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REPORT OF THE MEETING OF THE *AD HOC* COMMITTEE ON ORTHOPOXVIRUS INFECTIONS

Geneva, Switzerland
9 September 1994

Organized by: Programme on Viral, Bacterial Diseases and Immunology (BVI)

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Report of the Meeting of the
***Ad Hoc* Committee on Orthopoxvirus Infections**
Geneva, 9 September 1994

Introduction

Dr R.H. Henderson, Assistant Director-General, welcomed participants and opened the meeting on behalf of the Director-General. He indicated that the major objectives were to finalize recommendations about the fate of the last stocks of variola virus kept in the WHO Collaborating Centres for Smallpox and other Poxvirus Infections in the USA and the Russian Federation, and to make recommendations on the destruction or maintenance of cloned DNA fragments of variola virus genome, existing stocks of smallpox vaccine and seed virus for vaccine production.

Dr Henderson briefly reviewed the WHO activities since the global eradication of smallpox had been confirmed. He described the establishment of the WHO Committee on Orthopoxvirus Infections which had met annually between 1981 and 1986 to maintain surveillance activities and to oversee the post-eradication policy. At its last meeting, in 1986, the Committee recommended the establishment of an *Ad Hoc* Committee to oversee further activities, especially relating to the cloning (and later sequencing) of the variola virus genome, the retention of WHO stocks of smallpox vaccine and seed vaccine virus stocks, and ultimately to oversee the destruction of the remaining stocks of variola virus. Because of the responses received from the scientific and political communities concerning the recommendation to destroy the variola virus and in recognition of scientific advances made in obtaining nucleotide sequence information on the variola virus genome, WHO was now asking the *Ad Hoc* Committee to re-evaluate its previous recommendations.

Dr F. Fenner was appointed Chairman and Dr P.J. Greenaway Rapporteur.

Implementation of recommendations issued by the *Ad Hoc* Committee in December 1990

An overview the *Ad Hoc* Committee's previous recommendations and of the post-smallpox eradication programme since its last meeting in December 1990 was presented. The recommendations are given below, with comments pertaining to their current status:

Recommendation 1

All stocks of variola virus and materials containing variola virus must be destroyed by 31 December 1993.

The publication of this recommendation engendered a debate between those supporting and opposing the destruction of the variola virus. The issue was further discussed in an open international scientific forum organized during the IX International Congress of Virology held in Glasgow in August 1993. A summary of the debate as it appeared in the media, the scientific literature, during the Glasgow conference or otherwise communicated to WHO is attached below (see pages 5-6). The present meeting was convened to review the matter once again.

Recommendation 2

All recombinant plasmids and other related materials that contain variola virus DNA sequences should be destroyed at the same time as the variola virus stocks, provided that the Technical Committee (see recommendation 5 below) is satisfied that sufficient sequence information is available, and serious scientific objections have not been raised.

An inquiry among individual members of the *Ad Hoc* Committee indicated that the majority would reverse this recommendation. The recommendation is therefore brought for reconsideration by the Committee.

Recommendation 3

In the interim, all recombinant plasmids that contain variola virus DNA sequences should be registered with the World Health Organization (WHO). These plasmids may only be provided to requesting scientists after informing WHO and on the strict understanding that they must not be distributed to third parties or used in laboratories handling other orthopoxviruses.

All laboratories known to have worked with recombinant plasmids containing variola virus DNA sequences were informed of the recommendation and have reported their holding of such material to WHO. The inventory was included among the working papers of the meeting.

Recommendation 4

WHO should endorse the proposals made by representatives from the USA and USSR for determining the nucleotide sequences of the genomes of specific and representative variola viruses; the order of priority should be an Asian major strain, an American minor strain, an African major strain and an African minor strain.

The WHO Collaborating Centres for Smallpox and other Poxvirus Infections designated at the Centers for Disease Control and Prevention in Atlanta, Georgia, USA and at the Institute for Viral Preparations, Moscow, Russian Federation accepted and executed the sequencing project as a joint effort. The Institute of Molecular Biology, "Vector", Koltsovo, Novosibirsk Region, Russian Federation was approved by a WHO team to sequence variola virus genomes on behalf of the WHO Collaborating Centre for Smallpox and other Poxvirus Infections in Moscow. Close collaboration was established between these Institutes and the Collaborating Centre at CDC which also provided partial financial support for the project.

