JAMES RENNIE BEQUEST

REPORT ON EXPEDITION/PROJECT/CONFERENCE

Expedition/Project/Conference Title: Work Experience with WHO Collaborating Centre for Reference and Research on Influenza

Travel Dates: 23rd June to 9th August 2003 .....

Location: Melbourne, Australia .....

Group Member(s): Jennifer Badger .....

Aims: To gain experience in a range of virological techniques .....

OUTCOME (not less than 300 words):-

World Health Organisation Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia
Work Experience Report

Jennifer Badger
30th June to 8th August 2003

This work experience placement was set up between myself and the WHO collaborating centre for reference and research on influenza to allow me to gain valuable experience working in a laboratory, and to practice the varied techniques used for the isolation and identification of influenza strains. The experience I gained was in two main areas – serological techniques and molecular techniques. I also carried out several small projects during my time here.

Serology Techniques

The first two weeks of my placement were spent working in the serology laboratories, where the viruses are first grown, either in cell culture or eggs, and then several tests are used to characterise the viruses. These tests include haemagglutination assays, haemagglutination inhibition assays, neuraminidase inhibition assays and rapid diagnosis kits.

I spent a small amount of time during my placement working in the egg culture area, where I gained experience in both amniotic and allantoic inoculations of specific pathogen free eggs (SPF) and the methods of harvesting the egg fluids containing virus. All viruses used in the vaccines have to be grown in eggs, so this work is only done on original clinical samples (OCS) and the viruses obtained are used in the vaccine production.

I also spent some time in the cell culture laboratory where I assisted in the passaging of cells in cell culture. The cells used were both MDCK cells and MDCK suspension cells. I learned to make up the cell culture medium and then to passage the cells in this medium. I also gained some experience with the inoculation of the resultant cultures with influenza virus.

Much of my time in the serology laboratories was spent gaining experience in the use of haemagglutination assays (HA) and haemagglutination inhibition assays (HIA). The HA test is the main test used in the centre to check cell and eggs grown viruses for activity, and to titrate the

amount of virus in that sample. It is done using serial dilutions of the viruses and turkey red blood cells, as these have been shown to give the clearest results. The results of the HA tests are then used as an indication of the dilutions needed to use the viruses in the HIA test. In an HIA, unknown viruses are tested against a panel of antibodies against known viruses. The results are used to show how similar the unknown strains are to known viruses, based on the reaction profiles against the antibodies. These results are then used to show trends in virus strain, and any viruses with an unusual reaction profile are sequenced. I carried out both HA and HIA tests, under supervision and independently, on several occasions.

I also spent some time investigating the use of rapid diagnosis kits. These kits are produced by several manufacturers, and claim to be able to identify influenza in a sample in about fifteen minutes. I carried out a comparison of several tests – Quidel Quickvue, Binax Flu A/B and BD Flu A+B – and found that they were all quick and easy to use, but none showed good sensitivity to the samples.

In the Centre an ongoing investigation of the emergence of resistance to neuraminidase inhibitor drugs is being carried out. As part of the research, a novel neuraminidase inhibition assay is used. I spent a small amount of time observing this test and how it is used within the Centre.

Molecular Techniques

The middle two weeks of my placement were spent working in the molecular laboratories, where the viruses are further identified and sequenced. The techniques used include RNA extraction, reverse transcriptase-polymerase chain reaction (RT-PCR), agarose gel electrophoresis, RNA sequencing and multiplex PCR.

RNA extraction in the centre is done using the Qiagen viral RNA kit, which is used to extract RNA from clinical specimens and cultured influenza viruses. I carried out several extractions, both under supervision and independently and of both cell culture viruses and clinical samples.

RT-PCR was done using the Titan™ One Tube RT-PCR system, which carries out reverse transcription and PCR in the same tube. I carried out RT-PCR reactions on several occasions on samples that I had previously extracted. The PCR products were then washed using the QIAquick PCR purification kit, and then run on a 2% agarose electrophoresis gel. I gained much experience in the setting up, running and photography of such gels.

On several occasions I then went on to sequence the samples that I had extracted and amplified. Sequencing in the Centre is carried out using BigDye Terminator Cycle Sequencing. The dye terminators terminate the cDNA at all positions along the strand, giving strands of every possible length with a dye terminator at the end showing what base would be at that position. The samples are amplified with the dye terminators and then sent to another laboratory to be fully sequenced.

I also had the opportunity to carry out a multiplex PCR on original clinical samples during my time in the molecular laboratories. Multiplex PCR is carried out in a similar way to normal RT-PCR, but a variety of primers are used so that the samples can be typed according to their gel profile. For example, a sample can be run with primers which will amplify different sized fragments depending on if the virus is type A H1 or H3, or type B. When these samples are run on a gel, the size of the fragment shows what type the virus is. This can also be done to find out what subtype the type A viruses are.

Big Dye Terminators Investigation

As part of my work in the molecular laboratories, I carried out a small investigation into how the concentration of BigDye terminators in the sequencing reactions affects the quality of sequence obtained. When the sequencing reactions are carried out in the Centre, 6μl of BigDye

Terminators is used for each reaction. The investigation was carried out by doing several sequencing reactions with the same PCR product, but different volumes of BigDye in each one. The sequences obtained were compared and it was found that the volume of BigDye used makes very little difference to the quality of the sequence obtained. It was only when the volume was lower than 1µl per reaction that the sequence quality deteriorated. On the basis of this, it was decided to reduce the volume of BigDye terminators used in future sequencing reactions.

Xtrana Xtra-Amp RNA extraction kit

The second small investigation I carried out was into the use of the Xtrana Xtra-Amp RNA extraction kit. This is a new kit that had never been used in the Centre. The kit contains coated tubes for extraction and RT-PCR, lysis buffer, wash buffer and Amp enhance buffer. During extraction, the RNA binds to the tubes, which are then washed and the RT-PCR mix added directly to the tube. RT-PCR is then carried out in the same tube. I carried out an extraction and RT-PCR on two samples of the same virus, one egg grown, and one cell grown, but when the PCR product was run on an electrophoresis gel the result was negative. I carried out a second extraction with the same virus, but added in a drying step after the extraction because the literature with the kit suggested that excess wash buffer could inhibit PCR. However, the results were still negative. Due to time restraints, this investigation could not be continued.

FACS Analysis of suspension MDCK cells after infection with influenza virus

The last of my small projects was into FACS analysis of influenza infected MDCK suspension cells. The cells were infected with influenza for a certain length of time and then fixed and stained with fluorescent antibodies against nucleoprotein, haemagglutinin or neuraminidase. These cells were then run through the FACS machine to detect the presence of viral proteins. Unfortunately the results can back negative for viral proteins, and time restrictions prevented me investigating further.

Others

I was given the chance to attend a one-day workshop on immunological techniques at the Department of Microbiology and Immunology, Melbourne University. Several speakers gave talks on various new techniques in immunology, and the development in older techniques.

Acknowledgements

I would like to thank all of the staff of the WHO collaborating centre for reference and research on influenza for their kindness, patience and hospitality, and for teaching me so much in such a short time. I feel my trip has been a great success and look forward to putting my new skills into practice.

I would also like to thank the James Rennie Bequest fund for awarding me a grant which made this trip possible.