REPORT

WORKSHOP ON CELL CULTURE TECHNIQUES AND INTERNAL QUALITY CONTROL

Hong Kong
1-6 December 2003

Manila, Philippines
February 2004
REPORT

WORKSHOP ON CELL CULTURE TECHNIQUES
AND INTERNAL QUALITY CONTROL

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WORLD HEALTH ORGANIZATION
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NOTE

The views expressed in this report are those of the participants of the Workshop on Cell Culture Techniques and Internal Quality Control and do not necessarily reflect the policies of the World Health Organization.

Keywords:

Cell culture / Quality control / Laboratories / Poliomyelitis / Polioviruses

This report has been printed by the Regional Office for the Western Pacific of the World Health Organization for the participants in the Workshop on Cell Culture Techniques and Internal Quality, which was held in Hong Kong, from 1 to 6 December 2003.
SUMMARY

A Workshop on Cell Culture Techniques and Internal Quality Control was held at the Government Virus Unit of the Department of Health, Hong Kong (China) from 1 to 6 December 2003. The workshop was attended by 11 participants from national poliomyelitis laboratories (NPL), a scientist from NFL and two scientists from regional reference laboratories as resource persons and one WHO staff person.

The objectives of the workshop were:

1. to train participants (laboratory technicians) in quality assurance of cell sensitivity and virus isolation and in identification of poliovirus and non-polio enteroviruses from stool samples, with emphasis on in-house quality control, biosafety, containment and good laboratory practice;

2. to review established diagnostic techniques with an eye on quality assurance and facilitate exchange of information and experiences among laboratory network members; and

3. to increase awareness and appreciation for high standardized quality cell culture procedures.

The workshop focused on the theoretical and practical issues required for high quality cell culture techniques. The practical sessions of the workshop covered stool specimen processing and poliovirus and non-polio enterovirus (NPEV) isolation, observation of different forms of virus induced cytopathic effects as well as cell degradation due to other causes, cell sensitivity to Sabin poliovirus infection, maintenance of the RD-A and L20B cell lines including cell splits and counting, cell culture media and the freezing down and retrieval of cell lines from liquid nitrogen storage. The identification and isolation of single poliovirus serotypes by the poliovirus neutralization test (PNT) was covered through the provision of single and mixed enterovirus preparations, and the participants were required to isolate and confirm the identity of single serotypes. Daily microscopic observation of all cell preparations and tests were recorded and discussed with the workshop facilitators.

The lecture sessions covered all of the topics performed in the practical sessions as well as an update on the progress of the poliomyelitis global eradication programme with an emphasis on the need for timeliness of reporting of laboratory results. Presentations were made on internal quality control including record keeping, quality assurance and good laboratory practices. An outline of the current methods used by the regional reference laboratory for the intratypic differentiation (ITD) of polioviruses was presented. A questionnaire distributed to participants on the first day of the workshop was used for discussion of the correct interpretation of the PNT.

Consolidation of the techniques and theory learned by the workshop participants may occur through provision of a practical questionnaire on PNT within the next year. Requesting the national poliomyelitis laboratories to determine the virus TCID50 and challenge dose for the 2004 poliovirus isolation and identification PT would also be of benefit. The provision of worksheets in use at national poliomyelitis laboratories would assist other laboratories in implementation of quality control procedures. The theoretical and practical topics covered in the workshop may also be discussed at the next regional laboratory meeting.

The workshop participants were encouraged to remain in contact with each other and with the facilitators as a future resource for technical advice and support.
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1. INTRODUCTION

The eighth informal consultation of the global polio laboratory network held in Geneva in October 2002 recommended that network laboratory personnel be trained in cell culture techniques and mycoplasma-free maintenance of cell cultures, as problems with cell cultures are the most frequent cause of delays in obtaining virologic results. To implement this global recommendation, the WHO Western Pacific Regional Office held a workshop at the Government Virus Unit of the Public Health Laboratory Centre in Hong Kong (China) from 1 to 6 December 2003.

