as 'sentinel clinics' for the monitoring of sexually transmitted infections. In addition, the provision of specialised diagnostic services attracts referrals from other hospital-based and private pathology services. This referral work includes the diagnosis of sexually transmitted infections, identification of parasitic infections and the diagnosis of Legionella infection.

### Legionellosis

Diagnostic testing for Legionella infection constitutes part of the standard management of patients with acute, community-acquired pneumonia. One of the most convenient assays for diagnosis of the most commonly encountered infection (*Legionella pneumophila* serogroup 1) is detection of Legionella cell-wall antigen in urine, an assay that has been offered by our laboratory since 1995. Specimens are referred from public and private hospitals in Melbourne and country regions of Victoria, southern New South Wales and Tasmania. During the year there were 4072 tests performed, 7% fewer than in 2003. There were 46 positive patients.

Other means of testing include culture and PCR testing of respiratory specimens. The PCR test identifies either the *Legionella pneumophila* group or Legionella species (other than pneumophila). Additional tests allow the further speciation of the latter group to identify *L. longbeachae*, *L. micdadei* and *L. bozemanii*. PCR testing can also be used to detect Legionella in instances where antibiotic therapy has already been commenced and culture would be unlikely to yield a positive result. When compared with the number of urinary antigen tests performed, this test is probably under-utilised with only 212 tests performed in 2004. There were 15 positive results from 10 patients, with *L. pneumophila* detected in five, *L. longbeachae* in three, *L. micdadei* in one and Legionella species not further speciated in one.

**Figure 8: ID Serology Laboratory tests**



**Table No?: Comparison of molecular tests for HBV and HCV 2003-2004**

Test	2003	2004
HCV PCR	4278	4500
HCV Genotype	1289	1719
HCV Load	776	1502
HBV PCR and HBV Load	1303	3306

### Parasitology

The laboratory provides a diagnostic and reference service of enteric parasites. Assistance to other laboratories includes the provision of control specimens and in the identification of parasites. The latter aspect usually involves the differentiation of species of amoeba, with the definitive identification of *Entamoeba histolytica* causing problems for those laboratories that do not commonly encounter this pathogen.

Primary specimens are received from clinics that VIDRL services and from an immigrant health screening program conducted at the Royal Children's Hospital. The overall parasite detection rate was 22%, including 50 cases of *Giardia lamblia* and 43 of *Entamoeba histolytica*. The majority of *E.histolytica* was detected in specimens from gay men and is thought likely to be non-invasive (non-pathogenic).

### Sexually Transmitted Infections

The Roche Amplicor multiplex PCR for chlamydia and gonorrhoea constitutes the diagnostic test more frequently performed. This assay for gonorrhoea must be considered as a presumptive screening test only and a further PCR test to detect the cryptic plasmid of *Neisseria gonorrhoeae* is required to confirm any positive results. Both assays are also performed on a referral basis for other laboratories.

Over this year there has been a further 10% increase in requests for the multiplex chlamydia/gonorrhoea PCR. From 5252 tests performed there were 214 cases of chlamydia and 88 of gonorrhoea infection detected. (The latter figure excludes any cases where culture for gonorrhoea also proved positive). Combined figures for diagnosis of gonorrhoea by either culture or PCR totalled 332, this constitutes approximately 25% of the statewide notifications of infection to DHS. The majority of positive diagnoses were from males. 23% of chlamydia and 25% of gonorrhoea diagnoses were from anal swabs; the corresponding figures for pharyngeal swabs were <1% of chlamydia and 13% of gonorrhoea diagnoses.

### Quantiferon TB Gold Assay

The Quantiferon TB Gold assay is designed to detect latent TB infection by measuring cell mediated immunity to TB-specific antigens; in effect the test provides a quantitative and more specific alternative to Mantoux testing. Following commencement of testing in 2003, there has been a marked increase in requests for this test. During the year there have been 907 tests performed, with 113 positives.

### INFECTIOUS DISEASES SEROLOGY

The infectious diseases serology laboratory performs a range of tests covering viral, bacterial and parasitic diseases. During the year 49,135 tests were performed on specimens from 40,838 patients.

Confirmatory testing and routine diagnostic testing for syphilis is performed in the serology laboratory. During the year, 11,685 syphilis tests were performed. Screening consists of testing by rapid plasma reagin and enzyme immunoassay (EIA) total antibody tests. A positive result in one or both of these tests then requires further testing using the Treponema pallidum Particle Agglutination test and also an EIA IgM test when a primary infection is suspected.

Measles testing is performed for active surveillance and also routine diagnostic purposes; 2273 tests were performed.

Screening for respiratory infections is another major component of activity undertaken by the laboratory.

The main tests performed were:

Legionella	5779
Chlamydia psittaci/ pneumoniae	4741
Mycoplasma pneumoniae	3734
Influenza A/B	2247

Screening for parasites mainly on returned travellers and refugees is also performed. The two most common tests performed were

Schistosoma	1172
Strongyloides	1068

Figure 8 shows the most frequently requested tests performed in the laboratory.

### MOLECULAR TESTS FOR HEPATITIS B VIRUS AND HEPATITIS C VIRUS

The availability of a new HBV load assay with greater sensitivity and superior dynamic range has proved to be more useful clinically. This resulted in an increase in requests for this assay. Additionally, improvements in treatment for chronic hepatitis C saw a greater throughput of samples for genotype and load testing, the two major predictors of treatment response.

**Table 5**

PCR result	Serology positive. Evidence of recent infection.	Serology negative. No evidence of recent infection.	Serology previously positive. Evidence of reinfection or reactivation.	Serology previously positive. No evidence of reinfection or reactivation.
Positive	21	2	2	-
Negative	8	80	-	10

We found the *T pallidum* PCR to be a useful addition to serology for the diagnosis of syphilis, and confirmed the value of IgM serology for the detection of early cases. Further studies are required to fully assess the limitations of the assay.

## New Test Development

Several PCR assays were validated and introduced in 2004. These include mumps virus and influenza C virus PCRs. Preliminary results from testing a large number of respiratory specimens during the year show that influenza C virus is a very rare cause of respiratory illness in Victoria. The influenza C virus PCR will not be made available for diagnostic purposes but will be used during the investigation of discrete outbreaks of respiratory disease for which an aetiological agent cannot be identified.

Primers specific for the recently identified human coronavirus designated HCoV-NL63 have been incorporated into a multiplexed coronavirus PCR capable of detecting HCoV-OC43, HCoV-229E specific and HCoV-NL63. This test is available for outbreak investigations and as a fee-for-service diagnostic assay.

### PREPARATION OF MOLECULAR REAGENTS FOR HUMAN CORONAVIRUS SARS-COV

Last year we reported the cloning and expression of the coronavirus SARS-CoV genes encoding the structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N). The N clone has been found to form a multimeric complex that aggregated with cellular RNA. The C terminus RNA binding region was removed for further expression studies. The N and S expressed protein preparations have been used as a source of specific antigens for the panning of bacteriophage libraries. To date no unique binders to these proteins have been identified.

### PCR ASSAY TO DETECT PNEUMOCYSTIS INFECTION

Work is proceeding towards the validation of a PCR assay for detection of Pneumocystis infection. The detection methods currently used are histochemical and immunofluorescence staining techniques that are particularly labour-intensive. An additional complication is that the sensitivity of these tests is often compromised by the inadequacy of specimen volume. In these circumstances the PCR assay will constitute a more viable alternative approach. During the year there were 422 bronchoscopy (or induced sputum) specimens referred for this test, with 6% giving positive results.

### HEPATITIS A VIRUS PCR

New hepatitis A virus PCR primers were designed to replace the primers previously in use. The sensitivity and specificity of the assay was improved as a result. Furthermore with the new amplified PCR assay, sequence analysis of the PCR product allows for genotyping and phylogenetic analysis.

### HEPATITIS E VIRUS PCR

Hepatitis E virus (HEV) PCR testing was introduced at VIDRL in 1993 when the test was used to diagnose the first case in Australia. Subsequent work showed that HEV fell into two main genotypes, designated Mexican and Burmese. Taking into consideration sequence differences, an improved HEV PCR assay was developed this year. The test was used to help diagnose returned travellers with HEV infection after an outbreak in India.

### SYPHILIS PCR ASSAY

Since 2002, there has been a marked increase in the number of cases of locally acquired syphilis infection. Serology (RPR, TPPA, recombinant total Ab EIA +/- IgM EIA) has been the mainstay of diagnosis, as older direct detection methods are problematic.

A TaqMan real-time PCR assay was developed targeting the *Pol A* gene of *T pallidum*, based on published sequences. From February to December 2004, 331 specimens of various types from 303 patients submitted to the laboratory were tested. In 123 patients, adequate serology testing was performed to allow comparison with PCR results. Positive PCR results were confirmed by serology in 23 patients, of whom nine were HIV positive. See Table 6 for further details.

## New Test Validation

### HEPATITIS B VIRUS DNA QUANTIFICATION

From 1 May 2004, all test requests for quantitative hepatitis B virus (HBV) DNA or HBV load were performed by the Bayer VERSANT HBV DBNA 3.0 (bDNA) test. This test replaced the Digene Hybrid Capture II assay which had a lower limit of detection of 140,000 copies/ml. The Bayer assay lower limit is 2000 copies/ml, which increases the sensitivity by nearly 100 fold, and with an upper limit of  $10^9$  copies, the assay also has a superior dynamic range. Other advantages include reporting results in International Units (IU), allowing results to be compared with other HBV assays based on the International Standard. The layout of the HBV load report was changed to indicate both the copies/ml and IU/ml and contains the calculated  $\log_{10}$  values as well, which assists in determining changes in load during antiviral treatment. The report layout for HCV quantitative test, also done by Bayer bDNA technology, was modified to provide the  $\log_{10}$  values as for the HBV load report.

### REAL-TIME PCR ASSAYS FOR HEPATITIS B VIRUS AND HEPATITIS C VIRUS

The Roche TaqMan 48 system was evaluated for HBV and hepatitis C virus (HCV) quantification assays. For HCV, three methods were compared, Roche HCV MONITOR 2.0, TaqMan 48 HCV Assay and Bayer VERSANT HCV RNA 3.0. Good correlation was found between the Bayer VERSANT HCV RNA 3.0 and the HCV MONITOR 2.0 but deficiencies were found with the HCV TaqMan 48, which showed lower values for some genotypes. The HBV quantitative methods, TaqMan 48 HBV Assay and Bayer VERSANT HBV DNA 3.0 Assay correlated well.

## CHLAMYDIA PSITTACI AND PNEUMONIAE

Chlamydia is a gram-negative obligate intracellular bacteria that causes acute and chronic disease in mammalian and avian species. The genus Chlamydia is comprised of four species: *C. trachomatis*, *C. pneumoniae*, *C. psittaci* and *C. pecorum*.

*C. pneumoniae* is an important respiratory pathogen in humans and causes up to 10% of community-acquired pneumonia cases.

*C. psittaci* infects a diverse range of species from molluscs to birds to mammals and also causes severe pneumonia.

*C. trachomatis* is divided into 15 serovars. Serovars A, B, Ba and C are agents of trachoma, the leading cause of preventable blindness, endemic in third world countries. Serovars L1 - L3 are the agents of lymphogranuloma venereum. Serovars D-K are the common cause of sexually transmitted genital infection worldwide.

A validation program was undertaken for the expansion of Chlamydia testing in the serology laboratory. Previously only testing for *C. psittaci* was performed but, with the kit in use being withdrawn from the market, an opportunity to expand testing was made available. A comparative study was performed with sera previously tested at VIDRL for *C. psittaci* using a panel of negatives, positives and seroconversions and a small panel of samples positive and negative for *C. pneumoniae* sourced from the Alfred Hospital. Results were as follows.

#### Genus specific EIA

100% correlation with the negative sera (n=28)

94% correlation with the high positive sera (n=16)

86% seroconversions correlated, the two discrepant results gave negative to high negative results rather than a definitive seroconversion. (n= 14)

#### Immunofluorescence species specific assay

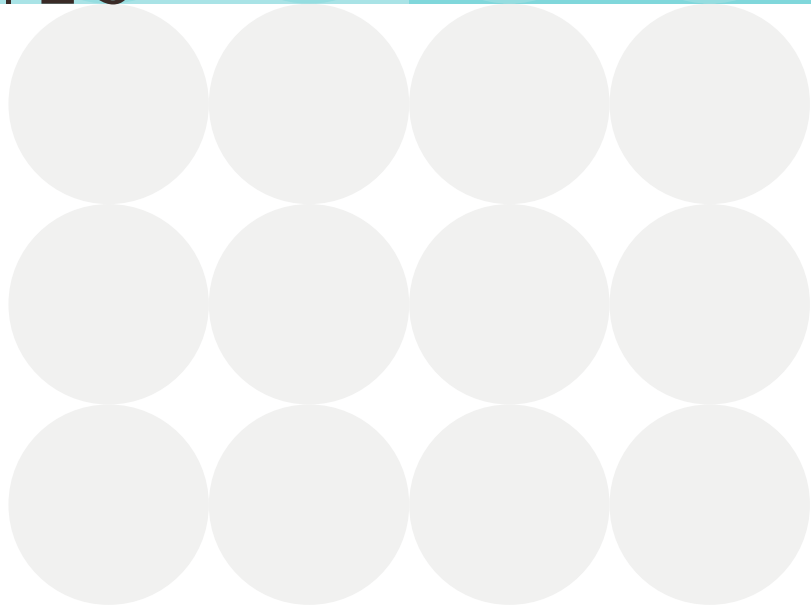
100% correlation with *C. pneumoniae* sera (n=10)

89% correlation with *C. psittaci* seroconversions (n= 9)

A test algorithm was set up to screen all respiratory samples using a genus specific EIA. Any samples testing positive by this method are then tested using a species specific indirect fluorescent antibody test to identify the causative species.



