Designation: E 1286 – 00

Standard Guide for Identification of Herpes Simplex Virus or Its DNA[1]

This standard is issued under the fixed designation E 1286; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

This guide covers the identification of herpes simplex virus (HSV) or its DNA and was developed by Subcommittee E48.02 on Characterization and Identification of Biological Systems. The objective is to describe laboratory characterization procedures that would be sufficient to verify that a biological preparation believed to contain primarily HSV (or HSV DNA) for use in any step of a biotechnology process actually does contain this virus (or its DNA).

This guide assumes a basic knowledge of virology and molecular biology.

1. Scope

1.1 This guide covers laboratory characterization procedures sufficient to identify purified specimens of HSV types 1 and 2 (HSV-1 and HSV-2) or HSV-1 DNA and HSV-2 DNA used in biotechnology. For cases in which identification of HSV DNA specimens is required, the characterization criteria of 6.2 and 6.3 of this guide are sufficient.

1.2 This guide does not cover the identification of HSV in HSV-infected host cells. To apply this guide to such a case, it would first be necessary to isolate the virus from such samples using standard techniques of HSV purification. This guide does not cover characterization of segments of HSV DNA or of vectors containing HSV DNA segments.

1.3 This guide does not cover the specific methodology used in the identification characterization. It does not address the question of degree of purity required for herpesvirus preparations: this would vary depending on the particular biotechnology use of the virus.

1.4 **Warning—**Laboratory work involving herpes simplex viruses can be hazardous to personnel. **Precaution:** Biosafety 2 level facilities are recommended **(1)**.[2] Safety guidelines shall be adhered to according to NCCLS M29–T2 and other recommendations **(1)**.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 1873 Guide for Detection of Nucleic Sequences by the Polymerase Chain Reaction Technique[3]

2.2 *NCCLS Standards:*

M29–T2 Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue—Second Edition; Tentative Guideline[4]

3. Terminology

3.1 Basic polymerase chain reaction (PCR) definitions apply according to the general PCR Guide E 1873 (Section 3).

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *capsomere*—a structural subunit of the outer protein shell (capsid) of a virus consisting of protein monomers.

3.2.2 *envelope*—a layer of cell membrane-derived lipoprotein that surrounds the protein coat (capsid) of some viruses.

3.2.3 *genome (of a virus)*—the genetic material consisting of nucleic acid (RNA or DNA).

3.2.4 *nucleocapsid*—the outer protein coat or shell (capsid) of a virus plus its inner core of nucleic acid and proteins.

3.2.5 *plaque*—a round, clear area in a layer of host cells caused by virus growth and resultant killing or lysis of the cells.

3.2.6 *restriction endonuclease*—a bacterial enzyme that cuts double-stranded DNA at positions consisting of specific short sequences of nucleotides.

4. Significance and Use

4.1 This guide is intended for use in a biotechnology laboratory whenever the necessity arises for identifying a biological preparation believed to contain primarily HSV or its

[1] This guide is under the jurisdiction of ASTM Committee E48 on Biotechnology and is the direct responsibility of Subcommittee E48.02 on Characterization and Identification of Biological Systems.

Current edition approved September 10, 2000. Published December 2000. Originally published as E 1286 – 89. Last previous edition E 1286 – 89 (1994).

[2] The boldface numbers in parentheses refer to a list of references at the end of this guide.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Available from the National Committee for Clinical Laboratory Standards, 940 West Valley Road, Suite 1400, Wayne PA 19087.

DNA. The characterization criteria used for the identification shall be performed by an individual trained in molecular virology.

4.2 This guide is not meant to be used in a clinical laboratory for the identification of HSV isolated from patient specimens.

5. Background Information About Herpes Simplex Virus

5.1 Herpes simplex virus is a common human virus that can cause primary and recurrent infections of the skin and mucous membranes **(2-4)**. It has been classified by the International Committee on Taxonomy of Viruses as (*a*) Family: Herpesvirus group (*Herpesviridae*) and (*b*) Subfamily: Herpes simplex virus group (*Alphaherpesvirinae*) **(2)**. There are two main immunologic variants of HSV, types 1 and 2 (HSV-1 and HSV-2). They are officially known as human (alpha) herpesvirus 1 and human (alpha) herpesvirus 2 **(2)**. HSV-1 has been isolated primarily from the oral cavity, eye, and skin vesicles above the waist. Herpes simplex virus recovered from the genitalia is predominantly type 2. HSV-1 and HSV-2 can be distinguished antigenically and biochemically.