1.1 Objectives

The workshop, in addition to cell culture techniques and mycoplasma-free maintenance of cell cultures, also covered laboratory quality assurance and good laboratory practices to facilitate the standardization of quality control and promote understanding of its importance as a performance indicator for laboratory accreditation. The sixth meeting on laboratory surveillance for poliomyelitis eradication in the Western Pacific Region, held in Manila in November 2002 emphasized the need to sustain the polio laboratory network as a fully functional component of the Expanded Programme on Immunization (EPI). The workshop assisted in this regard, through the exchange of information and experiences among network members.

1.2 Organization

The workshop was attended by 11 participants from seven selected national polio laboratories; three temporary advisers, two from regional reference laboratories—Victorian Infectious Diseases Reference Laboratory, Melbourne and National Institute of Infectious Diseases, Tokyo—and one from the Government Virus Unit of the Department of Health, Hong Kong (China); and a secretariat (Annex 1).

2. PROCEEDINGS

The workshop focused on the theoretical and practical issues required for high-quality cell culture techniques. The practical sessions of the workshop covered stool specimen processing and poliovirus and non-polio enterovirus (NPEV) isolation, observation of different forms of virus induced cytopathic effects as well as cell degradation due to other causes, cell sensitivity to Sabin poliovirus infection, maintenance of the RD-A and L20B cell lines including cell splits and counting, cell culture media and the freezing down and retrieval of cell lines from liquid nitrogen storage.

The identification and isolation of single poliovirus serotypes by the poliovirus neutralization test (PNT) was covered through the provision of single and mixed enterovirus preparations. Participants were required to isolate and confirm the identity of single serotypes. Daily microscopic observation of all cell preparations and tests were recorded and discussed with the workshop facilitators.

The lecture sessions covered all of the topics performed in the practical sessions as well as an update on the progress of the polio global eradication programme with an emphasis on the need for timeliness of reporting of laboratory results.
Presentations were made on internal quality control including record keeping, quality assurance and good laboratory practice. An outline of the current methods used by the regional reference laboratory for the intratypic differentiation (ITD) of polioviruses was presented. A questionnaire distributed to participants on the first day of the workshop was used for discussion of the correct interpretation of the PNT.

2.1 Workshop activities

Annex 2 shows the timetable of the workshop.

Day 1

The workshop participants and facilitators were introduced to the staff of the Hong Kong Government Virus Unit (GVU), headed by Dr Wilina Lim. The workshop was opened by Dr Leung Pak-yin, JP, Deputy Director of Health, Hong Kong (China), and the introductory speech from the WHO Regional Director for the Western Pacific, Dr Shigeru Omi, was read by Dr Kazunobu Kojima, the Regional Laboratory Co-ordinator, WHO Western Pacific Regional Office.

Lecture sessions. The first lecture covered the isolation and identification of polioviruses through use of the RD and L20B cell lines. A comparison of NPEV isolation rates between the RD and HEp2C cell lines was presented for discussion regarding the impact the discontinuation of the latter cell line may have on the NPEV isolation rate. The recently introduced methodology to determine cell sensitivity using authenticated Sabin poliovirus strains was presented. Avoidance of contamination of cell lines, particularly by mycoplasma was also covered. The second presentation was on stool specimen processing and the topics included the procedure for receipt of specimens, set-up and use of the Biosafety Cabinet (BSC), the steps involved in specimen processing and the records necessary to trace all steps of a specimen from the moment of receipt at the laboratory to final reporting. Photos of healthy cells and those with virus cytopathic effect (CPE) and other causes were presented for discussion. The photos were taken prior to the workshop and were presented to the participants on a compact disc for future reference and in-house training upon return to their laboratory.

