



WORLD HEALTH ORGANIZATION

FIFTY-FOURTH WORLD HEALTH ASSEMBLY
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Smallpox eradication: temporary retention of variola virus stocks

Report by the Secretariat

INTRODUCTION

1. In May 1999 the Health Assembly by resolution WHA52.10 authorized temporary retention up to, but not later than, 2002 of the existing stocks of variola virus at the current locations,¹ for the purpose of further international research. The Assembly requested the Director-General to appoint a new group of experts to establish what research, if any, must be carried out in order to obtain consensus on the timing for the destruction of the existing variola virus stocks.

2. In accordance with this resolution, a new group of experts was appointed; this WHO Advisory Committee on Variola Virus Research is composed of 16 members from all WHO regions. At its first meeting (Geneva, 6-9 December 1999), attended also by 10 advisers from fundamental applied research and regulatory agencies, the Committee agreed that a scientific subcommittee be established for the purpose of overseeing future research on variola virus, with members of this subcommittee to be drawn from the Advisory Committee on Variola Virus Research. A detailed report was submitted to the Executive Board at its 106th session in May 2000.² The committee's recommendation was that further limited research using variola virus stocks could be justified, but under no circumstances should this continue beyond the end of 2002.

MEETING OF THE WHO ADVISORY COMMITTEE ON VARIOLA VIRUS RESEARCH (GENEVA, 15-16 FEBRUARY 2001)

3. The main aims of the meeting were:

- to review progress on the agreed programmes of research on variola viruses;
- to determine whether this progress was sufficient to accommodate the planned destruction date in 2002;
- to identify any significant gaps in the present research programme; and

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² Document EB106/3.

- to advise, as appropriate, on other possible directions of research.

4. The Committee concluded that considerable progress had been made in several areas of research on variola virus: state of strain collections and viability of viral isolates, phylogenetic analysis, detection and differentiation of orthopoxvirus DNA, nucleotide sequence analysis of variola virus DNA, serological detection of variola virus, antiviral agents and animal models of smallpox.

5. **State of strain collections and viability of viral isolates.** The Centers for Disease Control and Prevention hold 451 viral isolates derived from several different national collections. Most are isolates of variola virus and a database has been created to link them with available diagnostic and epidemiological data. Of 49 strains, selected on the basis of geography, year of isolation and low passage history, that have been further analysed, 45 were shown to be viable. These isolates came from Asia (21), Africa (16), Europe (5), South America (2) and North America (1). Many showed uniform plaque morphology and grew to high titre in tissue culture. Of the 45 viable isolates, 37 came from *in vitro* cultured material and the remainder from scab (non-passaged) samples.

6. The samples currently held at the Russian Centre, VECTOR, began to be collected in Moscow in the mid-1950s. The collection was augmented with isolates obtained by the then WHO collaborating centre on smallpox in Moscow during the diagnostic studies that supported the smallpox eradication programme. The present collection includes primary material (scabs), frozen liquid cultures and lyophilized samples. Not all samples have been tested for viability; five primary scab isolates, four of nine frozen cultures and all six lyophilized strains have demonstrable viability. Difficulties have been experienced in obtaining support for further work but funding is now expected.

7. Cooperation between staff at the two collaborating centres has begun, in order to ensure that any future work on viral characterization, including the transfer of reagents, is adequately coordinated.

8. The Committee concluded that additional work may be needed to assess the viability of the stocks held in VECTOR, and that further molecular characterization of additional strains may be valuable in helping to identify strains from which further DNA sequences could be determined.

9. **Phylogenetic analysis with DNA amplification technologies.** Several techniques based on the polymerase chain reaction (PCR) to facilitate the characterization and phylogenetic analysis of variola virus isolates were described. These included restriction fragment length polymorphism of PCR products amplified with a variety of primers, and multiplex PCR analysis. As a general rule, primers complementary to sequences in the central conserved genomic region were used for the comparison of all orthopoxviruses whereas those complementary to sequences located towards the genomic termini were used to provide species- and strain-specific data. The Committee concluded that significant progress had been made in applying PCR technology to the investigation of phylogenetic relationships between the orthopoxviruses, particularly variola viruses.

