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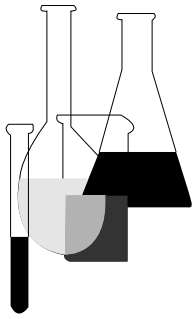
Toxicology Test Guidelines

OPPTS 885.3500
Cell Culture



INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

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OPPTS 885.3500 Cell culture.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP guideline 152A–16.

(b) **Purpose.** Cell culture tests provide information on the ability of viral pest control agents to infect, replicate in, transform or cause toxicity in, mammalian cell lines.

(c) **Definitions** The following definitions apply to this guideline:

Cytopathic effects (CPE) are any host cell damage or injury resulting from infection of the cell by a virus. These effects can be morphological or biochemical, and include but are not limited to, cell growth, attachment, morphology, nucleus size and shape, and cellular processes such as macromolecular synthesis.

Most infectious form (MIF) is the form or preparation of virus that gives optimal infection in the susceptible cell culture or organism. For non-occluded viruses, the MIF is the purified virus or purified tissues obtained from an infected host. For occluded viruses (e.g. baculoviruses, cytoplasmic polyhedrosis viruses, entomopox viruses) the MIF for cell culture or injection into an organism is extracellular virus found in cell culture medium or in infectious hemolymph. The MIF for susceptible insect hosts for infection by natural routes (feeding) is the viral occlusion body.

Transformation is the detectable modification of a host cell phenotype induced by the presence of viral nucleic acid. Transformed cells are considered to be infected by the virus.

Viral infectivity is the ability of viral genes to became established in a host cell genome, or the ability of viral genes to be expressed in a host cell (resulting in the production of virus-encoded nucleic acids).

Viral toxicity is the ability of a virus to inflict injury or damage to a host cell, where infection by, and/or replication of the virus are not necessarily required. Toxicity can also be the ability of non-viral components of a preparation to inflict injury or damage to a host cell.

(d) **Test standards**—(1) **Substance to be tested.** The purest, most infectious (MIF) of the virus should be used. Preparations of insect viruses should be free of insect hemolymph, unless it has been determined that the hemolymph is not toxic to the cell cultures used. The inoculum should be titered by the most sensitive assay available, and in the most permissive host system (cell culture or, if not available, host organism). For testing in the model systems, a minimum of five plaque-forming units (PFU) per cell is required when a plaque assay for the virus is available, or 7× the

LD50 units when a plaque assay for the virus is not available. If fewer units per cell or organism are used, justification/reasoning must be provided for using the lesser amount.

(2) **Cell cultures.** The following cells are recommended—one human line (e.g., WI38), one primary cell type (e.g. foreskin), one primate continuous line (e.g. monkey CV-1 or BSC-1), primary Syrian hamster embryo (SHE) cells (to provide data for the cell transformation assay, described under paragraph (d)(5) of this guideline). One other cell line is to be selected to evaluate potential concerns intrinsic to the specific viral pest control agent, and its intended use. Justification/reasoning must be provided for the selection of this latter cell line.

(3) **Toxicity evaluation.** Efficiency-of-plating tests should be performed with each cell line. For each cell line, approximately 200 cells are plated on each of 30 dishes. At 24 h postplating, 10 dishes per cell line are exposed to approximately 10^6 units of the virus. Appropriate vertebrate cell culture medium is added to 10 different dishes, and, if applicable, 10 dishes per cell line are exposed to invertebrate medium only. At 1 h postexposure, all cultures are fed with the appropriate vertebrate cell culture medium, and are incubated until control cultures have colonies consisting of at least 25 cells/colony. All cultures are fixed and stained, and colonies are enumerated.

(4) **Infectivity evaluation.** (i) Subconfluent cultures (containing approximately 2×10^5 cells on 25 cm^2 dishes) of each cell line are exposed to $>l \times 10^6$ units of the viral pest control agent. Appropriate controls include subconfluent cultures that receive no treatment, and those that are exposed to virus-free inoculation medium. At 7 days and at 14 days postinoculation, cells are to be subcultured.

(ii) Cell cultures are observed daily for 21 days postinoculation for appearance of CPE.

(iii) Cultures are quantitatively assayed for the virus on days 1, 2, 5, 7, 14, and 21 postinoculation.

(A) Cells (entire culture, or $>2 \times 10^5$ cells) are to be assayed in triplicate for viral antigen and nucleic acid.

(B) Cell culture fluid from replicate cultures is to be assayed for infectious virus, using an appropriate susceptible host model system.

(iv) Assays for fate of input virus and for presence of viral proteins and nucleic acid.

(A) The enzyme linked immunosorbent assay (ELISA), dot-immunobinding assay (see paragraph (g)(9) of this guideline, Hawkes et al., 1982, and Ram et al., 1982), protein blot immunoassay (Western trans-

fer) (see paragraph (g)(7) of this guideline and Volkman and Goldsmith, 1982) or similar assays are recommended for protein determination.