Recommendation 5

WHO should establish an expert Technical Committee to oversee the above DNA sequencing efforts; this committee should consist of a Chairman who is an expert in poxvirology, a representative from each of the sequencing laboratories, at least two other people with experience in the sequence analysis of large DNA molecules, and a member of the WHO secretariat.

The Technical Committee on Analysis of Nucleotide Sequences of Variola Virus Genomes was established in 1991 under the chairmanship of Professor Dumbell. The sequencing project advanced quickly and the nucleotide sequence of two strains, India-1967 and Bangladesh-1975, was nearly completed in December 1992 when the Technical Committee had its first meeting in Atlanta. A second and final meeting of the Committee was held in January 1994 in Geneva. In addition to the complete nucleotide sequence of strain Bangladesh-1975 and the complete genome coding sequence of strain India-1967, partial nucleotide sequences of variola virus DNA had been obtained from several other strains. The sequence information has been made available to the scientific community through the Genbank/EMBL data banks and several publications.

Recommendation 6

WHO should provide the financial resources and administrative support for the Technical Committee; an officer within the WHO Division of Communicable Diseases should continue to take responsibility for concluding the post-eradication activities.

Financial support has been available within WHO for some of the activities but it is appropriate to acknowledge the generous contributions of the Centers for Disease Control and Prevention. Core staff have been maintained within the WHO Division of Communicable Diseases.

Recommendation 7

WHO should establish a Commission to certify the destruction of all variola virus stocks and, when appropriate, all recombinant plasmids and other materials containing variola virus DNA sequences; this Commission should prepare a final report on post-eradication activities in time for presentation to the World Health Assembly in May 1994.

This recommendation is being reconsidered at this meeting.

Recommendation 8

The WHO Collaborating Centres for Smallpox and other Poxvirus Infections in Atlanta, Georgia, USA and Moscow, Russian Federation, should continue to serve as reference and research facilities for poxviruses.

The two Centres agreed to continue to serve in this function and were redesignated in 1991, for a period of three years with the following terms of reference:

- To maintain the capability in terms of both personnel and facilities for laboratory diagnosis of smallpox and other viruses in the group of orthopoxviruses;
- To maintain representative strains of variola virus;
- To determine the nucleotide sequence of the genome of at least one variola virus strain of special epidemiological significance;
- To register with WHO/HQ all recombinant plasmids containing variola virus DNA sequences produced in the laboratory and the distribution of these plasmids;
- To cooperate with WHO in implementing the recommendation of the Fourth Meeting of the Committee of Orthopoxvirus Infections and of the *Ad Hoc* Committee on Orthopoxvirus Infections to destroy the remaining stocks of viable variola virus before 31 December 1993;
- To submit an annual report to WHO on the relevant work of the Centre.

Recommendation 9

WHO should re-advise all countries that there is no necessity for vaccinating military personnel against smallpox.

Following the meeting of the *Ad Hoc* Committee, WHO Member States were informed of the recommendations issued with particular emphasis on the recommendations to destroy the remaining stock of variola virus. The recommendation that vaccination of military personnel should be discontinued in all countries was highlighted. WHO Headquarters received comments from six European Member States. They generally supported the recommendations but one country emphasized the continued need for smallpox vaccine. A few Member States informed WHO that they maintained a stock of smallpox vaccine and/or the seed virus and one, believing that variola virus was still needed for diagnosis, expressed concern about its destruction.

It was reported that a recent survey had indicated that approximately 61 million doses of smallpox vaccine were available and satisfactorily stored worldwide. Some 10 Institutes still retain seed stocks of the vaccine.

In answer to questions from members of the Committee, it was reported that WHO had undertaken an extensive survey throughout the world to confirm that no variola virus stocks existed outside of the designated laboratories. There is now good evidence to suggest that such stocks do not exist although no absolute guarantee can be given. Members of the Committee were satisfied that WHO had done everything feasible to ensure that no stocks of variola virus had been overlooked.

Since the declaration of global eradication some 170 rumours of smallpox have been reported to WHO Headquarters from all WHO regions. The incidence of these rumours has diminished during the post-eradication period; 3 have been reported so far in 1994. All rumours were investigated, none was confirmed.