10. **Detection and differentiation of orthopoxvirus DNA.** Several methods using DNA amplification technologies were described for the detection and subsequent diagnosis of orthopoxvirus infections. A major objective of this work is the real-time identification of smallpox viruses. The basic procedures used are similar to those already described for phylogenetic analysis of different variola virus isolates. The detection and differentiation of orthopoxvirus strains and individual strains of variola virus generally involve the generation of PCR-amplified products from both conserved and variable regions of the genome. Different groups have developed different platforms for the process of detecting amplified DNA products.

11. The Committee noted the enormous progress in this area. Although a major limitation to these procedures was the methods used to obtain the initial DNA samples, some reliable and rapid procedures with commercially available reagents are becoming available. The Committee also noted that the specificity of the procedures completely depended on the sequences of the primers used for amplification. The detection of nucleotide sequences in cowpox virus that were previously considered to be specific to variola virus, emphasized the point that the use of a single locus for PCR amplification is insufficient to provide unambiguous identification. Members of the Committee questioned the need for rapid analytical procedures that were sufficiently sensitive to differentiate between variola subspecies when the clinical management of infected individuals would be the same. It was recognized that the ability to detect the presence or absence of any orthopoxvirus in real time would be needed in emergency public health situations.

12. **Nucleotide sequence analysis of variola virus DNA.** The Committee was informed that the nucleotide sequences of three complete variola virus genomes were now available. Substantial parts of the genomes of three other variola virus strains – Congo 70, Somalia 77 and India 7124 – had also been determined. The full sequence of camelpox virus, the closest known relative of variola virus, was described. Many sequence data are also available for individual genes of various other orthopoxviruses.

13. The data obtained so far have confirmed the suspected evolutionary relationships between orthopoxviruses and have facilitated the further classification of different variola isolates into subspecies. At least six complete variola virus genome sequences should be available by the end of 2002. It may also be possible to obtain sequence information from scab material that has not been passaged *in vitro*. The Committee concluded that very good progress had been made in analysing the sequences of smallpox virus genomes.

14. **Serological detection of variola virus.** Work using monoclonal and polyclonal antibodies against vaccinia virus proteins in enzyme-linked immunosorbent assays to detect orthopoxviruses was described. With these reagents, such tests can detect various orthopoxviruses, including camelpox, cowpox, monkeypox, vaccinia and variola viruses. The relative sensitivity for detecting the different viruses varied, and strain differentiation was not possible with this method. Work is in progress to determine whether tests using these reagents can differentiate live variola major and minor strains.

15. The Committee concluded that much useful work was being done in this area and that it would be important for any generated monoclonal antibodies to be thoroughly characterized with respect to the variola proteins against which they react.

16. **Antiviral agents.** Cidofovir inhibits a wide variety of DNA viruses including orthopoxviruses. Its mechanism of action is through selective inhibition of the viral DNA polymerase. *In vitro* tests for antiviral activity show that cidofovir inhibits both vaccinia and 35 different isolates of variola virus. Each isolate had similar sensitivity to the drug and induction of resistance mutations does not appear to be a problem. Other orthopoxviruses could therefore be developed as surrogate models for antiviral drug testing after variola virus stocks have been destroyed. The Committee noted that considerable progress had been made.

17. **Animal models of smallpox.** Most work has been done on the aerosol infection of cynomolgus monkeys with the Yamada and Lee strains of variola virus. Clinical signs appear in infected animals by six days after infection. Clinical disease is apparent and the animals seroconvert but there is no mortality. The Committee concluded that this model was not suitable for assessing the efficacy of new vaccines or drugs. Further work is expected to be done with different strains of variola virus and

different primate species, including a possible collaborative project with scientists at VECTOR using baboons as the animal model.

18. The Committee concluded that progress was satisfactory but that the rate was slow. It noted the proposed work on the infection of baboons but indicated that additional work may be required to identify and characterize further surrogate models in comparison to those of variola virus infection so that a validated system for drug and vaccine assessments can be established.

19. Another meeting of the Committee is planned to be held at the end of 2001 and its recommendations will be submitted to the Executive Board at its 109th session in January 2002.

ACTION BY THE HEALTH ASSEMBLY

20. The Health Assembly is invited to note the report.

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