# RESEARCH AND DEVELOPMENT ACTIVITIES



# RESEARCH AND DEVELOPMENT ACTIVITIES

VIDRL undertakes a diverse range of research projects. These include studies of antiviral drug resistance and the transmissibility of viruses and viral variants. Investigations of the epidemiology of infectious agents of public health significance, and the development of potential new laboratory technologies are also important areas of study. Research is undertaken in all program areas.

The main areas of research activity in 2004 were:

- further development of an internet-based program for the analysis of hepatitis B virus sequence
- investigation of patients co-infected with HIV/HBV (an international collaborative study funded by the US National Institute of Health)
- hepatitis B virus vaccine escape mutants
- hepatitis B viral antiviral resistance
- social network analysis of hepatitis C virus in a cohort of injecting drug users (in collaboration with the Burnet Institute and funded by NHMRC)
- a pilot project to establish the clinical and laboratory investigation of undiagnosed encephalitis
- development of a real time PCR assay for the diagnosis of mycobacteria in birds (in collaboration with the Melbourne Zoo)
- an observation from serological testing of an increase in hepatitis E virus infection among Victorian travellers
- replication of SARS-CoV in cell-lines used in diagnostic virology laboratories
- molecular analysis of clinical isolates of acyclovir resistant herpes simplex virus.

In addition applied public health research included:

- investigation of possible hepatitis C virus transmission in a surgical setting
- demonstration that norovirus RNA can be excreted before and after acute symptoms (a project funded by a DHS public health research grant)
- estimation of influenza vaccine effectiveness from an outbreak where antiviral medication was used as a control measure
- demonstration of the usefulness of a medical locum service as a sentinel site for the surveillance of influenza-like illness
- a pilot study of influenza in patients from a paediatric hospital Emergency Department
- a review of the changing epidemiology of genital herpesvirus infection

## 2004 Activities

### INTERNET-BASED PROGRAM FOR HEPATITIS B VIRUS - SEQHEPB

As has previously been reported VIDRL has developed a web-based program (SeqHepB) to process data from hepatitis B virus (HBV)-infected patients for the purpose of providing support to healthcare professionals. An integrated database system was developed to manage the sequence data generated by the SeqHepB software program, as well as all the relevant clinical, drug history and pathology test results collected from patients. This was expanded to include phenotypic data generated from both VIDRL and published studies. The integration of data means comprehensive reports can be generated that contain all the relevant clinical and drug history, HBV pathological test results, viral genetic data and their clinical significance in relation to published references. In addition, complex queries have been built into the database system to allow comprehensive data mining such as finding novel associations between antivirals, resistance markers, and the natural history of HBV infections.

### HIV AND HEPATITIS B VIRUS CO-INFECTED PATIENTS

The Research and Molecular Development Division was awarded a NIH grant to investigate the virology and the clinical problems associated with HIV/HBV co-infection in cohorts of patients from Melbourne, Sydney and the Multi-Centre AIDS Cohort in USA. We have initiated as part of this grant a program of full HBV genome sequencing to analyse virological factors associated with co-infection and also antiviral resistance. A number of antiviral agents are effective against both HIV and HBV. Thus susceptibility to these agents needs to be monitored in both viruses to determine if mutations generated are different from mono-HBV infected patients and also if the frequency of these mutations is different in the co-infected cohort versus the mono-infected cohort.

## ANALYSIS OF VIRUS-VIRUS INTERACTIONS BETWEEN HIV AND HEPATITIS B VIRUS

While much is known of the biology of HIV and HBV, little is known of viral interactions between the two viruses. HIV encodes a number of small accessory proteins vpr, vpu, vif, nef, rev and tat. These proteins are associated with enhanced pathogenesis, transcription, replication regulation, cell signalling and the induction/repression of apoptosis during HIV infection. Although there is evidence of HIV infection of hepatoma cells *in vitro*, the presence of many HIV accessory proteins in infected serum and their ability to be taken up into cells may influence HBV replication. To investigate the interactions between HIV accessory proteins on HBV transcription several HBV promoter/luciferase reporter constructs were prepared. The level of HBV transcription from each promoter alone was compared to cells co-transfected with clones expressing different HIV accessory proteins. This work is a collaboration between VIDRL, the Department of Microbiology and Immunology, University of Melbourne, and the Institute of Medical and Veterinary Science, Adelaide.

## HEPATITIS B VIRUS VACCINE ESCAPE MUTANTS

During antiviral treatment, viruses may be selected that have an altered polymerase gene as well as an altered envelope gene. HBV has a complex genome in which the polymerase gene overlaps the gene encoding for the envelope proteins. Furthermore, two important regions for each gene overlap, i.e., in the polymerase gene the area encoding the reverse transcriptase in which resistance mutations are selected coincides with the major immunological region in the envelope protein. The characterisation of the mutated envelope protein in antigen/antibody binding studies is important in determining whether viruses encoding these mutations may have the potential to become a vaccine escape mutant and may have the ability to be transmitted to previously vaccinated individuals such as Health Care workers. A collaboration between CSIRO and the CRC for Diagnostics has been established to identify new antibody based reagents directed against HBV envelope proteins and the various HBV envelope mutations selected during antiviral resistance. In addition, this work is part of an ongoing collaboration with Dr Krawczynski of Hepatitis Branch at the Centre for Disease Control (CDC) Atlanta, USA.

## IDENTIFICATION AND CHARACTERISATION OF HEPATITIS B VIRUS RESISTANCE TO ADEFOVIR DIPIVOXIL AND ENTECAVIR

Last year we reported the initial characterisation of resistance to the nucleoside analogue adefovir dipivoxil (ADV). We have now extended this finding to identify a number of other resistance mutations associated with ADV resistance in patients who developed clinical and virologic evidence of breakthrough during antiviral treatment. This includes the primary resistance mutation at rtN236T and the mutation at rtA181T/V. We have now identified three clusters of mutations associated with ADV resistance and work on the phenotypic analysis of these mutations is in progress. Secondly in collaboration with BMS we have characterised the two groups of entecavir (ETV) resistance mutations using *in vitro* phenotypic assays. These two groups of mutations each consist of two unique mutations in combination with the lamivudine resistance mutations (i.e., ETV resistance Group 1: rtI169T+rtM205V+ rtL180M+rtM204I/V; ETV Group 2: rtT184G+ rtS202I+ rtL180M+ rtM204I/V). All four mutations were required for ETV resistance *in vitro*.

## MOLECULAR EPIDEMIOLOGY OF HEPATITIS C VIRUS IN INJECTING DRUG USERS

In collaboration with the Burnet Institute on an NH&MRC funded project, a cohort of injecting drug users was studied using social network analysis and molecular analysis of the HCV genome. Phylogenetic analysis of viral sequences allowed several clusters of infection to be identified, showing there was an epidemiological link present. Interestingly there was a low correlation between social network data and phylogenetic data which highlighted the difficulty in mapping networks of highly mobile injecting drug user populations. The ability to track recent HCV infections by molecular means may provide an opportunity for intervention strategies to be implemented.

**Figure 9: Highly reactive HEV ELISA results at VIDRL and total number of HEV ELISA tests performed at VIDRL per quarter January 1 1999 to June 30 2004**



### CLINICAL AND LABORATORY INVESTIGATION OF UNDIAGNOSED ENCEPHALITIS (CLUE STUDY)

Encephalitis is a potentially life-threatening illness for which no cause is found in a substantial proportion of cases. The recent emergence of Australian Bat lyssavirus, Nipah and Hendra viruses in our region emphasises the need for better awareness of pathogens causing encephalitis. VIDRL is participating in a new collaborative study that aims to describe the clinical features of patients with encephalitis in Australia and to investigate the aetiological agents involved.

Data will be collected on demographic factors, epidemiology, clinical features and outcomes from patients with encephalitis where aetiology is not established. A central specimen storage and database facility will be maintained so that data and specimens can be made available for future diagnostic studies for rare or newly emerging pathogens as new information or diagnostic technology becomes available. Following evaluation of an initial pilot study involving four Victorian hospitals in 2005, it is intended to expand the study to other Australian hospitals.

### INCREASE IN HEPATITIS E INFECTIONS DIAGNOSED IN VICTORIA

HEV is a major cause of enterically transmitted hepatitis worldwide. It is an important pathogen in Asia, the Middle East, and parts of Africa and Central America. Seroprevalence in non-endemic areas such as Australia is approximately 1-2%, in contrast to endemic areas where seroprevalence increases with age to range from 10 to 40% in adulthood. The incubation period for HEV is two to nine weeks, and the spectrum of disease ranges from subclinical infection through to fulminant hepatitis.

There was a seven-fold increase in the number of serum specimens highly reactive for anti-HEV IgG tested at VIDRL in the first half of 2004, from a six monthly mean for the last five years of 2.4, to 17 ( $p < 0.0001$ ). VIDRL is the only Victorian laboratory that tests for HEV. Patient interviews confirmed a clinically compatible illness in most cases and history of recent travel to HEV endemic countries. It could not be established why this increase in HEV infections diagnosed in Victoria had occurred but it was unlikely to be due to increased testing. The mean number of HEV serology tests performed at VIDRL per quarter over the previous five years was 51.6 (Figure 9). There were 57 and 59 tests performed in the first two quarters of 2004 which was not significantly different to previous quarters ( $p = 0.452$  and  $0.303$ ). The observation made at VIDRL of an increase in HEV infections among travellers may be repeated in other developed countries. Advising travellers to developing countries of preventative measures against HEV and other enterically transmitted diseases continues to be important.

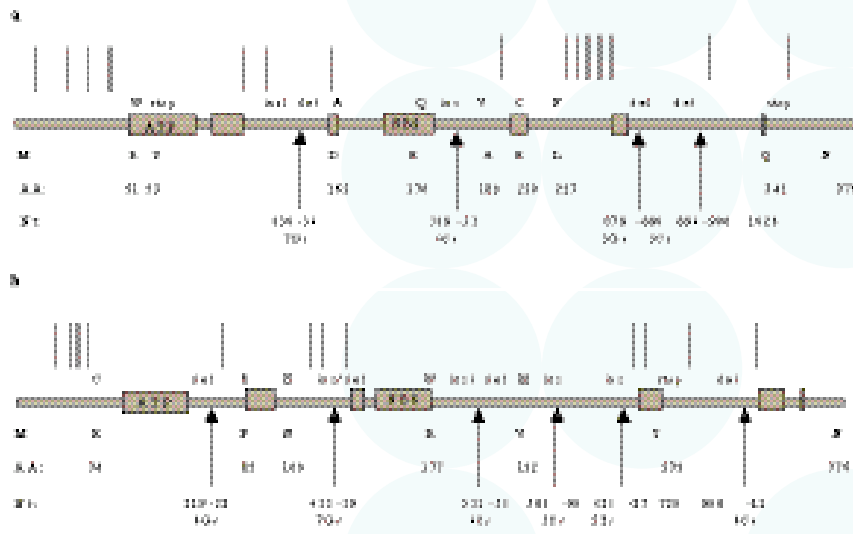
**Table 6: Susceptibility to SARS-CoV of cells used in diagnostic and research laboratories at VIDRL. Cells other than FRhK, PCMK, Vero and Vero E6 have not previously been reported as supporting replication of the virus**

Cell line	Species of origin	Cell type
<b>Virus replication detected:</b>		
BGM	monkey, buffalo green	kidney epithelium
COS	monkey	derivative of CV-1
CV-1	monkey, African green	kidney fibroblast
FRhK	monkey, rhesus	foetal kidney
LLC-Mk2	monkey, rhesus	kidney epithelium
MA-104	monkey, African green	kidney epithelium
MEK	monkey	embryonic kidney
PCMK	monkey, cynomolgus	primary kidney
Vero	monkey, African green	kidney epithelium
Vero E6	monkey, African green	clone of Vero
HEK-293	human	foetal kidney
Hep G2	human	liver hepatocellular carcinoma
Huh-7	human	liver hepatocellular carcinoma
RK-13	rabbit	kidney epithelium
<b>No virus replication detected:</b>		
A549	human	lung carcinoma epithelium
HEL	human	foetal lung fibroblast
HeLa-T	human	cervical epithelium
Hep-2	human	epithelium derived from HeLa-T
RD-A	human	Rhabdomyosarcoma
MDCK	canine	kidney epithelium
L20	murine	express poliovirus receptor

## REPLICATION OF SARS-COV IN CELLS USED IN DIAGNOSTIC VIROLOGY LABORATORIES

Given the potential for laboratory-associated SARS-CoV infections to occur, there is a need to be aware of cell lines permissive for infection with this virus. We investigated the susceptibility to SARS-CoV of 21 cell lines routinely used for diagnostic and/or research purposes at VIDRL. Cells were infected with the virus and observed for the development of specific cytopathic effects for up to 11 days. A quantitative, real-time PCR was used to distinguish on-going viral replication from in-put virus. Indirect immunofluorescence using serum from a patient infected with the SARS virus was used as a confirmatory assay. The study identified 10 previously unreported cell lines that were capable of supporting replication of SARS-CoV (Table 7). Continuous cell lines derived from monkey kidneys were particularly susceptible. Human liver and rabbit kidney lines also supported replication. The study demonstrated that the virus can replicate in cell lines commonly used for diagnostic and/or research purposes. The virus achieves high titres in several lines and cytopathic effects may be minimal. These findings are relevant to laboratory scientists undertaking virus isolation procedures on respiratory and enteric specimens collected from patients with atypical respiratory disease.

