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WHO Advisory Committee on Variola Virus Research

Report of the Seventh Meeting

Geneva, Switzerland
10 –11 November 2005

EPIDEMIC AND PANDEMIC
ALERT AND RESPONSE

World Health Organization

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1. Introduction and report of the Secretariat

1.1 Dr Mike Ryan welcomed participants to the seventh meeting of the Advisory Committee on Variola Virus Research and indicated that the WHA is showing an increased interest in the topics to be discussed. The Director General of WHO had made it clear that biosecurity and biosafety issues relating to work on live variola viruses had assumed greater significance during the past year. He has specifically requested additional advice from the Advisory Committee on the recommendation made at the last meeting regarding the expression of variola virus genes in other orthopoxvirus species.

1.2 Dr Cathy Roth indicated that the purpose of the meeting was to review the progress of current essential research that depended on access to live variola virus and to advise WHO on the continuing need for this research. She reiterated that this research should be output-oriented and not open-ended, open and transparent and necessary for public health. She reminded all researchers to provide the Secretariat with abstracts of the research done so their results could be disseminated to all interested parties.

1.3 Dr Daniel Lavanchy then stated that the proceedings of the Advisory Committee were confidential but that its final report would be a public document and posted on the WHO web site. He also indicated that the African Regional Office had asked for smallpox research progress, and review thereof, to be included as a special item on the Executive Board agenda and that it was possible that it might request the cessation of all further research in this area. He suggested that the Advisory Committee should take this into account during their deliberations.

1.4 Following agreement by members of the Advisory Committee, Professor Geoffrey Smith was appointed Chairman and Dr Peter Greenaway Rapporteur.

2. Update on variola virus strains in the two virus repositories

2.1 Professor Sergei Shchelkunov presented a paper describing the status of the variola virus repository held in the Russian collection at the Russian State Centre for Research on Virology and Biotechnology (VECTOR). Some 120 strains, including 17 clinical isolates, were held and tests had shown that 32 out of 55 of these were viable; it was now planned to assess the viability of the remaining strains in the collection.

2.2 Preparations of variola virus DNA were being conserved in three ways – as full length genomic DNA preparations, as extended PCR amplicons covering the full genome and as DNA fragments cloned in hybrid plasmids. All materials were associated with documentation describing derivation, method of preparation and storage. The most reliable method for the long term conservation of variola virus DNA sequences was considered to be cloning in plasmids.

2.3 In response to a question regarding the need for further work in this area, Professor Shchelkunov stated that the viability of all strains held in the collection needed to be assessed and their DNA conserved.

Action : Professor Shchelkunov to submit a proposal to the Advisory Committee describing essential research needed to support the conservation of genetic material in the variola virus strains held in the Russian collection.

2.4 The repository at the Centers for Disease Control and Prevention (CDC), Atlanta, United States of America contains items donated from five worldwide collections. The repository contains 451 semi-independent items: samples, isolates, or strains. Of the 451 items, 238 were viruses with recorded geographical location of identification and 228 had a known date of isolation. Added to this collection, in 2005, were the remainders of scabs possibly collected from an 1882 outbreak of smallpox in Boone County, Arkansas. Attempts at isolating viable virus were unsuccessful and DNA isolated from the material was inadequate for PCR analysis nor identification of any orthopoxvirus in the material. The material is currently being stored within the liquid nitrogen freezer in the maximum containment laboratory.

2.5 Dr Olsen indicated that a duplicate set of ampoules held in a secure back-up freezer were physically inventoried in April 2005 and transferred to a modern liquid nitrogen freezer. The genomes of 47 strains had been sequenced, and 45 of these had been analysed by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) analysis. The data obtained has been used to construct phylogenetic trees; ePCR/RFLP analysis suggests the strains group into two divergent clades.

2.6 Attempts to correlate biological properties of viable strains (comet formation by extracellular enveloped virus, EEV) with virulence (as reflected by case-fatality rates in the outbreaks from which the strains were derived) were described. No overall correlation was found across all strains within the clades, but association of comet phenotype was observed across clades. This work is relevant to the in vitro assessments of the potential efficacy of vaccines against variola virus. Specific proposals to do further research on this topic were not discussed.

2.7 It was noted that both Collaborating Centres were acting as guardians of the variola virus collections and that all research must be approved in advance by the Advisory Committee. It was also noted that requests for variola virus DNA samples had been made to the CDC by a variety of researchers. However, changes in the legislative requirements within the United States of America now needed to be clarified before distribution of variola virus DNA fragments could continue under the auspices of the 1994 recommendations of the Ad Hoc Orthopoxvirus Advisory Committee. Previous requests had been fulfilled in accordance with the recommendations made previously by the above mentioned Advisory Committee. It was reported that no distribution of variola DNA by VECTOR had occurred.

2.8 The WHO secretariat reminded the Committee that all such requests must be channeled through them so that an accurate record of all groups holding variola virus DNA could be maintained. It was proposed that this information should be made available to the Advisory Committee.

Action: WHO secretariat to provide information on the distribution of variola virus sequences to the next meeting of the Advisory Committee.

2.9 Clarification of the status of the hybrid viruses held in the CDC repository was requested. Representatives from the repository indicated that i) these viruses had not been destroyed, ii) no date had been set for their destruction based on security considerations as determined by United States government officials, and iii) they had been used during the last year for assessment of new diagnostic strategies and two viruses had been sequenced. Members of the Advisory Committee were then informed that although the total number of strains held by each repository had remained constant, the actual number of experimental samples that might contain variola virus, generated through various experiments, had now increased because of work in progress. A full inventory of the "items" in the repository freezers, as well as a full accounting of the working and master seed stocks was provided to the Committee Secretariat by the WHO CC at CDC in Atlanta at the meeting.

Action: Records of the stocks, including working stocks, of all variola virus samples held by each repository and submitted to the WHO secretariat as part of their normal reporting procedures will continue to be made available to the Secretariat of the Advisory Committee at its annual meetings.

3. Update on diagnostic assays

3.1 Dr Inger Damon described recent developments in diagnostic strategies utilizing real-time PCR. These could produce definitive results within 2–4 hours, using robotics to extract samples, after submission of a clinical specimen. Assay specificities are >99%, but the predictive value positive diminishes significantly in a no or low disease prevalence situation. Thus multiple different assays will be required to confidently make the diagnosis of the first case(s) of smallpox should it re-emerge. The robustness of the tests with respect to use for vaccine adverse event monitoring, diagnostic evaluation of pustular rashes and monkeypox testing was being investigated in a number of centres. Data on sensitivity, specificity, and positive and negative predictive values were presented. Relevant information was being gathered prior to a planned submission to the United States Food and Drug Administration (FDA) for review and subsequent approval of one of the assays.