Ten cases of monkeypox have been reported to WHO in the eight years since the end of the special surveillance programme on monkeypox. From three of them monkeypox virus was isolated. Eight of the 10 cases occurred in Gabon: three related cases in 1987 and five cases in neighbouring villages in January, May and June of 1991. One case occurred in Cameroon in 1990. The nine cases were further investigated and specimens were obtained from some for laboratory confirmation at the WHO Collaborating Centres. Monkeypox virus was isolated from one case in each outbreak in Gabon and the case in Cameroon. The most recent incident was reported from Zaire in 1992. It was reported by a local physician who provided photographs and offered his services for further investigation. Efforts by the Regional Office to contact the physician for follow-up failed.

Nucleotide sequencing of variola virus genomes

The *Ad Hoc* Committee then reviewed the reports of the Technical Committee on the Analysis of Nucleotide Sequences of Variola Virus Genomes and more recent data obtained.

The WHO Collaborating Centre for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, reported the complete nucleotide sequence (186,103 base pairs) of the DNA genome of variola major virus strain Bangladesh-1975. The collaborating group working in the Institute of Molecular Biology (IMB), Koltsovo, Novosibirsk Region, Russian Federation, together with the WHO Collaborating Centre for Smallpox and other Poxvirus Infections in Moscow, reported the sequence of 185,578 base pairs (bp) of the DNA genome of variola major virus strain India-1967, which includes the complete genome coding sequence (approximately 700 noncoding bp at each end of the viral DNA were not sequenced).

Partial nucleotide sequences of genomic DNAs of the following other variola major virus strains were reported as follows: Harvey-1944, 23,300 bp obtained at Oxford University and University of Cape Town, South Africa; Vellore-1964, 1,000 bp obtained in the University of Cape Town; Congo-1970, 30,000 bp, obtained at CDC, USA. Partial sequences of the genomic DNA of the following variola minor virus strains were reported: Butler-1952, 1,800 bp at CDC and Garcia-1966, 155,000 bp at IMB and CDC. Thus a total of about 600,000 bp of variola virus genomic DNA sequence has been determined to date.

The Technical Committee was impressed by the amount of nucleotide sequence data generated during the project and by the high level of collaboration between the study groups. They concluded that the data obtained were considerably in excess of the minimum of one complete genome sequence requested by the WHO *Ad Hoc* Committee on Orthopoxvirus Infections in 1990.

Data were presented from the Centers for Disease Control and Prevention with an update on recent studies concerning rapid diagnosis of orthopoxviruses. In particular, a type-specific diagnostic test was described which was based on amplification by polymerase chain reaction of part of the distinctive haemagglutinin gene present only in orthopoxviruses. This gene was selected because its hypervariable domain is type specific amongst orthopoxvirus species. The test uses a series of primers that differentiate North American orthopoxviruses from Eurasian and African orthopoxviruses and differentiate the species within these subgroups. This test was validated with DNA from clinical material (stored smallpox scabs) and infected tissue cultures. The test is in a format suitable for routine use and can be completed within one working day.

Variola virus stocks

The *Ad Hoc* Committee considered the arguments for and against the destruction of the variola virus stocks and a summary of the debate as it appeared in the media, the scientific literature, during the Glasgow conference or otherwise communicated to WHO is summarized below.

Arguments against destruction:

- all possibility of future studies on the variola virus will be lost (properties of viral genes and proteins, biological functions of the virus, pathogenesis, etc);

- destruction of the viruses in the two known repositories may not guarantee the complete removal of the virus from the Earth (preserved corpses of smallpox cases, forgotten or hidden stocks elsewhere).

Some suggested that destruction be agreed in principle but be postponed to allow more time for further analysis of the sequenced genomes of variola virus and for weighing of scientific merits when further advances in methods and understanding had occurred.

Arguments for destruction:

- the escape of the variola virus from the laboratories would be a serious risk as an increasing proportion of the population lack immunity to the disease due to cessation of vaccination and revaccination against smallpox more than 10 years ago;

- the sequence information and the availability of cloned DNA fragments of the full genome of several strains of variola virus allow most scientific questions about the properties of the viral genes and proteins to be resolved. The cloned DNA fragments of the virus genome are non-infectious and can be handled in safety;

- the decision to eradicate smallpox was a collective decision of the world community, based on public health considerations and all measures should be taken to ensure that smallpox does not again afflict mankind.