5.2 HSV DNA is synthesized in the cell nucleus. Viral particles are assembled in the nucleus, pass through the nuclear membrane to the cytoplasm (acquiring an envelope in the process), and are transported to the cell surface via the endoplasmic reticulum. HSV-1 and HSV-2 are highly cytopathic in cell culture and have a wide mammalian cell host range. The cellular response varies with the strain of virus used. Some strains cause marked clumping of cells, whereas other produce multinucleated giant cells by fusion of cell membranes. A number of strains produce characteristic plaques on suitable cell monolayers. Like other enveloped viruses, HSV is relatively unstable at room temperature and is readily inactivated by lipid solvents.

5.3 HSV virions have a diameter of 120 to 150 nm and a molecular weight of $>1000 \times 10^6$ daltons **(2)**. The outer membrane (or envelope) is primarily host-specific phospholipid acquired by budding through the host cell nuclear membrane. The nucleocapsid, 100 to 110 nm in diameter, has 162 capsomeres arranged as an icosahedron. The virus has greater than 24 virus-specific polypeptides including 5 major glycoproteins. Several different strains of HSV-1 and HSV-2 have been described and are available (for example, see American Type Culture Collection WEB site (Animal Virology Collection): http://www.atcc.org.

5.4 The genome of the virus consists of a single molecule of linear double-stranded DNA with a molecular weight of 96×10^6 daltons (about 148 kilobase pairs) **(2)**. The DNA exists in four isomeric forms. HSV-1 DNA shares about 50 % of its sequences with HSV-2. Isolated HSV DNA is infectious.

5.5 There are many uses of HSV or its DNA in basic and applied biotechnology. Examples of applied uses include the preparation of DNA probes and monoclonal antibodies for in vitro diagnostic testing and utilization of the virus in *in vitro* testing of antiviral substances.

6. Characterization Criteria for Identification

6.1 *Immunological Evidence*—Immunological evidence shall be provided such as demonstrating HSV envelope glycoprotein antigen in viral-infected host cells by immunofluorescent (IF) or immunoperoxidase staining, or Western blotting of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) gels (for example, see Refs **(3, 5 and 6)**). Enzyme immunoassay (EIA or ELISA) or radioimmunoassay (RIA) can also be used to demonstrate the presence of HSV envelope glycoprotein **(3, 5 and 6)**. HSV-1 can be differentiated from HSV-2, and different strains of the viruses can be distinguished, by the use of appropriate monoclonal antibodies for immunofluorescence or EIA. Any one of a number of published protocols can be used. It should be pointed out that, although different species of herpes viruses have distinct envelope glycoproteins, there are some shared antigenic determinants **(3)**.

6.2 *DNA Gel Electrophoresis*—Gel electrophoresis patterns of restriction endonuclease fragments of isolated HSV DNA are distinctive. They can be used to differentiate between HSV-1 and HSV-2 and to distinguish different strains of these viruses. Any one of a number of published protocols for isolation of the viral DNA can be used. Restriction enzyme analysis of the viral DNA shall be accomplished with reference to the current literature (for example, see Refs **(7-10)**).

6.3 *Polymerase Chain Reaction (PCR)*— PCR can also be used to detect and identify isolated HSV DNA.

6.3.1 For general information on detection of DNA by PCR see Guide E 1873.

6.3.2 Two satisfactory HSV-specific PCR primer pairs are HSV Viral Protein 16 (VP16) primers and HSV ribonucleotide reductase (RR) primers. These primer pairs are sensitive and do not amplify cellular sequences. However, they are not suitable for distinguishing between HSV-1 DNA and HSV-2 DNA.