**Figure 10:** Mutations identified in the tk gene of HSV-1 (a) and HSV-2 (b) isolates resistant to ACV (not to scale). ATP binding site (ATP), nucleoside binding site (NBS) and other regions of the TK gene conserved among herpesviridae are indicated by the grey boxes. The codon (AA) and nucleotide (Nt) numbers of the wild-type HSV sequence are noted below each gene. Mutational homopolymer runs and the Nts involved are indicated by vertical arrows. Amino acid changes and the relative positions of stop codons (stop), insertions (ins), deletions (del) and either an insertion or a deletion (ins/del) are indicated above each gene. Bars above each gene indicate the locations of polymorphisms.

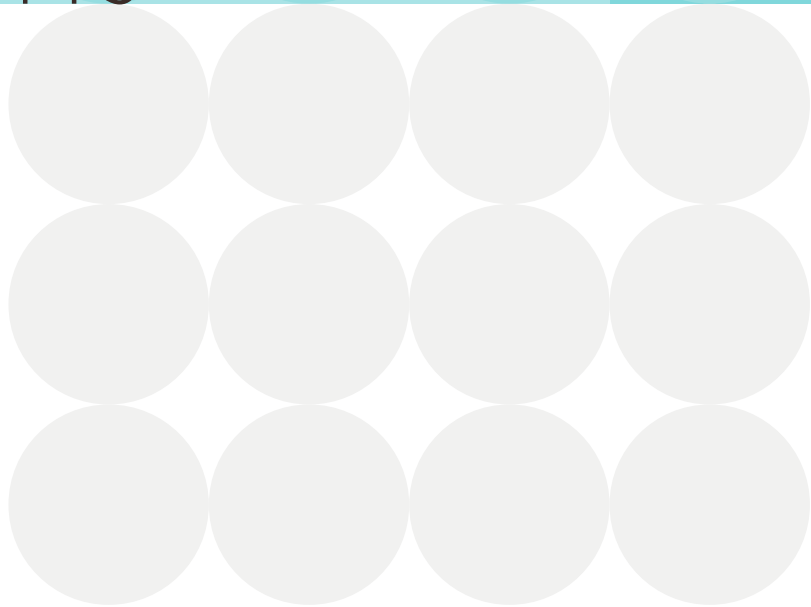


## MOLECULAR ANALYSIS OF CLINICAL ISOLATES OF ACYCLOVIR RESISTANT HERPES SIMPLEX VIRUS

We characterised the antiviral phenotype and genotype of 41 HSV strains from patients clinically resistant to acyclovir. Previously recognised mutations known to cause thymidine kinase (tk)-associated acyclovir resistance, in particular insertions and/or deletions at homopolymer stretches of Gs and Cs, were detected in 24 (59%) of the isolates. Previously described mutations in functional sites of this gene were identified in 7% of isolates. We identified novel stop codons or amino acid substitutions in a further 7% and 15%, respectively, of isolates. The precise location of these mutations in the tk gene are shown in Figure 10. Mutations in the DNA polymerase gene were identified in 12% of strains in which tk mutations were not detected. These results demonstrate the diversity of mechanisms involved in HSV resistance to acyclovir. They also contribute to a growing database of mutations known to contribute to acyclovir resistance, thereby enabling direct sequencing of PCR products obtained by PCR as a means to identify the presence or absence of clinically significant drug resistance mutations.



# COMMONWEALTH PROGRAMS



VIDRL holds three Australian national reference laboratory designations:

- National Poliovirus Reference Laboratory
- National Measles Reference Laboratory
- National High Security Quarantine Laboratory (NHSQL)

The main role of the National Poliovirus Reference Laboratory is to detect and characterise viruses from cases of acute flaccid paralysis (AFP). The national coordination of AFP surveillance is conducted by staff of the National Poliovirus Reference Laboratory and the Epidemiology Unit at VIDRL. Funding for these roles is from the DHS and the Commonwealth Department of Health and Ageing (DoHA).

The National Measles Reference Laboratory was first designated in 1999. It is the focus for Australia's contribution to the emerging global effort to control measles infection. A national reference service for genotyping of measles viruses is provided for molecular epidemiological purposes.

The NHSQL is a physical containment level 4 (PC4) facility funded through a service agreement with DoHA. As such, it provides Australia with laboratory testing facilities for the diagnosis of the quarantinable viral haemorrhagic fevers Lassa, Ebola, Marburg and Crimean Congo haemorrhagic fever, as well as infections exotic to Australia, including SARS and avian influenza.

## National Poliovirus Reference Laboratory

The Poliovirus Reference Laboratory is a member of the WHO global laboratory network for the eradication of poliomyelitis and is designated as the Australian National Laboratory. Specimens are received from patients suffering from acute flaccid paralysis (AFP), the most common clinical presentation of poliomyelitis (refer to section on Acute Flaccid Paralysis Surveillance).

Thirty AFP cases (64 specimens) from Australia were investigated in 2004. One of these cases involved a three-month old child from whom poliovirus 1 and poliovirus 2 were isolated from faecal specimens. Investigations using reverse transcription PCR and enzyme linked immunosorbent assay (ELISA) were performed in order to determine whether the viruses were wild, or Sabin-like vaccine strains. Results for the poliovirus 2 isolate showed it to be Sabin-like, but the poliovirus 1 isolate indicated the possibility of mutations within the viral genome. Nucleic acid sequencing of the VP1 gene determined 0.3% variation from the parental Sabin strain and the virus was classified as vaccine-like. The case was subsequently classified by the Polio Expert Committee as infant botulism, due to the isolation of *Clostridium botulinum* and the detection of botulinum toxin from the patient's faeces.

Non-polio enteroviruses and adenoviruses were also isolated from faeces. Coxsackie B5 was isolated from specimens of one AFP case and echovirus 18 from another. Adenovirus was isolated from specimens of seven AFP cases in 2004, representing 23% of all cases tested (including five from Victoria). A recent publication from Malaysian investigators describes an association of adenovirus type 21 with AFP. Serotyping of adenoviruses isolated in 2004 identified adenovirus types 1, 2, 4 and 5 from five cases while the specific serotype of the other two isolates could not be confirmed.

Vaccine-strain polioviruses may be isolated from clinical specimens (such as nasopharyngeal aspirates of recently immunised children) of cases other than AFP. These isolates are considered to be incidental. Five polioviruses were isolated, from sources other than AFP cases in 2004. All tested as Sabin vaccine-like.

## REVIEW OF ALL CASES OF ACUTE FLACCID PARALYSIS, 1996-2004

Acute flaccid paralysis (AFP) occurs in 0.1% to 1% of poliovirus infections. AFP surveillance has been used worldwide to monitor the control and eradication of circulating wild poliovirus. In Australia, VIDRL has been responsible for the testing of faecal samples from cases of AFP, between the ages of 0 and 15 years, since the 1995 commencement of the AFP surveillance program in Australia. All specimens are tested according to protocols developed by the World Health Organization.

Wild poliovirus or vaccine-derived poliovirus can be the cause of acute infection in AFP cases. Alternatively a vaccine-derived poliovirus may be associated with vaccine associated paralytic polio (VAPP). This association is estimated to occur in approximately 1 in 2.5 million doses of live attenuated poliovirus vaccine distributed, or it may be an incidental finding. An incidental poliovirus is found following immunisation with oral live attenuated vaccine but the poliovirus is not believed to be the cause of the clinical syndrome with which the patient presents.

Polioviruses, characterised as Sabin-like vaccine strains, were found in six (2%) of the 317 cases of patients with AFP. These cases were confirmed as being eligible for reporting to WHO from Australia between 1996 and 2004. Four AFP cases with a Sabin-like poliovirus isolated were diagnosed as infant botulism, one as a monofocal neuropathy with anterior horn cell involvement, the other as transverse myelitis. Each of these cases could have been classified as VAPP, if extensive laboratory testing and review had not been performed. This case series highlights the importance of continued AFP surveillance, even in polio non-endemic countries, when the world is approaching eradication of circulating wild poliovirus.

## Acute Flaccid Paralysis Surveillance

VIDRL is responsible for coordination of surveillance for acute flaccid paralysis (AFP) in collaboration with the Australian Paediatric Surveillance Unit. AFP is the most common clinical presentation of poliomyelitis and the WHO program for the eradication of polio relies upon surveillance for AFP in children less than 15 years of age. The surveillance co-ordinator requests clinical data from paediatricians who have notified cases of AFP and also assists with the shipment of specimens to the national polio laboratory for testing. The Polio Expert Committee (PEC) is convened quarterly to review the clinical data in conjunction with the laboratory test results, to determine if the illness is compatible with poliomyelitis.

In 2004, 48 cases of AFP in children less than 15 years old were notified, with an additional four cases in older patients. By early 2005, the PEC had reviewed the available clinical and laboratory data from 42 cases of AFP in children and classified 39 of the cases as non-polio AFP. The remaining three cases require further clinical information. Six cases have not been reviewed by the PEC as no clinical information has been received. The WHO benchmark for the notification of AFP cases is one per 100,000 children less than 15 years of age, which represents 40 cases per year for Australia. The 42 cases reviewed by the PEC represent a rate of 1.05. All Australian states except for Western Australia and Victoria reached or exceeded the target rate.

## National Measles Reference Laboratory

### VALIDATION OF DRIED BLOOD SPECIMENS FOR MEASLES SEROLOGY

Research continued to validate dried blood as an alternative to serum for measles serology. Venous blood and finger prick samples were collected from older Pacific Island community members who had probably been exposed to virus, and young Australian adults who we have previously found to be susceptible to measles virus infection or have low levels of vaccine induced immunity. To simulate possible temperature fluctuations during transport to the laboratory, dried samples (venous blood (DVB) and fingerprick (DFP)) were stored at 28°C (dry atmosphere) and 4°C for approx three weeks before being stored at 4°C.

Several reagents for eluting the dried blood from the filter paper were compared using the Bland –Altman method comparison analysis for quantitative comparison. It was determined that dried samples (DVB or DFP) stored at 28°C should be eluted with 5% dry milk reagent. The commercial assay using dried samples stored at 28°C and dried milk reagent to elute the blood from the filter paper had a sensitivity of more than 98% when compared to serum samples. This study also confirmed the adjustment factors, which were previously determined at VIDRL, for dried samples stored at 4°C. These adjustment factors allow the use of dried blood samples with the commercial test to determine whether people are protected against measles virus infection. However, determination of antibody titre using the alpha calculation described in the Dade Behring kit protocol for dried samples stored at 28°C may not be reliable.

### MEASLES MOLECULAR EPIDEMIOLOGIC SURVEILLANCE

The nucleoprotein genes of four measles viruses causing outbreaks in Australian states or territories, other than Victoria, were sequenced during this period, resulting in the identification of three genotypes. A single case of genotype D4 from NSW was linked to Bangkok. A virus of genotype G3 detected in Western Australia was linked to contact with Indonesia. India was the source of an importation of genotype D8 to South Australia, and a genotype D4 virus was received from Queensland where the source of the importation could not be determined.

## National High Security Quarantine Laboratory (NHSQL)

### PRODUCTION OF REAGENTS TO ENABLE DETECTION OF AVIAN INFLUENZA-RELATED VIRUSES

A highly pathogenic strain of avian influenza virus (H5N1) has circulated in avian species in Cambodia, Thailand and Vietnam since December 2003. A small number of cases of human infection have occurred, mostly involving close contact with avian species. No cases of human-to-human transmission have been recently documented. Co-infection of humans with this avian influenza virus and a currently circulating human influenza A virus strain provides the opportunity for a recombination event to produce a novel strain of influenza with pandemic capability. Such an event has major public health implications.