Practical sessions. The 11 participants were divided into five groups of two and one person paired with a staff member from the GVU. A set of practical notes for the workshop based on the WHO polio lab manual was distributed. Participants observed the splitting of cells from a flask and the performance of an accurate cell count. All participants were provided with a cell counting chamber for use in their laboratories as a routine procedure. The cells were used to prepare cell culture tubes and flasks and for incubation with authenticated Sabin virus strains to determine the cell sensitivity during the workshop. Stool specimens were processed and inoculated in tubes for observation and passaging over the following days. Media changes of cell cultures were performed during the workshop as appropriate. The GVU staff had also prepared tubes with CPE due to various causes for observation and discussion; these included toxicity, poliovirus and adenovirus infection and cells held too long in tubes.

Day 2

Lecture sessions. An update on the global polio eradication programme was presented with an outline of the countries still indigenous for circulating wild poliovirus and outbreaks of circulating vaccine-derived poliovirus (cVDPV). A comparison of NPEV isolation rates within the Western Pacific Region over the last two years initiated a discussion on the possible reasons for reported decreases in isolation rates by some laboratories. The NPEV isolation rate may be regarded as an indicator of cell sensitivity to enterovirus infection.
While an annual NPEV isolation rate of 10% is commonly quoted as the target figure, the actual figure can vary from country to country depending upon local environmental and social conditions. More importantly, the NPEV isolation rate for a particular lab should remain fairly constant from one year to the next. A significant decrease in the annual rate should be investigated to determine if technical issues are the cause. The recent changes to the accreditation checklist and performance indicators were reviewed to reinforce the need for timely reporting by all polio laboratories.

A presentation was made on the performance of the PNT. It was recommended that when a poliovirus is isolated independently in the RD and L20B cell lines, PNT should be performed in both cell lines. Emphasis was placed on the identification and confirmation of mixtures of poliovirus serotypes, as this has been the principal cause of incorrect results in the annual poliovirus proficiency test. Determination of the virus titre from the back titration and the Karber formula was reviewed. Two reasons to pick out and confirm mixtures of polioviruses were presented: (1) to confirm that the CPE is not due to breakthrough of a high titre virus and (2) to refer single isolates for ITD to the regional reference laboratory. The two methods recommended at the workshop for confirmation of picked out isolates were a full PNT or alternatively a simplified PNT with a 1/10 dilution of the isolate that does not include a back titration.

Practical sessions. The participants performed a cell split and count to prepare flasks and tubes for observation over the remainder of the workshop. The GVU staff had prepared a set of PNT plates for each group that contained mixtures of polioviruses and NPEVs for the participants to read and perform further tests during the course of the workshop to identify the viruses. Isolates were picked out and stored to perform a confirmatory PNT on day 3. Each group was also provided with a different mixture of polioviruses and then set up a PNT to follow during the week and determine what steps to take next. The cell sensitivity plates were read.

Day 3

Lecture session. A presentation was given on cell banking. This reviewed the establishment of a cell bank, good cell bank practices, freezing down and retrieval of cells, labelling and establishment of an inventory, risks and safety precautions and quality control.

Practical sessions. A confirmatory PNT was performed of the isolates picked out on day 2. This involved diluting the isolate 1/10 and challenging the virus with the same antisera pool as it was picked out from. The PNT plates of the poliovirus mixtures, set up on day 2, were read. A demonstration of freezing cells and storing them in liquid nitrogen was performed using a commercial freezing container that controls the rate of temperature decrease. Workshop participants were provided with a freezing tray for use in their own laboratories. The cell sensitivity plates were read.

Day 4

Lecture session. A lecture was given on cell culture techniques. Topics included the nature of the surface for cell attachment, constituents of cell culture media, issues related to the use of serum, growing cells in a closed or gaseous (CO₂) environment, incubator temperature, subculturing, sterility testing, antibiotics and quality control. The questionnaire was reviewed with the participants providing the answers and relating what steps they would take next to confirm a mixture of polioviruses and/or NPEVs using example PNT plates.