6.3.2.1 *HSV VP16 Primer Pair*—Sequence of VP16–a: GGACTCGTATTCCAGCTTCAC; Sequence of VP16–b: CGTCCTCGCCGTCTAAGTG. The optimum annealing temperature is 59.6°C. The PCR product length is 260 base pairs **(11)**.

6.3.2.2 *HSV RR Primer Pair*—Sequence of RR-a: ATGCCAGACCTGTTTTTCAA; Sequence of RR-b: GTCTTTGAACATGACGAAGG. Optimum annealing temperature is 56.2°C. Product length: 243 base pairs **(12)**.

7. Report

7.1 A concise, written report of the identification shall be prepared and shall include the following information:

7.1.1 Source of virus (or viral DNA) sample(s),

7.1.2 Other materials and methods used,

7.1.3 Results and data display, and

7.1.4 Discussion of results, conclusions, and references.

REFERENCES

(**1**) Richmond, J.Y., and McKinney, R.W., *Biosafety In Microbiological and Biochemical Laboratories*, 3rd ed., U.S. Department of Health and Human Services, Publication No. (CDC) 93-8395. U.S. Government Printing Office, Washington DC, 1993.

(**2**) Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., et al., eds., "Virus Taxonomy": *Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, Inc., New York, NY, 2000.

(**3**) White, D. O., and Fenner, F., *Medical Virology*, 4th ed., Academic Press, Inc., New York, NY, 1994.

(**4**) Luria, S. E., Darnell, Jr., J. E., Baltimore, D., and Campbell, A., *General Virology*, 3rd ed., John Wiley and Sons, New York, NY, 1978.

(**5**) Glaser, R., and Gotlieb-Stematsky, T., eds., *Human Herpes Virus Infections: Clinical Aspects*, Dekker, New York, NY, 1982.

(**6**) Taber, L. H., Brasier, F., Couch, R. B., Greenberg, S. B., Jones, D., and Knight, V., "Diagnosis of Herpes Simplex Virus Infection by Immunofluorescence," *Journal of Clinical Microbiology*, Vol 3, 1976, pp. 309–312.

(**7**) Skare, J., Summers, W. P., and Summers, W. C., "Structure and Function of Herpesvirus Genomes, I. Comparison of Five HSV-1 and Two HSV-2 Strains by Cleavage of Their DNA with EcoR1 Restriction Endonuclease." *Journal of Virology*, Vol 15, 1975, pp. 726–732.

(**8**) Skare, J., and Summers, W. C., "Structure and Function of Herpesvirus Genomes, II. EcoR1, Xbal, and HindIII Endonuclease Cleavage Sites on Herpes Simplex Virus Type 1 DNA." *Journal of Virology*, Vol 76, 1977, pp. 581–595.

(**9**) Morse, L. S., Buchman, T. G., Roizman, B., and Schaffer, P. A., "Anatomy of Herpes Simplex Virus DNA. IX. Apparent Exclusion of Some Parental DNA Arrangements in the Generation of Intertypic (HSV-1 × HSV-2) Recombinants," *Journal of Virology*, Vol 24, 1977, pp. 231–248.

(**10**) Roizman, B., and Tognon, M., "Restriction Endonuclease Patterns of Herpes Simplex Virus DNA: Application to Diagnosis and Molecular Epidemiology," *Current Topics in Microbiology and Immunology*, Vol 104, Cooper, M., Hofschneider, P. H., Koprowski, H., Melchers, F., Rott, R., Schweiger, H. G., Vogt, P. K., and Zinkernagel, R., eds., Springer-Verlag, New York, NY, 1983.

(**11**) Halford, W.P., Falco, V.C., Gebhardt, B.M., and Carr, D.J.J., "The Inherent Quantitative Capacity of the Reverse Transcription-Polymerase Chain Reaction," *Analytical Biochemistry*, Vol 266, 1999, pp. 181–191.

(**12**) Halford, W.P., and Schaffer, P.A., "Optimization of Viral Dose and Transient Immunosuppression Enable Herpes Simplex Virus ICPO-null mutants to Establish Wild-type Levels of Latency In Vivo," *Journal of Virology*, Vol 74, 2000, pp. 5957–5967.