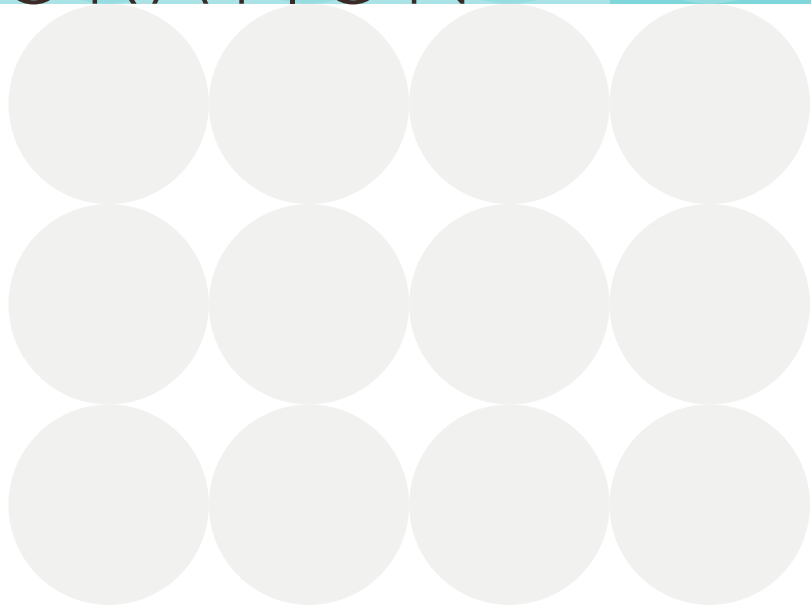
In response to this threat, the DoHA requested that the NHSQL produce and distribute control material to enable public health reference laboratories in each Australian state to rapidly identify cases of influenza infection involving H5 strains. In collaboration with the WHO Collaborating Centre for Influenza Virus Reference and Research, Parkville, several clinical isolates from humans infected with avian influenza virus were imported from the Public Health Laboratory, Hong Kong. Two strains were passaged in vitro under high security biocontainment in the NHSQL's physical containment level 4 (PC4) laboratory and high-titred RNA prepared to act as controls in PCR tests. This material was used to demonstrate that Australian public health laboratories have PCR tests capable of detecting currently circulating strains of influenza A H5N1. Infected cells were also harvested and used to confirm that immunofluorescence reagents used in most Australian diagnostic laboratories were capable of confirming infection with H5 strains of avian influenza.

New real time PCR assays were developed for Marburg, Rift Valley fever and the orthopox group of viruses this year. In addition a second round of "nested" amplification was added to the Lassa fever virus PCR assay. These changes have improved the analytical sensitivity and throughput capacity of assays for detection of these viruses at the NHSQL.

A senior NHSQL scientist visited the Bioterrorism Rapid Response and Advanced Technology Laboratory, Special Pathogens Branch and Poxvirus Branch at the Centres for Disease Control and Prevention (CDC) Atlanta, USA. The purpose of the visit was to observe procedures undertaken in these laboratories and to discuss testing strategies employed at CDC for risk group 4 viruses.



# INTERNATIONAL PROGRAMS/WHO COLLABORATION



# INTERNATIONAL PROGRAMS/WHO COLLABORATION

VIDRL holds the following World Health Organization (WHO) designations:

## WHO COLLABORATING CENTRE FOR VIRUS REFERENCE AND RESEARCH

The WHO Collaborating Centre in Virus Reference and Research is a designated centre of excellence in virology and encompasses much of the virological activities of VIDRL.

The Centre is responsible for:

- the detection and characterisation of viruses in clinical material;
- maintaining reference collections;
- training and consultancy in virology;
- the provision of infectious disease surveillance information;
- research and development in areas such as antiviral therapy, mechanisms of antiviral drug resistance and new technology for detection of viruses.

## WHO COLLABORATING CENTRE FOR BIOSAFETY IN MICROBIOLOGY

The WHO Collaborating Centre for Biosafety in Microbiology undertakes training in biosafety, provides biosafety related advice and support to institutions and participates in the work of the WHO Biosafety Advisory Group.

## WHO REGIONAL MEASLES REFERENCE LABORATORY

In 1999 VIDRL was designated the Australian reference laboratory for measles, and in the following year, was designated as the first WHO measles regional reference laboratory for the Western Pacific Region.

The objectives of the measles program at VIDRL are:

- to provide a timely diagnostic service for measles surveillance and outbreak investigation in Victoria;
- to provide a reference service for measles diagnosis for laboratories around Australia;
- to provide laboratory support for the improved understanding of the epidemiology of measles in Victoria and Australia;
- to provide a reference service for measles diagnosis and surveillance for laboratories within the WHO Western Pacific Region;
- to develop and evaluate alternative laboratory methods for measles diagnosis and surveillance that may be appropriate for resource poor countries.

## WHO REGIONAL POLIOVIRUS REFERENCE LABORATORY

The Poliovirus Reference Laboratory was established in 1994 and has played a major role in Australia's commitment to the WHO program for the global eradication of poliomyelitis.

The laboratory is accredited by the WHO and has the following responsibilities as a member of the global poliovirus laboratory network:

- national laboratory for Australia;
- national laboratory for the Pacific Island countries;
- national laboratory for Brunei Darussalam;
- one of two regional reference laboratories for the Western Pacific Region.

## WHO NATIONAL INFLUENZA CENTRE

The influenza surveillance program at VIDRL aims to assist in the early detection of influenza, define the distribution of influenza in the community and provide timely information regarding circulating strains. As a National Influenza Centre VIDRL:

- serves as the key point of contact with the World Health Organization
- collects appropriate patient specimens for influenza virus isolation
- undertakes initial identification of virus type and subtype
- forwards representative and potential variant virus isolates to the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne
- alerts the WHO Global Influenza Programme regarding any untypable influenza isolate
- alerts national authorities and the WHO immediately on the emergence of unusual outbreaks of influenza or influenza-like illness

## Collaborating Centre for Virus Reference and Research

### TERMS OF REFERENCE

- To play a leading role in the characterisation and classification of viruses in the centre's field of special interest
- To take an active part in collaborative studies
- To train virologists
- To report epidemiological information to WHO
- To provide a scientific and technological consulting and advisory function in virology, with special reference to rapid viral diagnosis
- To carry out and promote research and development on the use of antiviral agents active against medically important viruses

### ACTIVITIES IN 2004

The WHO Collaborating Centre for Virus Reference and Research encompasses under its terms of reference much of the virological work undertaken by VIDRL at the state and national level.

VIDRL's outbreak investigations, the activities of the HIV Laboratory, and those within the vaccine preventable diseases, respiratory pathogens, viral gastroenteritis and blood borne viruses and STD programs, also reflect activity under the terms of reference of the Centre. A full account of these activities is provided earlier in this report.

VIDRL retains a strong commitment to training virologists. This year, Dr Ben Cowie and Dr Jack Richards, trainees of the Royal College of Australasian Physicians, each completed a six-month attachment at the Centre. Dr Cowie and Dr Richards received training in clinical and diagnostic virology and undertook a research project.

VIDRL's research programs are diverse, but research related to antiviral therapy has always been a strong theme, as articulated in another of the Centre's terms of reference. Notable examples are projects involving aspects of hepatitis B antiviral therapy and antiviral research. A full account of this work is available in the Research Activities section. Collaborations continue to be a feature of VIDRL's research projects. A list of selected collaborators has also been included in the appendices to this report.

## Collaborating Centre for Biosafety in Microbiology

### TERMS OF REFERENCE

- To conduct biosafety training courses
- To implement biosafety training through workshops to 'train the trainer' and courses on laboratory technique
- To provide advice and support to institutions in establishing and operating biosafety programmes
- To participate in the work of the WHO Biosafety Advisory Group, which, in addition to its activities in the Communicable Diseases Department of WHO, provides technical advice to other United Nations agencies (UN Committee of Experts on Transport of Dangerous Goods, Universal Postal Union) and Non Government organisations (e.g. International Air Transport Association)

### ACTIVITIES IN 2004

The Australian Society for Microbiology (ASM) Standing Committee on Biosafety and the ASM Special Interest Group in Microbiological Safety were both established through the Centre. The former is a Standing Committee appointed by ASM to formulate biosafety policy for ASM, provide biosafety advice to ASM Council and to liaise with the Office of the Gene Technology Regulator and other relevant bodies and to provide guidelines. The Standing Committee held its annual meeting in October 2004 at which Ms Marie Gerrard represented the centre.

The Centre was represented by Dr Mike Catton at the WHO Biosafety Advisory Group Special Meeting on Polio Eradication Biosafety Issues, held in June 2004. The major task of this meeting was to develop recommendations for the safe containment of polioviruses, and safe manufacturing of poliovirus vaccine in the post-eradication era.

The Centre is represented by Mr Chris Birch and Ms Gerrard on the Institutional Biosafety Committee of the Melbourne Health Service, which oversees safe dealing with genetically modified organisms (SMD's) under Australian legislation which became law in June 2001. Both staff participated in laboratory inspections and certifications and reviewed research projects on GMOs.

In September 2004, Ms Gerrard visited Tan Toc Sen hospital Singapore. There she conducted training sessions and risk assessments for the pathology department in the wake of their involvement in the SARS outbreak. In preparation for this, Mike or Marie developed new training materials utilising WHO materials that have since been used successfully in VIDRL safety training seminars.

In October 2004, Ms Gerrard contributed to a Symposium on Biosafety at the Australian Society for Microbiologists Annual Scientific Meeting in Sydney.

Ms Gerrard was also appointed to the Standards Australia committee reviewing the Australian /New Zealand standard: "Safety in laboratories Part 3: Microbiological aspects and containment facilities AS/NZS 2243.3.2002".

## WHO Regional Measles Reference Laboratory Western Pacific Region

The expansion of measles molecular surveillance to developing countries where measles is endemic will help facilitate measles control. Limited infrastructure in these areas, in particular relating to specimen storage and control, is currently a barrier to such surveillance. We have now demonstrated that oral fluid (saliva) dried onto filter paper can be used for the detection and characterisation of measles virus (MV) strains. Using this approach, a positive MV by RT-PCR could be obtained from 67% of serologically confirmed acute measles cases. Mimicking certain environmental conditions and duration of transportation established that MV RNA remained detectable and suitable for nucleic acid sequencing in oral fluid spots for at least one week at temperatures up to 37°C. In the context of a measles outbreak in a remote region of the world where infrastructure is poor, oral fluid samples dried onto filter paper and sent to a specialised laboratory for testing will aid in the identification and characterisation of the causative MV strain.

## DETECTION OF RUBELLA IGM FROM DRIED VENOUS BLOOD SPOTS USING A COMMERCIAL ENZYME-LINKED IMMUNOSORBENT ASSAY

Measles elimination and rubella control programs are part of the immunisation strategies of many countries and have been endorsed by WHO. For both measles and rubella, confirmation of the diagnosis is critical to disease control. In countries with limited infrastructure and laboratory capacity, collection, transport and testing of venous blood samples may be difficult. We have adapted a commercial EIA designed for the detection of rubella IgM in serum to be used with dried venous blood spots (DVBS). Adaptations of the manufacturer's protocol included variations in incubation and washing procedures. Sixty DVBS were used, made at the time of collection of venous blood from the enhanced measles/rubella program in Victoria or from serum tested as part of a rubella outbreak in a Pacific Island nation. We compared the optical density (OD) from the Dade Behring Enzygnost Anti-Rubella-Virus/IgM EIA for the DVBS and the corresponding serum sample. The mean corrected OD was higher for the serum compared with the DVBS (0.199 and 0.148, respectively,  $p=0.0002$ ). However the sensitivity of the DVBS compared with serum for the categorisation of rubella IgM as positive or negative was 96.9% (83.8-99.9%) and the specificity was 100% (93.7-100%). We conclude that DVBS can be used to detect rubella IgM using a modified protocol in a commercial EIA.

## QUALITY ASSURANCE

As the world moves towards control of measles, confirmation of clinically diagnosed measles by IgM serology will become increasingly important. Proficiency testing is an important part of measles laboratory programs as both false positive and false negative results can occur with some of the commonly used measles IgM EIA.

VIDRL was invited by WHO to coordinate a global quality assurance program (QAP) for measles IgM serology. The aims of the panels are to assess the proficiency of laboratories in the WHO laboratory network testing for measles IgM, to identify problems with any assays routinely used in these laboratories and to check the accuracy of data reporting.

The first panel was distributed in 2001 to 46 laboratories and the program has continued to expand with 99 laboratories participating in the 2003 program.

Analysis and compilation of the final report for panel 00703 was completed this year. Interim reports of results achieved by each participating laboratory had previously been sent within approximately 10 working days of transmission of results to VIDRL. Interim reports are sent by email to the participating laboratory, the WHO regional co-ordinator and the WHO Global Project Leader. This enables a quick follow-up on a laboratory experiencing problems with its testing program.

### PANEL 00703

The 2003 measles panel was distributed globally to National and Regional Measles reference laboratories within the WHO measles network and 99 laboratories returned results for analysis.

The WHO regions included were Eastern Mediterranean, African, South East Asian, Europe and the Western Pacific.

Figure 11 shows the approximate site of the 99 laboratories that submitted results.

**Figure 11: Laboratories that submitted results in the measles proficiency panel**



### 00703

All samples were undiluted serum samples, comprising:

- 11 Measles IgM positive (sourced from 1999/2001 measles outbreaks in Victoria, Australia)
- 5 Measles IgM negative (VIDRL staff volunteers)
- 2 Parvovirus IgM positive (diagnostic sera)
- 2 Rubella IgM positive (diagnostic sera)

Ninety percent of laboratories achieved a score of 90% or greater. A score of 100% was achieved by 58% of laboratories. Compared to previous panels where 96% achieved >90% and >65% of participants were achieving scores of 100%, there was a slight decrease in overall performance. The Dade Behring Enzygnost IgM measles kit was used by 71% of laboratories.

### PANEL 00704

Panel 00704 was distributed in 2004.

All samples were undiluted serum samples, comprising

- 10 Measles IgM positive (sourced from 1999 measles outbreak in Victoria, Australia)
- 6 Measles IgM negative (VIDRL staff volunteers)
- 2 Parvovirus IgM positive (diagnostic sera)
- 2 Rubella IgM positive (diagnostic sera, 1 also positive for measles)
- 1 Dengue IgM positive (supplied by the WHO Measles laboratory Brazil)

This panel has so far been distributed to laboratories in the African and Eastern Mediterranean regions. Interim reports have been sent to participating laboratories and WHO regional and global co-ordinators.