The members of the *Ad Hoc* Committee on Orthopoxvirus Infections discussed the issues relating to the destruction of the last stocks of variola virus in depth. They agreed that the scientific arguments for retention were clear and that there was some potential for learning more about the virus, its virulence and pathogenicity; not all desirable studies could be done with cloned virus DNA. The lack of a convenient laboratory animal and the need to work in high security (P4) facilities limited the potential of such work, however. Those favouring early destruction of the virus indicated that many of the scientific questions raised could be better addressed using other orthopoxviruses, or were of lower priority than a variety of issues pertaining to agents still causing widespread disease. It was noted that the scientific community had been consulted widely and that many supported destruction including the Executive Board of the International Union of Microbiological Societies, the Presidium of the Russian Academy of Medical Sciences, the Council of the American Society for Microbiology and the Board of Directors of the American Type Culture Collection.

The Committee unanimously agreed that all remaining stocks of variola virus, including whitepox virus, viral genomic DNA, clinical specimens and other materials containing infectious variola virus held in the WHO Collaborating Centres for Smallpox and other Poxvirus Infections in the Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and in the Institute for Viral Preparations, Moscow, Russian Federation should be destroyed. There was debate over the date on which destruction should occur however. Those favouring early destruction considered that the genomic sequence data from several strains of variola virus, with the availability of other sequences cloned in bacterial plasmids, satisfied the need for an archival record of the virus. They noted that these cloned DNA fragments would provide sufficient reference material to resolve any future diagnostic problem involving suspected smallpox and allowed for future studies of properties of variola virus genes and proteins. They also stressed that escape of variola virus from the laboratory would be a serious risk to the increasing proportion of the population that lacks immunity to smallpox. They noted that the decision to eradicate smallpox was a collective decision of the world community, based on public health considerations, and health officials and others in many countries were concerned about the continued retention of stocks of variola virus, especially as some of these countries had made considerable efforts to destroy their own variola virus stocks.

Members of the *Ad Hoc* Committee in favour of postponing destruction of the virus recommended that the archival storage of variola virus be continued in the two Collaborating Centres. They considered that the rapid advances in science and technology now occurring would enable new questions to be addressed in the future and that it was therefore too early to take this irrevocable step. They urged that serious consideration be given to storing the virus for a further five years.

The majority (8/10) of the members of the *Ad Hoc* Committee on Orthopoxvirus Infections recommended that the date for the destruction of the remaining stocks of variola virus and other materials as listed above should be on Friday 30 June 1995 in both Centres subject to agreement by the World Health Assembly in May 1995.

Members of the *Ad Hoc* Committee then recommended procedures for destruction of the variola virus. These are as follows:

Stored ampoules with variola virus, including whitepox virus, virus-containing specimens, and viral genomic DNA, should be destroyed by autoclaving following submission of a full written risk assessment of the procedure. The autoclaving process should involve as few personnel as possible, all of whom should have had a smallpox vaccination within the previous three years. Autoclaving should be done in a fully validated double ended autoclave.

Virus-containing samples should be autoclaved without opening of the ampoules or other primary containers. Autoclaving should be done at 120°C at one atmosphere pressure for 45 minutes. This procedure should be performed twice. Maintenance of the holding temperature (120°C) for the specific time (45 minutes) at the centre of the autoclave load must be validated using electronic thermal probes. All autoclaved materials should then be disposed of by incineration.

Documentary evidence giving an inventory of the samples destroyed and the conditions of destruction should be provided to WHO Headquarters. A WHO statement confirming destruction should be issued when it has been confirmed that the samples autoclaved under the prescribed conditions match the known inventory.

The Commission for certification of destruction of variola virus at each Centre should be composed of:

(a) two representatives of WHO: a member of the *Ad Hoc* Committee on Orthopoxvirus Infections with experience in the destruction of variola viruses and a member of the WHO Biosafety Group;

(b) the person responsible for destruction of variola virus strains and specimens in the WHO Collaborating Centre for Smallpox and other Poxvirus Infections.

The members of the Commission should verify the identity of all samples provided for destruction and should confirm that all the prescribed procedures have been adhered to.

The text of a Certificate verifying destruction of variola virus should be as follows:

*We, the undersigned, have conducted a complete inventory of all variola virus strains, all clinical specimens and laboratory materials that might contain variola viruses (including whitepox viruses) and viral genomic DNA at the * WHO Collaborating Centre for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA * Moscow Institute for Viral Preparations, Moscow, Russian Federation (* as appropriate). All strains, clinical specimens, genomic DNA and laboratory materials so identified have been destroyed under our supervision by the method of destruction recommended by WHO.*

Signatories:

The Director of the Centers for Disease Control and Prevention and the Director of the Moscow Institute for Viral Preparations should attach to the corresponding certificates a statement confirming that no materials containing variola viruses or viral genomic DNA remain in any laboratory of their Institute.

