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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 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. The resolution requested the Director-General to continue the work of the Advisory Committee, with periodic review of research accomplishments and outcomes, and to report annually on the progress in the research programme and relevant issues to the Health Assembly, through the Executive Board.

3. This document provides a report of the Committee's fourth meeting (Geneva, 20 and 21 November 2002), at which progress was reviewed on research using live *Variola virus* that had been conducted since its last meeting.[2]

FOURTH MEETING OF THE WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH

4. Overall, the committee judged that progress during the past year in approved research using live *Variola virus* had been considerable, but that further research was still needed before consensus could be reached on a date for the destruction of the remaining stocks of virus. This additional research should continue to be carefully monitored and reviewed under the auspices of WHO, and steps should continue to be taken to ensure that all approved research is outcome-focused, time-limited and periodically reviewed.

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

[2] See document A55/21 for a report to the Health Assembly on the third meeting.

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

(a) systems for inventorying clinical material and *Variola virus* isolates and DNA at the two repositories should be standardized and improved, and information generated by these systems should be shared between the two groups and reported to WHO at least once each year;

(b) records on material used for work in progress should be available for inspection and audit. The volumes of live virus suspensions generated as a result of this work should be kept to the minimum needed for successful completion of approved research;

(c) where possible, information on the origin, biological properties, passage history and other characteristics of the material held in each repository should be included as part of the inventory. WHO agreed to seek information from archival records to facilitate this work;

(d) viral isolates whose retention has no scientific justification (particularly chimeric viruses held in the American collection) should be destroyed after the original donating countries have been informed of this intention;

(e) DNA sequence analyses should be performed on additional authenticated clinical material without prior cloning;

(f) further work should be done to refine the primate model of human smallpox in order to facilitate its better use in assessing candidate vaccines and antiviral agents;

(g) a technical panel, comprising relevant safety experts, should be urgently convened to consider revision of existing guidelines on the simultaneous handling of variola viruses and other orthopoxviruses;

(h) the technical panel should be further charged to draw up guidelines on the distribution of fragments of cloned viral DNA, taking into account advances made in technologies for nucleotide synthesis, and on the modification of other orthopoxvirus genomes to resemble more closely *Variola virus* gene sequences;

(i) laboratories conducting approved research should produce written annual progress reports for eventual dissemination to the wider scientific community. Wherever possible, this research should be published in the open peer-reviewed literature.

6. **Viral strains in the two repositories**. Of the 120 strains of *Variola virus* in the Russian collection, 55 isolates had been selected for further investigation, involving viability studies, biological characterization and genome analysis. To date, 39 isolates have been studied, of which 29 were found to be viable. DNA had been isolated from the non-viable isolates. Analysis of the 451 isolates in the American collection revealed that some of these are *Monkeypox virus*, *Camelpox virus* or chimeric viruses prepared by recombination of variola viruses with other orthopoxviruses. The geographical origin and year of isolation were known for 229 isolates; 50 of these were selected for further study on the basis of year and region of isolation, passage history and clinical information, and 46 were shown to be viable.

7. The Committee agreed on the need for better auditing of viral isolates in both collections, and recommended that systems for preparing inventories should be improved and standardized. Information from such audits should be shared between the two repositories and submitted in both

printed and electronic formats to WHO at least once each year. The Committee further recommended that records of samples containing virus that were being used for work in progress should be available for inspection and audit.

8. The Committee recommended that, where feasible, the inventory should include information on the origin, biological properties, passage history and other characteristics of the material held in each repository, and WHO agreed to assist by searching archival records for information on the derivation of some isolates. The Committee further recommended that isolates whose retention could not be scientifically justified, in particular chimeric viruses held in the American collection, should be destroyed and that the original donating countries should be informed of this intention.

9. Some concern was expressed about the volumes of suspensions of live virus being generated as a result of approved research. The Committee recommended that these volumes be kept to the minimum needed for successful completion of the studies agreed within the framework of the Committee's recommendations.

10. The Committee recalled the recommendation by the Global Commission for the Certification of Smallpox Eradication in 1979 that "[r]esearch on poxviruses other than variola or whitepox viruses should not be performed under circumstances where there is any possibility of cross-contamination with these two agents".[1] As demands for handling *Variola virus* in specific experiments, for example, concurrent testing of multiple orthopoxviruses in assays of antiviral activity, have subsequently changed, the Committee recommended that a technical panel, including safety experts, be convened to assess the safety issues and issue updated guidelines.

11. **Sequence analysis of *Variola virus* DNA**. Work on sequence analyses of DNA from various *Variola virus* strains had progressed and 10 full-length genome sequences were now available. Researchers at the American repository plan to sequence at least three more complete genomes. Work at the Russian repository had focused on obtaining data on a limited number of variable genes from a large number of isolates. This work has enabled phylogenetic relationships between specific genes contained within the sequenced genomes to be analysed by various criteria. The results clearly showed that variation in nucleotide could not be used as a marker of pathogenicity among isolates derived from outbreaks with different fatality rates. The Committee further noted the potential use of nucleotide sequence analyses in forensic testing for identifying strains and their origins should *Variola virus* be deliberately released.

12. Results to date showed no variation in nucleotide sequence between material from primary scab isolates and that from the same samples after two passages. The Committee recommended that additional material derived from authenticated clinical material and without prior cloning be considered for sequence analyses.

13. A validated and certified library of DNA fragments from two complete genomes of cloned *Variola virus* had been completed, and similar work using five additional virus strains was planned. The Committee acknowledged that this work would provide material for the long-term preservation of *Variola virus* genomes but questioned whether the procedures would allow faithful representation of variability within a strain.