### WHO Regional Poliovirus Reference Laboratory Western Pacific Region

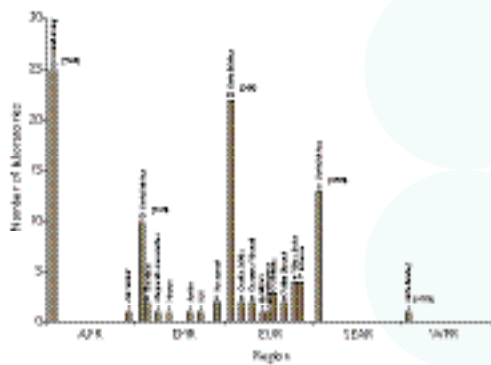
The poliovirus reference laboratory at VIDRL is a member of the WHO global network of laboratories for the eradication of poliomyelitis. Specimens from cases of acute flaccid paralysis (AFP) are referred from Brunei Darussalam and the Pacific Island countries for poliovirus testing. In 2004, testing of 29 specimens from 15 AFP cases from these countries was performed and, while no polioviruses were detected, non-polio enteroviruses were isolated from two cases. Adenoviruses were isolated from two further cases.

The VIDRL polio laboratory is also a Regional Reference Laboratory for the Western Pacific Region. Reference laboratory responsibilities involve the further testing of poliovirus isolates referred from national laboratories in the Western Pacific region. National laboratories also refer untyped enterovirus isolates for further identification. The Regional Reference Laboratory is a cell bank for the Western Pacific region and supplies national laboratories with the WHO approved cell lines, RD-A and L20B, for poliovirus isolation.

In 2004, 51 specimens of poliovirus isolates were referred from Malaysia and the Philippines. A further 326 specimens and isolates were referred from Papua New Guinea and Viet Nam (Ho Chi Minh City) for retesting, as part of an ongoing laboratory quality assurance program. No wild polioviruses were detected from any of the samples.

The Western Pacific region has remained free of endemic wild poliovirus since the declaration by WHO in October 2000.

**Figure 12: Assays in use by WHO regions that participated in the 00703 proficiency panel**



## Collaborations with WHO

### EXPANDED PROGRAM ON IMMUNIZATION

The Head of the Polio Reference Laboratory conducted an on-site accreditation review of the national polio laboratories of Viet Nam located in Hanoi and Ho Chi Minh City.

### INFLUENZA PANDEMIC PREPAREDNESS IN THE WESTERN PACIFIC REGION

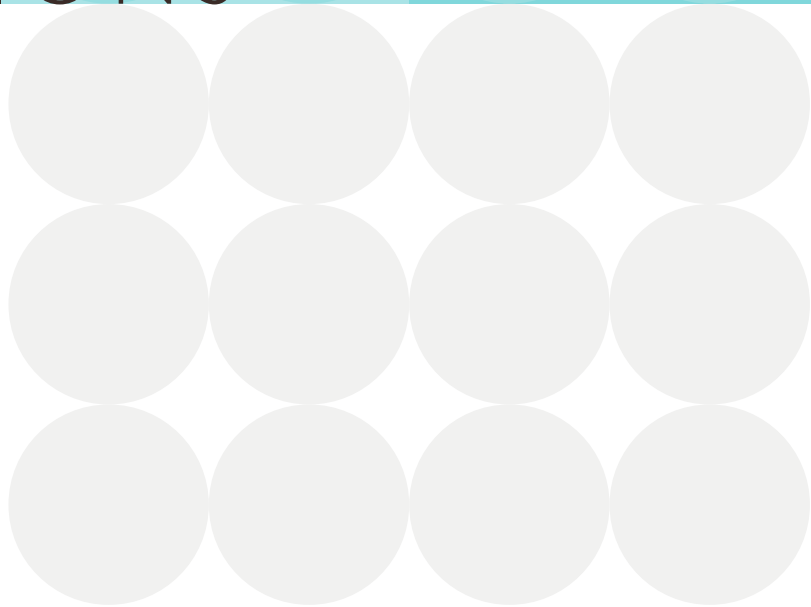
Working with staff from WHO Headquarters in Geneva and the Western Pacific Regional (WPR) Office in Manila, Dr Heath Kelly, working as a consultant for the WPR, developed a draft checklist for influenza pandemic preparedness. The checklist was divided into what were considered to be essential and desirable sections. It was intended that countries (member states) which had not commenced pandemic planning could use the checklist as a guide to commence planning, or member states that were well advanced with planning could check the comprehensiveness of their plans. After discussion with representatives from countries that had experienced avian influenza and/or SARS, the checklist was modified to reflect some of the experiences of these countries. It was subsequently edited by staff from WHO Headquarters and circulated for comment. A final document was published in May 2005.

## MEETING ATTENDANCE

This year VIDRL staff chaired the WHO Workshop on Laboratory Surveillance of Measles in the Western Pacific Region, Manila, and the WHO Meeting on the Standardization of the Nomenclature for Describing the Genetic Characteristics of Wild-Type Rubella Viruses, Geneva. VIDRL was also represented at the WHO Biosafety Group Special Meeting on Polio Eradication Biosafety Issues, Geneva.



# OPERATIONS



# OPERATIONS

During 2004 there were two main areas of activity. The first was the development of the sequence analysis software, and the second the ongoing development of the Medipath system.

## SEQUENCE ANALYSIS SOFTWARE

The Medipath based sequence analysis module for HIV continued to be refined during 2004. The State HIV Reference Laboratory has been using Medipath for a number of years to record and report significant mutations associated with resistance to antiviral therapy. A number of modules have been developed for the Medipath System for monitoring, analysing and reporting these mutations in association with other test results. The sequence analysis engine identifies the mutations of diagnostic importance and generates the appropriate report via a web browser interface.

Assistance was also provided to the Molecular Research and Development Division regarding the ongoing development of the HBV sequence analysis software that was commissioned in 2001. The changes introduced in 2004 were associated with the 'HBV full genome' analysis required for a new study funded by the NIH, USA.

## MEDIPATH LABORATORY COMPUTER SYSTEM

The specimen registration and account generation programs were extensively modified to automatically enforce new data consistency checks relating to key dates and referred specimen details, as well as enforcing new billing requirements associated with the introduction on May 1<sup>st</sup> of the new rule change allowing publicly owned pathology laboratories to claim for the Referred Specimen charge. These changes to the programs also required extensive updating of our Sender – Doctor file to enable the classification of referring organisations for triggering the new billing rules. Following agreements with a number of referring organisations, the billing programs were further modified to 'automatically' manage the billing of 'private patients' in public hospitals.

Many changes were made to the file maintenance modules to improve the speed and flexibility in making 'system-wide' changes to billing schedules, billing options, abbreviation codes, worksheets, patient file merge details, and audit reporting. The electronic reporting of test results was further modified to accommodate various clinic requirements and to provide customised data file formats for their in-house databases. These changes required significant modifications and extensive testing to ensure reliable delivery of the required data.

Changes to the billing module to manage full episode consolidation of Medicare and other rebateable invoice types were carried out to further improve the reliability of the system when generating invoices. Institutional customers were provided with the option of electronic invoices as well as the option for receiving them by an encrypted email service.

New data extraction programs were developed for a wide range of purposes, most of the new programs being associated with the extraction of patient result data which was subjected to further analysis.

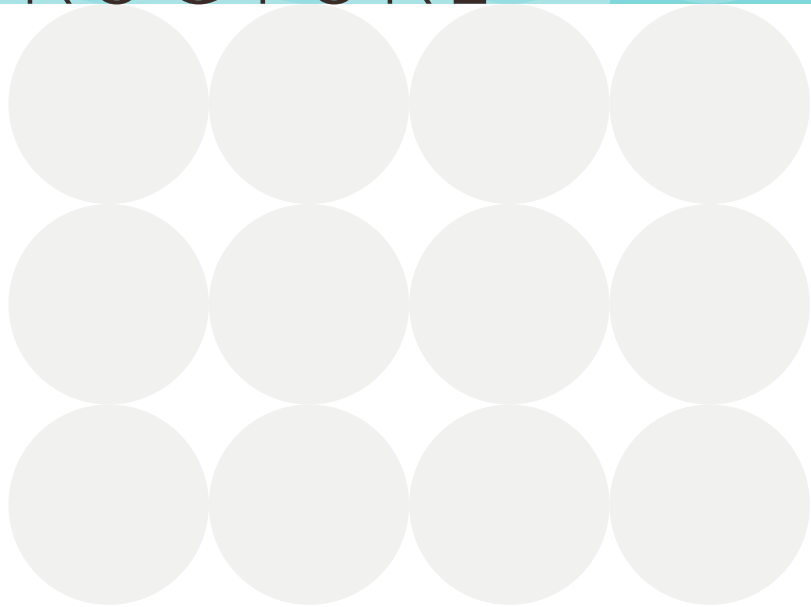
The Result Enquiry program was further enhanced with additional options for re-sending electronic copies to other than the referring organisation, as well as an audit option for confirming the delivery and collection time of electronic copies.

Extensive preparations were undertaken for the introduction of a 'Patient Alias' system which would allow for the same patient file to be known by different patient personal identifiers depending on the referring organisation. This was initially expected to be completed by late 2004 but due to the large number of changes required for the full implementation of this service, it will not now be completed until sometime in the first half of 2005.

An instrument interface for Bayer 340 DNA analyser was completed for downloading assay results and importing them in the Medipath results file.



# WORKS AND INFRASTRUCTURE



# WORKS AND INFRASTRUCTURE

## PC3 HIV CULTURE LABORATORY

This laboratory is operated at -50 Pascals relative to atmospheric pressure. The negative pressure is controlled by variable speed fans in the air-conditioning system. On 9th December 2004 the air-conditioning system was shut down for service. Shortly after an apparently normal air-conditioning start-up laboratory staff noticed white dust and plasterboard 'chunks' on the laboratory floor. Plasterboard wall sheets on the west wall had 'bulged' out from the support studs and noggins such that hand pressure could press the board back at least 10 mm. The breaks in the plasterboard were seen at the positions of power and data points. ThAdvice was sought from architects and consulting engineers before repairs were carried out. Expert opinion concluded the cause to be defective workmanship at the time of the laboratory construction.

Repairs to the wall structure and internal surfaces were carried out and the laboratory is once more operational. A more complete review and upgrade of the laboratory will be undertaken on an elective basis in the near future subject to the availability of funding.

## JANE BELL HOUSE BRICKWORK

A brickwork crack high on the Jane Bell House brick wall facing Wreckyn Street has been monitored since 1998. The characteristics displayed by the crack have remained stable over 2003 and 2004, however advice from a structural engineer will be sought in 2005.

## SECURITY

The VIDRL building security system was upgraded this year. Software received an incremental upgrade, and a new more powerful computer workstation was installed to provide increased capacity. Physical security of the computer room was also improved via additional electronic access controls.

Water has entered the basement on several occasions prior to VIDRL's occupancy of the building. VIDRL has installed several anti-flooding measures to protect equipment including a bund, a sump and automatic pump. Sand bags are provided to give further protection in cases of severe flooding. A new water detection device has been installed this year to give an early warning of water in the basement by auto-dialling the Laboratory Manager on-call.

The small computer room on Level 2 houses the Building System computer and the Building Security computer. These computers control many important building functions. The provision of an electronically controlled lock with card access has improved security for this room.

The freezer area in the VIDRL basement contains 30 ultra-low temperature freezers. The area has been secured by a strong fence/wall and a door supplied with an access card lock. The door is locked using a magnetic lock located at the top of the door. Following review of the system an additional drop bolt has been added that is controlled electronically and located at the bottom of the door, to provide a more secure closure.

## PLUMBING

Leaking hot water pipes continue to be encountered in the ceiling space. Three pipe repairs were made and the electric trace heaters removed.

