WORLD HEALTH ORGANIZATION

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Smallpox eradication: destruction of variola virus stocks

Report by the Secretariat

1. The WHO Advisory Committee on Variola Virus Research was established pursuant to resolution WHA52.10, which authorized the temporary retention of existing stocks of variola virus at the two current locations[1] up to but not later than, 2002 and subject to annual review by the Health Assembly. The resolution also requested the Director-General to appoint a group of experts to determine what research, if any, must be carried out in order to reach consensus on the timing of destruction of virus stocks.

2. In resolution WHA55.15 the Health Assembly authorized the further temporary retention of the existing stocks of live virus on the understanding that all approved research would remain outcome-oriented and time-limited, and its accomplishments and outcomes would be periodically reviewed. The resolution requested the Director-General to continue the work of the Advisory Committee and to report annually to the Health Assembly, through the Executive Board, on progress in the research programme and relevant issues.

3. This document provides a report of the Committee's fifth meeting (Geneva, 4 and 5 November 2003), at which progress was reviewed on research that had been conducted since its last meeting with live variola virus.[2]

FIFTH MEETING OF THE WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH

4. Overall, the Committee judged that significant progress had been made during the past year, particularly in further characterization of the isolates held in the two collections, the development of diagnostic tests for smallpox, and in understanding the genomic diversity of variola virus. Although the primate model of human smallpox had been refined, further improvement was needed before it could be used in assessing the efficacy of new antiviral drugs and safer vaccines. The Committee noted that several specific gaps in knowledge had been filled, but concluded that additional research

[1] Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, United States of America, and the Russian State Centre for Research on Virology and Biotechnology (VECTOR), Koltsovo, Novosibirsk Region, Russian Federation.

[2] Reports of the Committee's meetings and abstracts summarizing recent research are accessible at the following web site: http://www.who.int/csr/disease/smallpox/research/en/.

was needed before consensus could be reached on a date for destruction of the remaining stocks of virus.

5. The Committee made the following **recommendations**.

(a) Inventories of viral material in the two repositories should be updated according to the standardized format established in collaboration with WHO; the Committee should review progress in implementing this recommendation at its next meeting.

(b) Viral isolates whose retention has no scientific justification (notably chimeric viruses held in the CDC collection in the United States of America and isolates shown to be non-viable) should be destroyed and that action recorded in the inventory; this recommendation does not preclude the preparation of DNA samples for subsequent archiving if the material is considered potentially useful for future studies. WHO should be responsible for implementation of this recommendation, in collaboration with authorities at the two repositories.

(c) Non-variola virus orthopoxviruses retained in the CDC collection should not be included in the inventory and should be either held separately, in the biosafety level 4 facility, or destroyed.

(d) Details on methodologies for smallpox diagnostic tests, being developed in authorized research programmes, should be made available on request to all Member States.

(e) In order to validate diagnostic tests, additional research is needed on procedures to extract variola virus DNA from authentic clinical samples; material from infected non-human primates or historical samples should be used for this purpose.

(f) The primate model of human smallpox needs further refinement in order to facilitate the development of antiviral drugs and vaccines.

(g) Research leading to the development of new antiviral drugs and safer vaccines should be given high priority.

(h) WHO should prepare and make widely available guidelines for assessing the quality, safety and efficacy of new generation smallpox vaccines.

(i) Several unresolved safety issues pertaining to proposed research using live variola virus or variola virus genes need further expert consideration, through the mechanisms of the WHO Biosafety Advisory Group and the Ad Hoc Committee on Orthopoxvirus Infections, before approval of such research could be recommended.

6. **Viral strains in the two repositories**. Of the 120 strains of variola virus in the collection held at VECTOR in the Russian Federation, 55 isolates had been tested for viability and 32 could be propagated. Analysis of DNA from 21 isolates demonstrated their clustering into three large groups (African, Asian and Alastrim strains). Of the 451 isolates in the CDC collection, 49 had been tested for viability and 45 could be propagated. DNA analysis of the 45 isolates likewise demonstrated their clustering into large groups.

7. The Committee repeated the recommendation, made at its fourth meeting,[1] that chimeric viruses (prepared by recombination of variola viruses with other orthopoxviruses) held in the CDC collection should be destroyed and their destruction noted in the inventory. This recommendation would not preclude the preparation of genomic DNA samples for subsequent archiving. The Committee also recommended that isolates shown to be non-viable be destroyed, with a note thereof in the inventories. This recommendation would not preclude the isolation of DNA, if that were considered useful for future studies.

8. WHO had devised a standard electronic format for documenting and updating the inventories, including information on origin, biological properties, passage history and other characteristics of the isolates, and records of material used for work in progress, and would make it available shortly. Standardized inventories would facilitate inspection and audit of the repositories, regularly conducted by WHO.

9. **Diagnostic tests and methods of detection**. CDC scientists have successfully developed and compared two real-time polymerase chain reaction methods for the generic detection of orthopoxvirus DNA and specific detection of variola virus DNA. These methods were deployed successfully during a recent outbreak of monkeypox in the United States of America. CDC staff has also established coded DNA panels, including DNA from variola virus and other orthopoxviruses, for the evaluation of diagnostic tests that rely on the identification of viral DNA.

10. Work on the detection of variola virus conducted by scientists in the United Kingdom of Great Britain and Northern Ireland had also resulted in a technique that can distinguish DNA from variola virus from that of other orthopoxviruses.