Practical sessions. The PNT plates of the confirmatory pick-outs and the mixture of polioviruses were read along with the cell sensitivity plates and observation of the tubes and
flasks prepared on day 2. A demonstration of reviving cells from liquid nitrogen and culturing into flasks was performed.

Day 5

Lecture sessions. A lecture was given on laboratory quality assurance. This reviewed the purpose of a quality assurance programme, what such a program entails, staff training records, equipment maintenance records, documentation including SOPs, worksheets, inventories and the concept of traceability of specimens from receipt to reporting. The use of the authenticated Sabin strains was reviewed and a nomenclature for virus isolate pick-outs was presented. Further example PNT plates were presented for practice to determine the virus titration using the Karber formula. A presentation was also given on the ITD methods used at the regional reference laboratories in the Western Pacific Region. This included the ability of the methods to detect vaccine-derived poliovirus (VDPV) and examples of virus isolates from the Western Pacific with discordant ITD.

Practical sessions. The PNT and cell sensitivity plates and the flasks and tubes were observed. Discussion with the participants of their observations and results occurred throughout the practical sessions.

Day 6

Lecture session. The final lecture was on good laboratory practice (GLP). The participants were first asked to list what issues they considered to be part of GLP. Topics included biosafety, quality assurance, physical layout of the laboratory, clothing, liquid nitrogen, laboratory techniques, cell banking and media, the polio lab manual and good communication.

Practical session. The participants made their final observations and then presented a summary of their results and conclusions to the facilitators. All results and conclusions were appropriate.

Dr Kazunobu Kojima presented the closing remarks and thanked the participants for actively participating in the sessions. He thanked Dr Bruce Thorley and staff of the Victorian Infectious Diseases Reference Laboratory, Melbourne and Dr Hiroyuki Shimizu and staff of National Institute of Infectious Diseases, Tokyo for their full support for the workshop, particularly for planning and modifying the schedule/agenda, providing technical expertise (instructions and instructions/lectures) as well as seasoned experience in poliomyelitis laboratories, providing the necessary materials for the workshop (preparation of photos of cells, questionnaires, and presentation materials. Dr Wilina Lim and the staff of the Government Virus Unit, Department of Health, Hong Kong (China) were also gratefully acknowledged for their thorough preparation of the practical materials and dedication throughout the period of the workshop.

3. CONCLUSIONS

The workshop ran smoothly throughout both the practical and lecture sessions. The Public Health Laboratory Centre was an excellent venue for the workshop and the warm hospitality of Dr Wilina Lim and all the GVU staff is gratefully acknowledged. All workshop participants were dedicated in their application to every task and were enthusiastic to understand all issues. The topics covered during the workshop were relevant to the needs of the participants and covered cell culture, quality assurance and poliovirus isolation. The
participants were positive in their feedback regarding the workshop and gained from the open discussion on the cell culture and PNT questionnaire. The workshop schedule was fairly tight. A longer period for the workshop would have improved the assimilation of workshop agenda items.

The main conclusions of the workshop were as follows:

(1) Consolidation of the techniques and theory learned by the workshop participants may occur through provision of a practical questionnaire on PNT within the next year.

(2) Requesting the national polio laboratories to determine the virus TCID₃₀ and challenge dose for the 2004 poliovirus isolation and identification PT would also be of benefit.

(3) The provision of worksheets in use at national polio laboratories would assist other laboratories in the implementation of quality control procedures.

(4) The theoretical and practical topics covered in the workshop may also be discussed at the next regional laboratory meeting.