## EQUIPMENT PURCHASES

The following items of equipment were purchased this year:

Equipment	Section
Sonicator - Misonix	Molecular R&D
Slide Stainer - Hema Tek 2000	Haematology
Seqscape V2.1 Initial Licence	Molecular R&D
Fast Prep Extraction Instrument	Mycobacterium Reference Laboratory
Incubator - Carbon Dioxide	Bacteriology
Biological Safety Cabinet Class 2	Molecular R&D
ThermalCycler - Cooled Gradient Palm Cycler	Viral Identification
Incubator - Forma Water jacket	Molecular R&D
Gel Doc EQ System PC	Viral Identification
Thermocycler - MJ Research PTC-200 dual block	Molecular R&D

# APPENDICES

## Appendix 1

### PUBLICATIONS

1. **Aitken CK, Bowden S, Hellard M, Crofts N.** Indications of immune protection from hepatitis C infection. *J Urban Health* 2004; 81:58-60.
2. **Aitken C, McCaw R, Jardine D, Bowden S, Higgs P, Nguyen O, Crofts N, Hellard M.** Change in hepatitis C virus genotype in injecting drug users. *J Med Virol* 2004; 74:543-554.
3. **Aitken CK, McCaw RF, Bowden DS, Tracy SL, Kelsall JG, Higgs PG, Kerger MJ, Nguyen H, Crofts JN.** Molecular epidemiology of hepatitis C virus in a social network of injection drug users. *J Infect Dis* 2004; 190:1586-1595.
4. **Angus P, Locarnini S.** Lamivudine-resistant hepatitis B virus and ongoing lamivudine therapy: stop the merry-go-round, it's time to get off! *Antiviral Therapy* 2004; 9:145-148.
5. **Ayres A, Locarnini S, Bartholomeusz A.** HBV genotyping and analysis for unique mutations. *Methods Mol Med* 2004; 96:125-149.
6. **Bartholomeusz A, Schaefer S.** Hepatitis B virus genotypes: comparison of genotyping methods. *Rev Med Virol* 2004; 14:3-16.
7. **Bartholomeusz A, Tehan B, Chalmers D.** Comparisons of the HBV and HIV polymerase and antiviral resistance mutations. *Antiviral Therapy* 2004; 9:149-160.
8. **Basuni AA, Butterworth L, Cooksley G, Locarnini S, Carman WF.** Prevalence of HBsAg mutants and impact of hepatitis B infant immunization in four Pacific Island countries. *Vaccine* 2004; 22:2791-2799.
9. **Beard MR, Locarnini S.** Monitoring gene expression using DNA microarrays during hepatitis infection. *Methods Mol Med* 2004; 96:55-64.
10. **Bowden S, Jackson K, Littlejohn M, Locarnini S.** Quantification of HBV covalently closed circular DNA from liver tissue by real-time PCR. *Methods Mol Med* 2004; 95:41-50.
11. **Brussen KA, Stambos V, Thorley BR.** Annual report of the Australian National Poliovirus Reference Laboratory, 2003. *Commun Dis Intell* 2004; 28:339-344.
12. **Chibo D, Druce D, Sasadeusz J, Birch C.** Molecular analysis of clinical isolates of acyclovir resistant herpes simplex virus. *Antiviral Research* 2004; 61:83-91.
13. **Dimech W, Bowden DS, Brestvac B, Byron K, James G, Jardine D, Sloots T, Dax EM.** Validation of assembled nucleic acid-based tests in diagnostic microbiology laboratories. *Pathology* 2004; 36:45-50.
14. **Genobile D, Gatson J, Tallis GF, Gregory JE, Griffith JM, Vulcanis M, Lightfoot D, Marshall JA.** An outbreak of shigellosis in a child care centre. *Commun Dis Intell* 2004; 28:225-229.
15. **Gilpin C, Fyfe J.** MIRU: the national tuberculosis genotyping strategy in Australia. *Microbiology Australia* 2004; 25:34-35.
16. **Gish R, Locarnini S.** Studying the treatment of chronic hepatitis B viral infection in special populations. *Methods Mol Med* 2004; 96:465-498.
17. **Goller JL, Dimitriadis A, Kelly H, Marshall JA.** Norovirus excretion in a health-care worker without major symptoms of gastroenteritis: infection control implications. *Aust NZ J Public Health* 2004; 28:88-89. 18. **Goller JL, Dimitriadis A, Tan A, Kelly H, Marshall JA.** Long-term features of norovirus gastroenteritis in the elderly. *J Hosp Infect* 2004; 58:286-291.
18. **Goller JL, Dimitriadis A, Tan A, Kelly H, Marshall JA.** Long-term features of norovirus gastroenteritis in the elderly. *J Hosp Infect* 2004; 58:286-291.
19. **Greig JE, Carnie JA, Tallis GF, Ryan NJ, Tan AG, Gordon IR, Zwolak B, Leydon JA, Guest CS, Hart WG.** An outbreak of Legionnaires' disease at the Melbourne Aquarium, April 2000: investigation and case-control studies. *Med J Aust* 2004; 180:566-72.
20. **Guy R, Andrews R, Kelly H, Leydon J, Riddell M, Lambert S, Catton M.** Mumps and rubella: a year of enhanced surveillance and laboratory testing. *Epidemiol Infect* 2004; 132:391-398.
21. **Guy RJ, Di Natale R, Kelly HA, Tobin S, Robinson P, Tallis G, Hampson AW.** Influenza A outbreaks in aged-care facilities: staff vaccination and the emerging use of antiviral therapy. *Med J Aust* 2004; 180:640-642.
22. **Jazayeri M, Basuni AA, Sran N, Gish R, Cooksley G, Locarnini S, Carman WF.** HBV core sequence: definition of genotype-specific variability and correlation with geographic origin. *Vaccine* 2004; 11:448-501.
23. **Jazayeri MS, Basuni AA, Cooksley G, Locarnini S, Carman WF.** Hepatitis B virus genotypes, core gene variability and ethnicity in the Pacific region. *J Hepatol* 2004; 41:139-146.
24. **Johnson P, Hayman J, Jenkin G, Stinear P, Globan M, Fyfe J.** *Mycobacterium ulcerans*: an unwelcome visitor. *Microbiology Australia* 2004; 25:20-22.
25. **Kelly H, Atta J, Andrews R, Heller D.** The number needed to vaccinate (NNV) and population extensions of the NNV: comparison of influenza and pneumococcal vaccine programs for people aged 65 years and over. *Vaccine* 2004; 22:2192-2198.
26. **Kelly H, Birch C.** The causes and diagnosis of influenza-like illness. *Aust Fam Phys* 2004; 33:305-309.

27. Kelly H, Worth L, Karapanagiotidis T, Riddell M. Interruption of rubella virus transmission in Australia may require vaccination of adult males: evidence from a Victorian sero-survey. *Commun Dis Intell* 2004; 28:69-73.
28. Laskos L, Ryan C, Fyfe J, Davies J. The RpoH-mediated stress response in *Neisseria gonorrhoeae* is regulated at the level of activity. *J Bacteriol* 2004; 186:8443-8452.
29. Lim LL, Druce J, Street AC. Human herpesvirus type 8-associated episodic multisystem illness in an HIV-infected patient in the absence of hemophagocytic lymphohistiocytosis. *Clin Infect Dis* 2004; 38:1640-1641.
30. Lee J-Y, Locarnini S. Hepatitis B virus: pathogenesis, viral intermediates and viral replication. *Clin Liver Dis* 2004; 8:301-29.
31. Locarnini S. Molecular virology of hepatitis B virus. *Semin Liver Dis* 2004; 24:S1:3-10.
32. Locarnini S, Hatzakis A, Heathcote J, Keefe E, Liang TJ, Mutimer D, Pawlotsky J-M, Zoulim F. Management of antiviral resistance in patients with chronic hepatitis B. *Antiviral Therapy* 2004; 9:679-693.
33. Marshall JA, Hellard ME, Sinclair MI, Fairley CK, Cox BJ, Catton MG, Kelly H, Wright PJ. Failure to detect norovirus in a large group of asymptomatic individuals. *Public Health* 2004; 118:230-233.
34. Marshall J, Kelly H, Wright P. Asymptomatic carriage of norovirus. A reply to the comments of Duizer et al. *Public Health* 2004; 118:456-457.
35. Norder H, Courouce A-M, Coursaget P, Echevarria JM, Lee S-D, Mushahwar IK, Robertson BH, Locarnini S, Magnus LO. Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes and HBsAg subtypes. *Intervirology* 2004; 47:289-309.
36. Shaw T, Locarnini S. Entecavir for the treatment of chronic hepatitis B. *Expert Rev Anti Infect Ther* 2004; 2:853-871.
37. Shaw T, Locarnini S. A parsimonious method for screening drug combinations for antihepatidnaviral activity using a parametric dose-response surface approach. *Methods Mol Med* 2004; 96:387-413.
38. Shaw T, Locarnini S. Problems inherent to antiviral therapy. In: Milestones in Drug Therapy. Eds. Colacino JM, Heinz BA. Birkhauser, Basel, Switzerland, 2004.
39. Shaw T, Locarnini S. Hepatitis B. In: *Encyclopedia of Gastroenterology*. Ed. Johnson L. Elsevier, Amsterdam, the Netherlands, 2004.
40. Shaw T, Bowden S, Locarnini S. Rescue therapy for drug resistant hepatitis B: another argument for combination chemotherapy? *Gastroenterology* 2004; 126:343-347.
41. Shimizu H, Thorley B, Paladin FJ, Brussen KA, Stambos V, Yuen L, Utama A, Tano Y, Arita M, Yoshida H, Yoneyama T, Benegas A, Roesel S, Pallansch M, Kew O, Miyamura T. Circulation of type I vaccine-derived poliovirus in the Philippines in 2001. *J Virol* 2004; 78:13512-13521.
42. Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, Plym M, Pokornowski K, Yu C, Angus P, Ayres A, Bartholomeusz A, Sievert W, Thompson G, Warner N, Locarnini S, Colonna RJ. Clinical emergence of entecavir resistant hepatitis B virus requires additional substitutions in prior lamivudine resistant virus. *Antimicrobial Agents Chemotherapy* 2004; 48:3498-3507.
43. Tran T, Druce J, Catton M, Kelly H, Birch C. Changing epidemiology of genital herpes simplex virus infection in Melbourne, Australia, between 1980 and 2003. *Sexually Transmitted Infect* 2004; 80:277-279.
44. Turner J, Tran T, Birch C, Kelly H. Higher than normal seasonal influenza activity in Victoria, 2003. *Commun Dis Intell* 2004; 28:175-180.
45. Werle-Lapostolle B, Bowden S, Locarnini S, Wursthorn K, Petersen J, Lau G, Trepo C, Marcellin P, Goodman Z, Delaney WE 4th, Xiong S, Brosgart CL, Chen SS, Gibbs CS, Zoulim F. Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; 126:1750-1758.
46. Whitfield K, Kelly H. Differences by state in notification of cases presenting with acute flaccid paralysis since certification of Australia as polio free. *J Paed Child Health* 2004; 40:466-469.
47. Wightman F, Walters T, Ayres A, Bowden S, Bartholomeusz A, Lau D, Locarnini S, Lewin S. Comparison of sequence analysis and a novel discriminatory real-time PCR assay for detection and quantification of lamivudine-resistant hepatitis B virus strains. *J Clin Microbiol* 2004; 42:3809-3812.
48. Zanati SA, Locarnini SA, Dowling JP, Angus PW, Dudley FJ, Roberts, SK. Hepatic failure due to fibrosing cholestatic hepatitis in a patient with pre-surface mutant hepatitis B virus and mixed connective tissue disease treated with prednisolone and chloroquine. *J Clin Virol* 2004; 31:53-57.

## Appendix 2

### PRESENTATIONS AT MEETINGS

1. Ayres A, **Bartholomeusz A**, Yuen L, Locarnini S, Lewin S, Crowe S, Roberts S, Sasadeusz J, Matthews G, Dore G, Thomas D, Thio C. Characterisation and clinical significance of lamivudine resistant hepatitis B virus (HBV) mutations in HIV and HBV co-infected individuals. 4th Australasian hepatitis C meeting and ASHM, August 31 – September 3, 2004, Canberra, Australia.
2. **Bartholomeusz A**, Locarnini S, Colledge D, Ayres A, Kuiper M, Chalmers D. Molecular modelling of hepatitis B virus polymerase: comparison of adefovir, entecavir and lamivudine resistance. International Society for Antiviral Research, May 2-6, 2004, Tucson, Arizona, USA.
3. **Bartholomeusz A**, Locarnini S, Thompson G, Ayres A, Kuiper M, Chalmers D. Molecular modelling of APPENDIX 1 - hepatitis B virus polymerase: characterisation of adefovir resistance. 4th Australasian hepatitis C meeting and ASHM, August 31 – September 3, 2004, Canberra, Australia.
4. **Bartholomeusz A**, Locarnini S. Development of bioinformatics programs for patient management. 4th Australasian hepatitis C meeting and ASHM, August 31 – September 3, 2004, Canberra, Australia.
5. **Bartholomeusz A**, Locarnini S, Ayres A, Thompson G, Angus P, Sievert W, Sasadeusz J, Chalmers D, Kuiper M. Molecular modelling of hepatitis B virus polymerase and identification of three clusters of adefovir resistance mutations. Molecular Biology of HBV, October 24-27 2004, Woods Hole, Massachusetts, USA.
6. **Bartholomeusz A**, Locarnini S, Ayres A, Thompson G, Sozzi V, Angus P, Sievert W, Sasadeusz J, Chalmers D, Kuiper M. Molecular modelling of hepatitis B virus polymerase and adefovir resistance identifies three clusters of mutations. AASLD, October 29-November 2, 2004, Boston, Massachusetts, USA.
7. **Bartholomeusz A**. Molecular modelling of entecavir resistant mutations in the hepatitis B virus polymerase selected during therapy. AASLD, October 29-November 2, 2004, Boston, Massachusetts, USA.
8. **Bartholomeusz A**, Locarnini S, Ayres A, Yuen L, Lewin SR, Sasadeusz J, Matthews G, Dore G, Thomas D, Thio C. Characterisation of lamivudine resistant hepatitis B virus (HBV) mutations in HIV-HBV co-infected individuals. HIV And Hepatitis Co-infection, December 1 – 4, 2004, Amsterdam, Netherlands.
9. **Bartholomeusz A**, **Locarnini S**, Thompson G, Sozzi V, Angus P, Sievert W, Sasadeusz J, Chalmers D, Cooper M. Molecular modelling of HBV polymerase and adefovir resistance identifies three clusters of mutations. 55th Annual Meeting of AASLD, October 29-November 2, 2004, Boston, USA.
10. **Catton M**. SARS: The international picture. Patient Care Technology Group Annual Conference 2004: A Driving Force, March 19, 2004, Melbourne, Australia.
11. **Catton M**. Role of the laboratory and preparedness for disease outbreaks. Royal Australasian College of Physicians Annual Scientific Meeting, May 18, 2004, Canberra, Australia.
12. **Catton M**. Nucleic acid tests (NAT) in diagnostic virology. 21st National Reference Laboratory Workshop on Serology, August 17-20, 2004, Melbourne, Australia.
13. **Catton M**. Measles IgM serology proficiency testing in global laboratory network. WHO Laboratory Surveillance for Measles Elimination, August 24-25, 2004, Manila, Philippines.
14. **Catton M**. Rubella surveillance in Australia. WHO Meeting of Standardization of the Nomenclature for Describing the Genetic Characteristic of wild-type Rubella viruses, September 2-3, 2004, Geneva, Switzerland.
15. **Catton M**. Southeast Asian patients with chronic hepatitis C: Response of unusual genotypes to therapy. Asian Pacific Digestive Week, October 4-7, 2004, Beijing, China.
16. **Catton M**. Impact of molecular diagnostics on the practice of microbiology. National Healthcare Group Annual Scientific Congress, October 9-10, 2004, Singapore.
17. **Catton M**. SARS and Avian flu: evolution and transmission. The Tasmania Branch of the ASM scientific meeting and AGM, November 20, 2004, Launceston, Australia.
18. **Locarnini S**. Hepatitis B: Mutants, variants, mixed infections, unusual situations. Update 2004, February 8-10, 2004, Whistler, Canada.
19. **Locarnini S**. Viral resistance: Mechanism and lesson for physician. 1st International Liver Symposium on HBV, February 12-14, 2004, Seoul, Korea.
20. **Locarnini S**. Rationale for combination treatment in chronic hepatitis B Hong Kong. Shanghai International Liver Congress, February 14-19, 2004, Shanghai, Hong Kong.