(B) The dot hybridization procedure (see paragraphs (g)(1), (g)(2), and (g)(6) of this guideline), Southern hybridization procedure (see paragraphs (g)(7) and (g)(8) of this guideline) or other similar assay are recommended for protein determination.

(v) To serve as controls, for each cell culture, cells inoculated with a preparation of the inactivated test virus should be analyzed as described for the active test virus, and for each series of tests, the inoculum should be tested in the permissive cell line or host organism as a positive control and for direct reference to the data obtained from the vertebrate cell lines.

(5) **Cell transformation assay.** (i) The ability of the viral pest control agent to transform primary Syrian hamster embryo (SHE) cells is to be determined, using an appropriate assay system. If other test systems are used, justification/rationale must be presented to show that the alternate systems are appropriate.

(ii) Transformation of SHE cells with Simian adenovirus 7 (SAV 7) serves as the positive control. SHE cells treated with cell culture medium alone, and SHE cells treated with a killed preparation of the inactivated viral pest control agent serve as appropriate negative controls. The inactivation procedure must be demonstrated as effective in preventing transformation. An efficiency of plating test with SHE cells is considered an appropriate toxicity control.

(iii) If the data show that the test virus modifies the cell phenotype, cells from cultures derived from morphologically transformed colonies are to be inoculated into hamsters, and tumorigenesis in the host animal is to be evaluated.

(iv) This assay may not be required if, in the infectivity evaluations (under paragraph (d)(4) of this guideline), it is conclusively demonstrated that viral nucleic acid is not persistent in any of the test cell lines employed.

(e) **Data reporting and evaluation.** The following information should be provided for each test:

(1) **CPE in the cell monolayers.** (i) The appearance of CPE should be described in such a way that virus-induced cell destruction is differentiated from nonspecific effects.

(ii) Cultures should be inspected with the aid of a microscope to provide evidence of CPE that should be recorded as:

(A) 1+ = suggestive of virus-induced morphologic changes.

(B) 2+ = definitive morphologic changes.

(C) 3+ = more than 50 percent cell degeneration.

(D) 4+ = complete cell destruction.

(iii) The TCID50 value calculated by an appropriate statistical method. For computation of the infectivity results, only cultures showing a >2+ CPE are considered to be infected.

(2) **Toxicity evaluation.** (i) Details of all procedures used, including appropriate reagents and materials, and assay sensitivities and limitations.

(ii) Efficiency of plating data of cultures receiving virus, and cultures receiving vertebrate media (control cultures) and invertebrate media.

(iii) Assessment of mitotic process prevention or of interference with chromosomal replication, as indicated for example, by significant reductions in efficiency of plating.

(3) **Assay of culture fluid.** Details of procedures used, including a discussion of all data that indicate viral replication.

(4) **Data from assays of input viruses.** (i) Details of procedures used for detection of viral antigens and nucleic acids and their persistence in culture, including appropriate reagents and materials, and assay sensitivities and limitations.

(ii) Intracellular concentration of viral antigens and viral nucleic acids, reported as a function of cell number (e.g., viral genome number/cell).

(5) **Cell transformation assay.** (i) Details of the protocols used for the cell transformation assay, and reference to the assay used, if published.

(ii) Control value data, including deficiency of plating results.

(iii) Tumorigenesis data in test animals, if this study is required.

(6) **General information to be provided for all tests.** (i) The source of each cell line used.

(ii) Evidence for lack of adventitious agents in cell lines.

(iii) Information on genetic stability of continuous cell lines, and on donors of primary cells.

(f) **Tier progression.** The requirement for further studies as indicated by the data from these studies also will be based on data from the other test requirements in this tier.

(1) If the data show that the viral pest control agent is not cytotoxic, nor does it infect, replicate in, or transform any cell culture, no further testing is required.

(2) If the data show that the viral pest control agent preparation is toxic to any of the test cell cultures, but does not infect, replicate in, or transform any of the cell cultures:

(i) The toxic components of the preparation may have to be identified.

(ii) An acute toxicity study may be required with the toxic components.

(3) If the viral pest control agent infects any of the test cell cultures, reproductive and fertility effects, oncogenicity, immunodeficiency, and primate infectivity/pathogenicity studies may be required.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Bishop, D.H.L. The application of RNA finger printing and sequencing to viral diagnosis. *Current Topics in Microbiology and Immunolology* 104:259–271 (1983).

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(8) Southern, E., Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98:503 (1975).

(9) Tijssen, P., Laboratory techniques in biochemistry and molecular biology. Practice and theory of enzyme immunoassays. Elsevier (ISBN 0–444–80633–4) (1985).