[1] *The global eradication of smallpox: final report of the Global Commission for the Certification of Smallpox Eradication, Geneva, December 1979*. Geneva, World Health Organization, 1980. Recommendation (15).

14. **Polymerase chain reaction-based analysis of orthopoxvirus DNA.** The Russian team has initiated analyses by polymerase chain reaction (PCR) and extended restriction fragment length polymorphism PCR on 24 cell-culture isolates and eight scab samples with a view to using these procedures to characterize different strains, isolates or gene-dependent microheterogeneities. This work has revealed surprising variations between isolates from the same outbreak. The American group has conducted similar work using capillary electrophoresis of restriction fragment length polymorphisms as an alternative to gel electrophoresis techniques.

15. **PCR-based diagnostic assays**. Variations of the PCR technique have been developed specifically to detect *Variola virus* in samples containing minute amounts of DNA. One extensively evaluated procedure can differentiate *Variola virus* from other orthopoxviruses that infect human beings and other infectious agents that cause rashes resembling those in smallpox. The procedure is considered sufficiently sensitive to detect as few as 50 copies of the *Variola virus* genome in specimens obtained during the prodromal phase of infection.

16. The Committee encouraged the sharing of details of the new PCR-based detection methods with the international community as soon as possible, but noted that their validation outside the two facilities could present problems owing to the unavailability of short DNA fragments from cloned *Variola virus*, as a result of observance of current guidelines on the supply of this material. The Committee recommended that the proposed technical panel (see paragraph 5(g) and (h)) should be charged with drafting appropriate guidelines, taking into account advances that have been made in the technologies associated with nucleotide synthesis.

17. **Serological assays.** Several difficulties, including problems in producing *Variola virus*-specific monoclonal antibodies, have impeded the development of sensitive methods for the detection of *Variola virus* antigens. The Committee considered it unlikely that the sensitivity of serological assays would approach that of PCR methods, and concluded that serological assays would contribute little to the early diagnosis of *Variola virus* infection.

18. **Animal models.** Experiments have shown the ability of strains of *Variola virus*, when administered at high doses, to cause lethal infection in cynomolgus monkeys. Use of lower doses resulted in a slightly delayed onset of symptoms, thereby offering a possibly more useful model for testing candidate vaccines and antiviral agents. However, the high doses needed to induce disease in these animals result in a bypassing of the prodromal stage and direct onset of the viraemic stage, with infected animals invariably dying of a disease that resembles haemorrhagic smallpox. For these reasons, the Committee concluded that the model was not ideal and that further work was needed to improve its utility in the assessment of candidate vaccines and drugs.

19. **Antiviral drug development.** The Committee noted the considerable efforts being made in both public institutions and private companies to identify new compounds active against *Variola virus*. The Russian facility had screened 2432 compounds for inhibitory activity, and had identified six new compounds for further testing in animal models. Similar drug discovery research was under way in both the United Kingdom of Great Britain and Northern Ireland (with testing of promising leads to be conducted in the American facility) and the United States of America, where a total of 40 lead compounds with promising properties had been identified.

20. In the current primate model, cidofovir protected monkeys when given 24 hours before infection but failed to protect when administered 12 hours after infection. The severity of the challenge needed to induce disease might explain these results. Data from experiments using *Cowpox virus* and *Vaccinia virus* in mice indicate that the effectiveness of cidofovir highly depends on the dose of challenge virus

and that post-infection protection could be conferred only when low doses were used to initiate infection.

21. The Committee noted that cidofovir was not an ideal drug because of its nephrotoxicity and the need to be administered parenterally, and therefore welcomed studies of three pro-drug derivatives of cidofovir that can be orally administered. Results against *Cowpox virus* infection in mice demonstrated lower toxicity and higher plasma concentrations of these drugs than with cidofovir. Testing in the primate model of smallpox, however, will not take place for another six months.

22. **Vaccine development.** The Committee discussed four vaccine development programmes. Work in the United Kingdom aimed at developing second generation subunit vaccines was still at the stage of identifying suitable protective antigens. Work had been conducted in both the United Kingdom and United States to assess modified *Vaccinia virus* Ankara as a candidate live attenuated vaccine. Research in the United States to develop DNA vaccines using sequences coding for four antigens was at an early stage.

23. **General discussion.** Concerning passive immunotherapy, the Committee noted the absence of strong evidence to support the beneficial use of vaccinia immune globulin. However, some work was being done to develop transgenic animals capable of producing humanized vaccinia immune globulin.

24. In discussion of safety issues the Committee considered the possible distribution of short fragments of *Variola virus* DNA for use in validating PCR diagnostic procedures, site-directed mutagenesis of *Vaccinia virus* DNA to make it more similar to that of *Variola virus*, the insertion of foreign genes into *Variola virus*, and the simultaneous use of *Variola virus* and other orthopoxviruses. It noted that, while existing guidelines preclude such work, considerable technological advances since the guidelines were issued may have altered their relevance. However, the Committee felt that it lacked the specific expertise to address these important matters and recommended the urgent convening of a technical panel, with appropriate expertise, to review the issues and offer guidance to WHO.

25. In general, the Committee was encouraged by the considerable progress being made in research using *Variola virus* and recommended that this work should continue. Laboratories conducting approved research were requested to produce written annual progress reports that can eventually be disseminated to the wider scientific community. The Committee further recommended that all research be published in the open peer-reviewed literature.

ACTION BY THE EXECUTIVE BOARD

26. The Executive Board is invited to note the report.

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