21. **Locarnini S.** Current state of knowledge. AASLD Single Topic Conference, March 12-13, 2004, Chicago, USA.
22. **Locarnini S.** Prevalence of HBsAg mutants and impact of hepatitis B infant immunization in four pacific island countries. 4th Australasian Hepatitis C Meeting, August 31-September 2, 2004, Canberra, Australia.
23. **Locarnini S.** Hepatitis B virus antiviral drug resistance: Public health and pathogenesis aspects. Asian Pacific Digestive Week 2004, October 4-7, 2004, Beijing, China.
24. **Locarnini S.** The role of viral mutations in pathogenesis and treatment of hepatitis B and C. Falk Foundation Conference, October 12-17, 2004, Frieberg, Germany.
25. **Locarnini S,** Shaw T, Sozzi T, Edwards R, Currie G, Brosgart C, Xiong S. HBV mutants associated with clinical resistance to adefovir dipivoxil display only small decreases in antiviral sensitivity. 55th Annual Meeting of AASLD, October 29-November 2, 2004, Boston, USA.
26. **Marshall J.** Electron microscope identification of poxvirus in clinical samples in Victoria, Australia, 1997-2001. The 18th Australian conference on Microscopy and Microanalysis, February 2004, Geelong, Australia.
27. **Marshall J.** Norovirus research and MRSA surveillance – findings and future directions. Will this affect infection control practice? Forum held at the Function Centre, The Royal Melbourne Hospital, May 5, 2004, Melbourne, Australia.
28. **Middleton T.** HIV-2 viral load testing. Plenary at the 21st National Reference Laboratory Workshop on Serology, August 17-20, 2004, Melbourne, Australia.
29. **Middleton T.** Transmission and evolution of antiretroviral drug resistant HIV strains between 1996 and 2003 in Victoria, Australia. ASHM, September 2-4, 2004, Canberra, Australia.
30. **Middleton T.** Transmission of antiretroviral drug resistant HIV strains between 1996 and 2003 in Victoria, Australia, and their subsequent evolution in untreated individuals. XIII International HIV Drug Resistance Workshop: Basic Principles and Clinical Implications, June 8-12 2004, Canary Islands, Spain.
31. Vietheer PTK, Netter HJ, Sozzi T, **Bartholomeusz A.** Hepatitis B virus lamivudine resistant mutations abrogate secretion of hepatitis delta virus. Molecular Biology of HBV, October 24-27 2004, Woods Hole, Massachusetts, USA.
32. Vietheer PTK, Netter HJ, Sozzi T, **Bartholomeusz A.** Hepatitis B virus lamivudine resistant mutations abrogate secretion of hepatitis delta virus. AASLD, October 29-November 2, 2004, Boston, Massachusetts, USA.
33. Warner N, Ayres A, Shaw T, Leung NWY, Kuiper M, Chalmers D, Locarnini S, **Bartholomeusz A.** Functional analysis of the hepatitis B virus rL801 mutant selected in patients with severe hepatitis during long term lamivudine therapy. 4th Australasian hepatitis C meeting and ASHM, August 31 – September 3, 2004, Canberra, Australia.
34. Warner N, **Locarnini SA,** Colledge D, Edwards R, Angus P, Sievert W, Kuiper M, Chalmers D, Bartholomeusz A. Molecular modeling of entecavir resistant mutations in the hepatitis B virus polymerase selected during therapy. 55th Annual Meeting of AASLD, October 29-November 2, 2004, Boston, USA.

## Appendix 3

### COLLABORATORS

**Dr Campbell Aitken**, Epidemiology and Social Research Unit, Macfarlane Burnet Institute for Medical Research and Public Health, Prahran, Victoria

**Dr Ross Andrews**, Clinical Epidemiology and Biostatistics Unit, The Royal Children's Hospital, Melbourne, Victoria

**Professor Peter Angus**, Liver Transplant Unit, Austin and Repatriation Medical Centre, Heidelberg, Victoria

**Dr Michael Beard**, Institute of Medical and Veterinary Science, Adelaide, South Australia

**Dr Noel Bennett**, Chairman, Victorian Arbovirus Task Force, Melbourne, Victoria

**Dr Beverley-Ann Biggs**, Department of Medicine, University of Melbourne, Parkville, Victoria

**Dr Helen Billman-Jacobe**, University of Melbourne, Parkville, Victoria

**Dr Thomas Bock**, Department of Gastroenterology and Hepatology, University of Hanover, Hanover, Germany

**Dr Anna Braue**, St Vincent's Hospital, Melbourne, Victoria

**Professor Graham Brown**, Head, Victorian Infectious Diseases Service, Royal Melbourne Hospital, Parkville, Victoria

**Dr Graham Byrnes**, School of Population Health, University of Melbourne, Parkville, Victoria

**Dr Bill Carman**, Specialist Virology Centre, Gartnavel General Hospital, Glasgow Scotland

**Sonia Caruana**, Department of Medicine, University of Melbourne, Parkville, Victoria

**David Chalmers**, Victorian College of Pharmacy, Parkville, Victoria

**Dr Patrick Charles**, Austin Hospital, Heidelberg, Victoria

**Dr Suzanne Crowe**, Macfarlane Burnet Institute for Medical Research and Public Health, Prahran, Victoria

**Dr Robert de Man**, Erasmus University Hospital, Rotterdam, The Netherlands

**Dr Tony Della-Porta**, Managing Director, Biosecurity and Biocontainment Consultants, Geelong, Victoria

**Dr Paul Desmond**, Department of Gastroenterology, St Vincent's Hospital, Melbourne, Victoria

**Dr Greg Dore**, National Centre in HI Epidemiology and Clinical Research, The University of NSW, Sydney, NSW

**A/Professor Elizabeth Elliot**, Australian Paediatric Surveillance Unit, Sydney, New South Wales

**Professor Christopher Fairley**, Melbourne Sexual Health Centre, Carlton, Victoria

**Rebecca Guy**, Macfarlane Burnet Institute for Medical Research and Public Health, Prahran, Victoria

**A/Professor John Hayman**, Department of Anatomy and Cell Biology, Monash University, Clayton, Victoria

**Dr Margaret Hellard**, Macfarlane Burnet Institute for Medical Research and Public Health, Prahran, Victoria

**Dr Peter Hudson**, Scientific Director, CSIRO Health Sciences and Nutrition, Parkville, Victoria

**Professor Harriet Isom**, Department of Microbiology and Immunology, Penn State College of Medicine, Pennsylvania, USA

**Dr Grant Jenkins**, Department of Microbiology, Monash University, and Monash Medical Centre, Clayton, Victoria.

**A/Professor Paul Johnson**, Deputy Director, Infectious Diseases Department, Austin and Repatriation Medical Centre, Heidelberg, Victoria

**Dr Nor Shahidah Khairullah**, Malaysia Liver Foundation, Kuala Lumpur, Malaysia

**Professor Krzysztof Krawczynski**, Center for Disease Control and Prevention, Atlanta, Georgia, USA

**Mike Kuiper**, Victorian Partnership for Advanced Computing, Carlton South, Victoria

**Professor Hsiang-Fu Kung**, Institute of Molecular Biology, University of Hong Kong, Hong Kong, China

**Dr Stephen Lambert**, School of Population Health, University of Melbourne, Parkville, Victoria

**Dr George Lau**, Division of Gastroenterology and Hepatology, Queen Mary Hospital, Hong Kong, China

**Mr Andrew Lawrence**, Microbiology Department, Women's and Children's Hospital, North Adelaide, South Australia

**Professor Sharon Lewin**, Department of Infectious Diseases, Alfred Hospital, Prahran, Victoria

**Dr Vladimir Loparev**, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

**Dr Johnson Mak**, Macfarlane Burnet Institute for Medical Research and Public Health, Prahran, Victoria

**Dr Geert Maertens**, Infectious Diseases Program Manager, Innogenetics, Gent, Belgium

**Professor Michael Manns**, Department of Gastroenterology and Hepatology, Medical School of Hannover, Hannover, Germany.

**Laurent Marsollier**, Unite de Genetique bacterienne, Institut Pasteur, Paris, France

**Professor G. McCaughan**, Director, The AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Camperdown, NSW

**Professor Peter McIntyre**, National Centre for Immunisation Research and Surveillance, Children's Hospital at Westmead, Sydney, NSW

**A/Prof Raina MacIntyre**, National Centre for Immunisation Research and Surveillance, Children's Hospital at Westmead, Sydney, NSW

**Dr Nicholas Medland**, Clinical Director, The Centre Clinic, Victorian AIDS Council/Gay Men's Health Centre, St Kilda, Victoria

**Dr Anne Mijch**, Department of Infectious Diseases, The Alfred Hospital, Prahran, Victoria

**Dr Steve Oberste**, Respiratory and Enteric Viruses Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

**Dr Françoise Portaels**, Institute of Tropical Medicine, Antwerp, Belgium

**Dr Stuart Roberts**, Department of Gastroenterology, Alfred Hospital, Prahran, Victoria

**Dr Priscilla Robinson**, La Trobe University, Melbourne, Victoria

**Dr Norman Roth**, Prahran Market Clinic, Prahran, Victoria

**Natalie Rourke**, Veterinary Surgeon, Melbourne Zoo, Parkville, Victoria

**Dr Joe Sasadeusz**, Victorian Infectious Diseases Service, Royal Melbourne Hospital, Parkville, Victoria

**Dr Hiroyuki Shimizu**, Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan

**Dr William Sievert**, Department of Medicine, Monash Medical Centre, Clayton, Victoria

**Dr David Smith**, Pathcentre, Perth, Western Australia

**A/Prof Joseph Smollich**, Cardiology Department, Monash Medical Centre, Clayton, Victoria

**Dr Tim Stinear**, Department of Microbiology, Monash University, Clayton, Victoria

**Dr Hans Tillmann**, Department of Gastroenterology and Hepatology, Medical School of Hannover, Hannover, Germany

**Dr Chris Trautwein**, Department of Gastroenterology and Hepatology, Medical School of Hannover, Hannover, Germany

**Dr Kumar Visvanathan**, Department of Medicine, University of Melbourne, Royal Melbourne Hospital, Parkville, Victoria

**Dr Leon Worth**, Peter Macallum Cancer Institute, Melbourne, Victoria

**A/Professor Peter Wright**, Department of Microbiology, Monash University, Clayton, Victoria

**Professor Hui Zhuang**, Department of Medicine, Beijing Medical University, Beijing, China

## Appendix 4

### VISITORS 2004

**Ms Raudzah Baharuddin**, Senior Medical Laboratory Technologist, Institute for Medical Research, Kuala Lumpur, Malaysia

**Dr David Brown**, Director, Enteric and Respiratory Virus Laboratory, Public Health Laboratory Service, London, England

**Professor Paul Chan**, Microbiology Department, The Chinese University of Hong Kong, Hong Kong

**Peter Cheng**, Scientific Officer, Government Virus Unit, Public Health Laboratory Centre, Hong Kong

**Dr Paul Coleman**, Associate Research Fellow, DD Core Research and Development, Abbott Laboratories, Illinois, USA

**The Honourable Zhang Fenglou**, Vice Minister of Health, China, and accompanying delegation

**Dr Nicholas Hellmann**, Chief Medical Officer, Roche Molecular Systems Inc, California, USA

**Dr James Hnatyszyn**, Manager, Scientific Affairs, Bayer Diagnostics, California, USA

**Professor John Horvath AO**, Chief Medical Officer, Department of Health and Ageing, Canberra, Australia

**Professor Dato Mohd Amin Jalaludin**, Dean of Medicine, University of Malaysia, Malaysia

**Dr Kazunobu Kojima**, Regional EPI Laboratory Coordinator, WHO Regional Office for the Western Pacific, Manila, Philippines

**Dr Bob Lanford**, Southwest Foundation for Biomedical Research, Department of Virology and Immunology, San Antonio, Texas, USA

**Dr John McHutchison**, Director, Gastroenterology/Hepatology Research, Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina, USA

**Ms Mary Murnane**, Deputy Secretary, Department of Health and Ageing, Canberra, Australia

**Ms Lesley Podesta**, Department of Health and Ageing, Canberra, Australia

**Professor Dr Solko Schalm**, Department of Hepatology and Gastroenterology, Erasmus MC University Medical Centre, Rotterdam, The Netherlands

**Lori Spencer**, Technical Support Manager, Thermo Electron Corporation, Colorado, USA

**Professor Joseph Sung, Chairman**, Department of Medicine and Therapeutics; Director, Centre for Emerging Infectious Diseases, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong

**Dr Senya Toyama**, The Director for Life Sciences, Bureau of Science and Technology Policy, Cabinet Office, Japan

**Salina Wait**, Malaysian Liver Foundation, Malaysia

**Dr Ken-ichi Yayou**, Deputy Director for Life Sciences, Bureau of Science and Technology Policy, Cabinet Office, Japan

## APPENDIX 5

### APPOINTMENTS

#### Dr Chris Birch

Member, Primary HIV Working Group  
(*National Centre in HIV Epidemiology and Clinical Research*).