The certificate signed by the WHO Commission members validating that the variola virus stocks have been destroyed and the confirming statement of the Director of the Institute should be transmitted to the World Health Organization by the most senior health official of the Russian Federation and the USA. They should be accompanied by a statement, signed by that health official, affirming that there are in the country no known remaining strains of variola virus, clinical specimens or uncloned viral genomic DNA.

Cloned DNA fragments of variola virus genome

The *Ad Hoc* Committee revised the recommendation of the last meeting about destruction of all recombinant materials that contain variola virus DNA sequences. Taking into account that

cloned DNA fragments of variola virus genome are themselves not infectious and provide a useful resource and tool for analysing variola virus genes and protein structure and function, the majority (9/10) of the members of the *Ad Hoc* Committee recommended that such cloned material be kept. The Committee also recommended the establishment of two international repositories for the storage, maintenance, distribution and monitoring of the cloned DNA fragments of variola virus genome - one at the WHO Collaborating Centre for Smallpox and other Poxvirus Infections, Centers for Disease Control and Prevention, Atlanta, Georgia, USA and the second at the Russian State Research Centre of Virology and Biotechnology, (RSRCVB), Koltsovo, Novosibirsk Region, Russian Federation. These repositories would maintain duplicate material.

The terms of reference of the international repositories for storage, maintenance and distribution of cloned DNA fragments of the variola virus genome should be as follows:

1. Storing all clones containing variola virus specific DNA sequences under appropriate conditions to ensure their preservation (as ethanol precipitated DNA at -20° C).

2. Preparing archival quality material and validating the identity of each sample by nucleotide sequence determination.

3. Maintaining a register of the clones, including full descriptive and available sequence information.

4. Acting as a resource centre for information exchange on variola virus - specific clones.

5. Distributing clones to appropriate research laboratories that request them, if the following conditions are met:

   (i) The request has been submitted to the international repository through WHO/ Headquarters.

   (ii) The clones will not be further distributed to third parties.

   (iii) An annual report on the status of variola-specific DNA clones will be made to the international repository.

6. Permitting studies on clones containing variola virus DNA sequences on condition that:

   (i) The clones will not be used for insertion of variola DNA into vaccinia virus or related poxviruses.

   (ii) No laboratory (except the International repositories) shall be permitted to hold clones representing more than 20% of the variola virus genome at any one time.

The Committee recommended that special measures be adopted for the handling of cloned DNA fragments of variola virus genome, as follows:

All work with cloned DNA fragments containing variola virus genetic information (greater than 100 nucleotides long) should only be done following a written risk assessment and in accordance with locally agreed national guidelines.

The insertion of variola virus genome sequences into genetic material of other orthopoxviruses is prohibited. No other orthopoxviruses should be handled in the laboratory rooms where material containing cloned variola virus genome sequences are studied.

All by-products containing cloned DNA fragments of variola virus genomes and other related materials must be disposed of by autoclaving at 120°C for 30 minutes.

Following these deliberations the *Ad Hoc* Committee reviewed the programme of work currently in progress and made the following recommendations:

- haemagglutinin gene sequences from further specimens of scab material and from at least one Botswana strain of variola virus (including possible DNA mapping) should be obtained before 30 June 1995 (CDC);
- the cloning and sequence analysis of the Garcia strain of variola virus (apart from the terminal hairpin loops) should be completed (CDC and RSRCVB);
- archival quality cloned material should be prepared for distribution by the international repositories (CDC and RSRCVB);
- protocols for conducting the validated diagnostic test for variola virus identification should be published and made widely available (CDC).

Smallpox vaccine

The *Ad Hoc* Committee revised the recommendations of the fourth meeting of the Committee on Orthopoxvirus Infections held in 1986 regarding the elimination of the emergency stock of smallpox vaccine and retention of the seed virus stock for preparation of the vaccine. The Committee recommended that the 500,000 doses of smallpox vaccine, presently in storage at -20°C at WHO, be retained indefinitely and be retested for potency every five years. While there is no cause to believe that humans will ever again be infected with variola virus, this vaccine reserve will serve to provide protection should the unforseen occur, such as the occurrence of a mutant, more readily transmissible monkeypox virus. The risk appears negligible but the costs of retaining such stocks are likewise negligible.