11. Work on variola virus-specific monoclonal antibodies has not yet resulted in useful assays; new strategies, including the combination of several monoclonal antibodies, were being explored.

12. The Committee agreed that progress in the development of diagnostic tests and methods of detecting variola virus was, overall, good. Additional work, however, should be conducted to evaluate diagnostic tests with material from authentic smallpox lesions from infected non-human primates or historical samples. The effectiveness of the procedures to extract DNA from such material also needed to be confirmed. Scientists working on diagnostic tests for smallpox should be given access to non-infectious variola virus material for use in the validation of tests.

13. **Sequence analysis**. Work progresses on sequence analyses of DNA from various variola virus strains. Russian researchers have sequenced five genes from a wide range of orthopoxviruses, including variola virus. Dendrograms constructed from the results illustrate the close relationship between different isolates of the same orthopoxvirus species with the exception of cowpox virus isolates, which appear more heterogeneous when compared to each other. The Committee noted, however, that difficulties might arise in establishing phylogenetic relationships on the basis of only a few genes. The sequencing of 26 variola virus genomes has been completed by researchers in the United States of America. A rapid sequencing method that can facilitate confirmation of previously known genomes is under development. In addition, this research has highlighted unique features of the variola virus genomes. New computer software is being developed for the analysis and visualization of conserved and variable variola virus sequences.

[1] See document EB111/5.

14. **Animal models**. Work on a primate model of human smallpox continues. Experimentally infected macaques develop an invariably lethal haemorrhagic disease similar to haemorrhagic smallpox, and recent research has shed more light on the pathology of infection. The Committee agreed that additional work was needed on routes of infection and on increasing the virulence of variola virus for monkeys by sequential passage. A reliable animal model of human smallpox would be needed to fulfil the licensing requirements for new antiviral drugs and vaccines.

15. **Antiviral drug development**. Antiviral treatment with cidofovir has been shown to protect infected monkeys from death when given 24 hours before infection. When monkeys were infected with lower doses of variola virus, more representative of the pathogenesis of authentic smallpox, cidofovir conferred protection when given two days after infection in experimental conditions compared with control animals which displayed large lesions with some deaths. These results were also validated with the monkeypox virus challenge model. Tests of cidofovir conjugated with lipids, designed as new oral formulations, have shown increased potency *in vitro* and in mice challenged with normally lethal doses of cowpox virus.

16. The considerable efforts being made to identify new compounds continue. Drug discovery programmes in the Russian Federation and the United States of America have used computer-assisted and *in vitro* assays to screen a large number of compounds and have identified several new lead compounds for further testing in animal models.

17. **Vaccine development**. Work in the United States of America continued to assess modified virus Ankara strain of vaccinia virus as a candidate live attenuated vaccine. Scientists obtained promising results using the monkeypox virus challenge model to test the ability of an attenuated modified virus Ankara strain for protecting monkeys compared with a standard smallpox vaccine. A second line of research on candidate subunit vaccines with different gene products is being pursued in a number of countries. A third line of research, with an additional attenuated vaccinia virus strain, is being conducted in China. This work, along with that in the United Kingdom, drew attention to the need for guidelines to assess the quality, safety and efficacy of new generation smallpox vaccines for Member States engaged in this important research.

18. **Recommendations from the technical subcommittee**. As recommended by the Committee at its fourth meeting, a technical panel, comprising relevant safety experts, had been convened electronically to consider existing guidelines on safe research practices. Four issues were considered: the simultaneous handling of variola virus and other orthopoxviruses; the generation of recombinant variola viruses expressing "reporter" genes, which encode easily-assayed proteins; the insertion of variola virus genes or "variola-like" gene sequences into other orthopoxviruses; and the distribution between laboratories of fragments of variola virus DNA.

19. In evaluating the draft recommendations of the subcommittee, the Committee considered both safety issues and the scientific value of proposed experiments in meeting the urgent need for new antiviral drugs and safer vaccines.

20. The Committee in general agreed with two of the subcommittee's draft recommendations. The simultaneous handling of variola virus and other orthopoxviruses within the same biosafety level 4 laboratory was not considered to represent a significant problem, provided that all infected materials were properly decontaminated or disposed of at the end of the experiment. It was further considered that fragments of variola virus DNA not exceeding 500 base pairs in length could be freely distributed between laboratories for use as positive controls in diagnostic kits. The Committee recommended against the *in vitro* synthesis of double-stranded DNA fragments larger than 500 base pairs.

21. Some members of the Committee expressed serious reservations about recommended permission to conduct, in specified conditions, experiments designed to generate recombinant variola viruses and to express variola virus genes in other orthopoxviruses. The Committee therefore decided to seek further expert advice on all four issues through the mechanisms of the WHO Biosafety Advisory Group and the Ad Hoc Committee on Orthopoxvirus Infections before reaching conclusions about the safety of such experiments.

22. **General conclusions**. The Committee was encouraged by the rapid progress being made towards the goal of developing new antiviral drugs and safer vaccines. However, substantial additional research was still needed. Laboratories conducting approved research should receive adequate support in order to reach the remaining research objectives in the shortest possible time.

ACTION BY THE EXECUTIVE BOARD

23. The Executive Board is invited to note the report, which will be transmitted to the Health Asembly.

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