Member and Virologist, 'HIV Connect' Virology Group (*United Kingdom Medical Research Council HIV European/Australian HIV Drug Trial Programme*)

Assessor, National Association of Testing Authorities, Sydney

Member, Expert Consensus Panel for HIV Drug Resistance, Bayer Health Care

Reviewer for *Archives of Virology*, *Emerging Infectious Diseases* and *Journal of Infectious Diseases*.

Member, HIV Advisory Board, Bristol-Myers Squibb, Australia

#### Dr Scott Bowden

Honorary Senior Lecturer, Microbiology Department, Monash University

Honorary Senior Lecturer, Department of Applied Biology, RMIT University

Member, Melbourne Liver Group

Member, Advisory Board Hepatitis C Council of Victoria

Vice-President, Australian Centre for Hepatitis Virology

#### Dr Mike Catton

Member, Victorian Arbovirus Taskforce (*Department of Human Services, Victoria*).

Member, Influenza Vaccine Committee (*Therapeutic Goods Administration, Canberra*).

Reviewer, Communicable Diseases Intelligence (*CDNA, Canberra*).

Member, Public Health Laboratory Network (*CDNA, Canberra*).

Assessor, National Association of Testing Authorities, Sydney.

Member, Measles Elimination Advisory Committee (*Commonwealth Department of Health and Ageing, Canberra*).

Member, Commonwealth Panel of Expert Advisors for Crisis Management (*Emergency Management Australia*).

Member, RCPA Quality Assurance Standing Committee (*Royal College of Pathologists of Australasia, Sydney*).

Member, National Influenza Pandemic Action Committee (*Commonwealth Department of Health and Ageing, Canberra*).

Member, Infectious Diseases Working Group (*Commonwealth Department of Health and Ageing, Canberra*).

Member, Influenza Pandemic Planning Committee (*Department of Human Services, Melbourne*).

Member, Biosafety Advisory Group (*WHO, Geneva*).

Member, Victorian Enhanced Measles, Mumps, Rubella Surveillance Working Party (*Department of Human Services, Victoria*).

#### A/Prof Heath Kelly

Associate Professor, School of Population Health, University of Melbourne.

Member, Victorian Enhanced Measles, Mumps, Rubella Surveillance Working Party (*Department of Human Services, Victoria*).

Member, Editorial Group (*Victorian Infectious Diseases Bulletin*).

Member, Advisory Board (*Victorian Public Health Training Scheme*).

Chair, Organising Committee (PHA National Immunisation Conference, Queensland, 2004).

Chair, Victorian influenza surveillance sub-committee for pandemic preparedness.

Member, Influenza working party for the Australian Technical Advisory Group on Immunisation.

Invited reviewer for the following national and international journals: *Medical Journal of Australia*, *Journal of Paediatrics and Child Health*, *Australian Family Physician*, *Hepatology*, *Australian and New Zealand Journal of Public Health*, *Communicable Diseases Intelligence*, *Vaccine*, *Journal of Clinical Virology*, *Clinical Infectious Diseases*, *Scandinavian Journal of Infectious Diseases*.

#### Dr David E. Leslie

Member and Laboratory representative, Tuberculosis Advisory Committee, (*Department of Human Services, Victoria*)

STI / BIVI surveillance group, (*Department of Human Services, Victoria*)

RCPA Serology QAP Back-up Report Reviewer

#### Jennie Leydon

Member, Enhanced Measles, Mumps, Rubella Surveillance (*Department of Human Services, Victoria*).

Member, Quality Assurance Serology Scientific Advisory Network (*Royal College of Pathologists of Australasia, Sydney, NSW*)

Assessor, National Association of Testing Authorities, Sydney

#### Professor Stephen Locarnini

Consultant, Medical Advisory Committee (*Australian Animal Health Laboratory, Geelong*)

Member, Melbourne Liver Group

Member, Blood Borne Viruses Consortium, Victoria

Member, Australian Centre for Hepatitis Virology

Member of the National Centre in HIV Epidemiological and Clinical Research: Working Group on Viral Hepatitis

Advisory Committee, Gilead Sciences

Advisory Committee, Idenix Pharmaceuticals

Member of Hepatitis C Sub-Committee of the Ministerial Advisory Committee on AIDS, Sexual Health and Hepatitis

Editorial Board for the following international journals: *Hepatology*, *Antimicrobial Agents & Chemotherapy*, *Antiviral Research*, *Antiviral Chemistry and Chemotherapy*, *Viral Hepatitis Reviews*, *Antiviral Therapy*, *Journal of Clinical Virology* and *International Antiviral News*

#### Joseph Manitta

Honorary Senior Lecturer, Medical Laboratory Sciences, RMIT University.

Assessor, National Association of Testing Authorities, Sydney.

#### Dr Bill Maskill

Member, Pathology Messages Working Group Standards Australia Health Informatics IT – 14-6-5.

#### Rhonda McCaw

Member, Organising Committee for Victorian Hepatitis C Awareness Week (HAPPE).

Member, Reference Committee, Access Information at the Alfred Hospital.

#### Dr Michaela Riddell

NHMRC Sidney Sax Postdoctoral Fellow.

Member, Enhanced Measles, Mumps, Rubella Surveillance (*Department of Human Services*).

Invited Reviewer, *Clinical and Diagnostic Laboratory Immunology*, *Journal of Clinical Microbiology*, *Australian and New Zealand Journal of Public Health*.

Report reviewer, RCPA Serology QAP.

#### Dr Bruce Thorley

Member of Polio Expert Committee, Australia.

## APPENDIX 6

### STAFF IN 2004

#### Director

Dr Mike Catton

#### Laboratory Manager

Rob Pringle

#### Business Manager

Renato Raimondi

#### Finance

Mouhamed Kaya I

#### Medical Registrar

Dr Pat Charles

Dr Ben Cowie

Dr Jack Richards

#### Medical Microbiologist

Dr David Leslie

#### Information Systems

Dr Bill Maskill

Rob Warren

Dallas Wilson

#### Executive support

Pam Nagle

Sandra Maunders

Lisa Clayworth

Wendy Leong-Shaw

Sharon Mackenzie

#### General Office

Kay Hunt

Arron Lau

#### Data Entry/Billing

Adele Attard

Christine D'Angelo

Shanti Lamb

Jan McSevery

Edith Spiteri

Dana Wexelbaum

#### Specimen Reception

Margherita D'Angelo

Sam Djukic

Roma Hagos

Naomi Tomlinson

Lynn Metcalf

Julie Vatsilas

#### CSSD

Brigitte Ford

Wendy Staal

#### Maintenance

Vicki Miftari

Don Sheppard

#### Division of Virology

Head of Division: Dr Mike

Catton

#### Serology

Dr Alan Breschkin

Louise Carolan

Stav Corby

Shannon Curley

Adam Enriquez

Marie Gerrard

Theodora Kaminis

Theo Karapanagiotidis

Jennie Leydon

Suellen Nicholson

Maria Ramirez

Sue Tanner

Megan Wheeler

#### Honorary Appointment

A/Prof John Hayman

A/Prof Paul Johnson

Dr Anthony Della Porta

Dr Noel Bennett

#### Viral Identification

Dr Chris Birch

Abdul Amiri

Louise Carolan

Doris Chibo

Julian Druce

Nicole Gardiner

Matthew Kaye

Renata Kostecki

Tracey Middleton

Jessica Morris

Jodie Nicholls

Gina Papadakis

Brenna Scholes

Roseena Taqi

Thomas Tran

Brett Trounson

#### Electron

#### Microscopy/Gastroenterology

Dr John Marshall

Anna Dimitriadis

#### Division of Epidemiology

Head of Division: A/Prof Heath

Kelly

#### Epidemiology

Michaela Riddell

Hazel Clothier

Joy Turner

Janin Hoffman

#### Polio Reference Laboratory

Bruce Thorley

Kerri-Anne Brussen

Aishah Ibrahim

Jason Roberts

Vicki Stambos

David Tyssen

#### Division of Research and Molecular Development

Head of Division: Professor

Stephen Locarnini

#### Molecular Microbiology

Dr Scott Bowden

Rachel Hammond

Kathy Jackson

Rhonda McCaw

Lilly Tracy

Liza Cabuang

#### Molecular Pharmacology

Dr Tim Shaw

#### Molecular R&D

Dr Angeline Bartholomeusz

Anna Ayres

Danni Colledge

Jo Dean

Ben Dewar

Ros Edwards

Dr Margaret Littlejohn

Nadia Warner

Geoff Thompson

Margaret Nash

Tina Sozzi

Sally Rodgers

Lilly Yuen

Alex Thompson

Lucy Selleck

#### Division of Microbiology

Head of Division: Dr David

Leslie

#### Malaria/Exotic Pathogens

#### /Biochemistry

Joe Manitta

Wayne Metcalf

Anna Zorzetti

#### Mycobacterium

#### Reference Laboratory

Aina Sievers

Christine Birch

Kerrie Lea

Gina Papadakis

Gwen Styger

Wendy Ong

#### Mycobacterium

#### Molecular Research

Dr Janet Fyfe

Maria Globan

Kerry Raios

#### Bacteriology/Parasitology

Dr Norbert Ryan

Francesca Azzato

Christine Birch

Aaron Lock

Melissa Pontre

Sandra Robertson

Jason Sends

\* includes part-time staff and staff who joined or left VIDRL during 2004

## Appendix 7

### ABBREVIATIONS

ADV	Adefovir Dipivoxil	MAC	<i>Mycobacterium avium</i> complex
AFP	Acute Flaccid Paralysis	MIRU	Mycobacterial Interspersed Repeating Units
BCG	Bacillus Calmette-Guerin	MMLS	Melbourne Medical Locum Service
CI	Confidence Interval	MRI	Magnetic Resonance Imaging
CSIRO	Commonwealth Scientific and Industrial Research Organisation	MRL	Mycobacterium Reference Laboratory
DFP	Dried Fingerprick	MSHC	Melbourne Sexual Health Centre
DHS	Department of Human Services, Victoria	MV	Measles Virus
DoHA	Commonwealth Department of Health and Ageing	NH&MRC	National Health and Medical Research Council
DVB	Dried Venous Blood	NHSQL	National High Security Quarantine Laboratory
DVBS	Dried Venous Blood Spots	NIH	National Institutes of Health
ED	Emergency Department	OD	Optical Density
EIA	Enzyme immunoassay	PC3	Physical Containment Level 3
ELISA	Enzyme-Linked Absorbent Assay	PC4	Physical Containment Level 4
ETV	Entecavir	PCR	Polymerase Chain Reaction
GPs	General Practitioners	PEC	Polio Expert Committee
HBV	Hepatitis B Virus	QAP	Quality Assurance Program
HCV	Hepatitis C Virus	RT	Reverse Transcriptase
HEV	Hepatitis E Virus	RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
HIV	Human Immunodeficiency Virus	SARS	Severe Acute Respiratory Syndrome
HIV-2	Human Immunodeficiency Virus Type 2	SARS-CoV	Severe Acute Respiratory Syndrome Human Coronavirus
HSV	Herpes Simplex Virus	TB	Tuberculosis
HSV-1	Herpes Simplex Virus 1	tk	Thymidine kinase
HSV-2	Herpes Simplex Virus 2	VAPP	Vaccine Associated Paralytic Polio
ILI	Influenza-Like Illness	VE	Vaccine Effectiveness
ITS	Intergenic Spacer Region	VIDRL	Victorian Infectious Diseases Reference Laboratory
IU	International Units	VIDS	Victorian Infectious Diseases Service
		WHO	World Health Organization