The *Ad Hoc* Committee recommended that the Lister Elstree strain of vaccinia virus should continue to be kept as the smallpox vaccine seed virus stock. It was also recommended that the seed virus, in quantities of about 3 litres should be kept at -20°C by the WHO Collaborating Centre for Smallpox Vaccine at the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. The seed virus should be tested every 5 years and, if the titre decreases, additional passages of the seed virus should be done at that Institute. The Collaborating Centre should provide seed virus and national reference smallpox vaccine when required.

It was noted that smallpox vaccine can be prepared using tissue cultures of rabbit kidney cells. The detailed protocol for this process had been developed by the National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands. The *Ad Hoc* Committee recommended that WHO should obtain this protocol for subsequent distribution to production units for use in case of emergency.

Summary of Recommendations

1. The members of the *Ad Hoc* Committee unanimously recommended that all stocks of variola virus, including all whitepox viruses, clinical specimens, and other materials containing infectious variola virus or viral genomic DNA, held in the WHO Collaborating Centres for Smallpox and other Poxvirus Infections at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and the Moscow Institute for Viral Preparations, Moscow, Russian

Federation, should be destroyed by autoclaving followed by incineration. Subject to agreement by the World Health Assembly, the majority view (8/10 members) was that destruction should occur in both Centres on Friday 30 June 1995. A minority view (2/10 members) was that destruction of variola virus stocks should be delayed for a maximum of five years for scientific studies.

2. Variola virus genomic DNA should be destroyed in all laboratories holding such material when the variola viruses are destroyed in the Collaborating Centres for Smallpox and other Poxvirus Infections.

3. The certificate signed by WHO Commission members validating that the variola virus stocks have been destroyed and the confirming statement of the Director of the Institute should be transmitted to the World Health Organization by the most senior health official of the Russian Federation and the USA. The certificate and the statement should be accompanied by an attestation, signed by that health official, affirming that there are in the country no known remaining strains of variola virus, clinical specimens or viral genomic DNA.

4. The majority of the *Ad Hoc* Committee (9/10) recommended that stocks of cloned fragments of variola virus DNA should be retained. Two designated WHO International Repositories for the storage, maintenance, distribution and monitoring of the cloned DNA fragments of variola virus genome at the Centers for Disease Control and Prevention, Atlanta, Georgia, USA and at the Russian State Research Centre of Virology and Biotechnology, Koltsovo, Novosibirsk Region, Russian Federation should be established. Each repository should hold a duplicate set of archived material. Requests for access to these stocks should be made through WHO Headquarters and the stocks should only be released under specified conditions which guarantee that they will be handled under nationally agreed guidelines. Work should not be done in laboratory rooms that are concurrently handling orthopoxviruses. Insertion of variola virus genome sequences into genetic material of other orthopoxviruses is prohibited.

5. The Centers for Disease Control and Prevention, Atlanta, Georgia, USA should publish their detailed protocols for undertaking validated tests for differentiating variola major and Alastrim strains from each other and from other orthopoxviruses.

6. A prioritised programme of work should be undertaken before 30 June 1995 to obtain certain additional DNA sequences, as indicated above, and to complete the cloning of DNA for the Garcia-1966 strain of variola virus. Sets of clones containing variola DNA sequences should be prepared and fully archived for distribution to the designated repositories. The Committee hopes that DNA sequences for other species of orthopoxviruses will be obtained by laboratories interested in poxvirus studies.

7. A stock of smallpox vaccine (500,000 doses) should continue to be kept by WHO Headquarters in case of emergency. This should be stored at -20°C and retitrated every 5 years.

8. Smallpox vaccine seed virus (vaccinia virus strain Lister Elstree) should continue to be kept at -20°C by the WHO Collaborating Centre for Smallpox Vaccine at the National Institute for Public Health and Environmental Protection, Bilthoven, The Netherlands. Seed virus should be tested every 5 years and, if necessary, additional passages of seed virus should be done at this Institute.

9. These recommendations should be submitted for consideration at the 95th Executive Board meeting (January 1995) and at the 48th World Health Assembly (May 1995).

List of participants

Members of the *Ad Hoc* Committee

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[1] Unable to attend