(5) The workshop participants are encouraged to remain in contact with each other and with the facilitators as a future resource for technical advice and support.
### INFORMATION BULLETIN NO. 2

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<td>concentration for seeding (flasks, tubes, plates)</td>
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<td>plate during PNT 1 hour incubation</td>
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<td>PNT to confirm single serotype</td>
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<td>Day 1 Reading: inoculated samples;</td>
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<td>Day 2 Reading: Cell sensitivity (Day 1 Practical 1)</td>
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<td>Freeze/thaw +ve cultures, passage</td>
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<td>Day 1 Reading: PNT plates (Day 2 Practical 4)</td>
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<td>confirmatory PNT plates</td>
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<tr>
<td>1530-1600</td>
<td>COFFEE BREAK</td>
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**TENTATIVE TIMETABLE**
<table>
<thead>
<tr>
<th>Time</th>
<th>Thursday, 4 December</th>
<th>Time</th>
<th>Friday, 5 December</th>
<th>Time</th>
<th>Saturday, 6 December</th>
</tr>
</thead>
<tbody>
<tr>
<td>0830-1030</td>
<td>Media preparation</td>
<td>0930-1000</td>
<td>Laboratory quality assurance (global requirements; maximum of 15 passages per cell line, mycoplasma testing at RRL)</td>
<td>0930-1000</td>
<td>Practical 11: Final reading/observations</td>
</tr>
<tr>
<td></td>
<td>Sterilization/Filtration</td>
<td></td>
<td>Proper use of equipment</td>
<td></td>
<td>Day 4 Observation of TC flask, tubes (Day 2 Practical 3)</td>
</tr>
<tr>
<td></td>
<td>Storage/expiry dates</td>
<td></td>
<td>Review of questionnaire</td>
<td></td>
<td>Day 5 Observation of inoculated cultures and passages (Day 1 Practical 2)</td>
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<tr>
<td></td>
<td>Record keeping</td>
<td></td>
<td>Review of earlier lectures</td>
<td></td>
<td>Day 4 Reading: PNT (Day 2 Practical 4)</td>
</tr>
<tr>
<td></td>
<td>Buffering systems – different incubator conditions</td>
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<td></td>
<td>Day 3 Reading: PNT (mixtures confirmatory neuts) (Day 3 Practical 6)</td>
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<tr>
<td></td>
<td>Commercial preparations (Liquid, Powder)</td>
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<td>Trypsin</td>
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<td>1030-1100</td>
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<tr>
<td>1100-1200</td>
<td>Practical 7: Revival of cells from liquid N₂, into flasks.</td>
<td>1030-1200</td>
<td>Practical 8: Observation of revived cells (Day 4 Practical 7)</td>
<td>1100-1200</td>
<td>Practical 10: Compilation of results by participants, discussions and review</td>
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<td></td>
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<td>1200-1300</td>
<td>Media change of revived cells</td>
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<td>Day 3 observation of TC flask, tubes (Day 2 Prac 3)</td>
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<td>Media change of flasks to 2% MEM if almost confluent</td>
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<td>1200-1300</td>
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<td>1300-1500</td>
<td>Practical 8:</td>
<td>1400-1700</td>
<td>Practical 10:</td>
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<td>Day 2 Observation of TC flask, tubes (Day 2 Practical 3)</td>
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<td>Day 4 Observation of inoculated cultures and passages (Day 1 Practical 2)</td>
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<td>Media change to 2% MEM if almost confluent (discussion)</td>
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<td>Freeze/thaw +ve cultures, passage</td>
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<td></td>
<td>Day 3 Observation of inoculated cultures (Day 1 Practical 2)</td>
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<td>Day 3 Reading: PNT (Day 2 Practical 4)</td>
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<td>Freeze/thaw +ve cultures, passage</td>
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<td>Day 2 Reading: confirmatory PNT of mixtures (Day 3 Practical 6)</td>
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<td>Day 2 Reading: PNT (Day 2 Practical 4)</td>
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<td>Day 1 Reading: confirmatory PNT of mixtures (Day 3 Practical 6)</td>
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<tr>
<td>1600-1700</td>
<td>(12) Closing ceremony</td>
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