



Standard Guide for Performing the Mouse Lymphoma Assay for Mammalian Cell Mutagenicity¹

This standard is issued under the fixed designation E 1280; 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 was developed at the request of ASTM Subcommittee E47.09 on Biomarkers in order to aid toxicologists, geneticists, biochemists, other researchers, and interested persons in the understanding, performance, and analysis of the mammalian cell mutagenicity test that uses the TK^{+/–}-3.7.2C strain of L5178Y mouse lymphoma cells. In this rapidly changing area of toxicology, it is not intended for this guide to replace, alter, or diminish the usefulness of presently available protocols and procedures.

1. Scope

1.1 The purpose and scope of this guide is to present background material and to establish criteria by which protocols and procedures for conducting the L5178Y/TK^{+/–}-3.7.2C mouse lymphoma mutagenicity assay (commonly referred to as the mouse lymphoma assay, (MLA)) can be properly understood and evaluated. This guide is also intended to aid researchers and others to gain a better understanding of the critical elements involved with mammalian cell mutagenicity testing. More specifically, this guide is intended to provide for researchers the accomplishment of the following goals:

1.1.1 Provide an understanding of the critical procedures (steps) in the performance of this mammalian cell mutagenicity test.

1.1.2 Provide generalized criteria by which researchers can evaluate if they are properly performing, utilizing, and interpreting this assay.

1.1.3 Provide criteria by which individuals responsible for evaluating MLA data can determine if the experiments have been properly performed and interpreted.

1.1.4 Provide a basis from which new procedures and developments in testing procedures can be evaluated.

1.1.5 Provide an understanding of the types of genetic damage (that is, gene and chromosome mutation) that may be detected in this mammalian cell mutagenicity test.

2. Terminology

2.1 Definitions:

2.1.1 *clastogen*—any agent that is capable of inducing chromosome breaks.

2.1.2 *gene mutation*—any heritable change whose physical extent is restricted to the limits of a single gene.

2.1.3 *mutagen*—any physical or chemical agent capable of inducing a mutation.

2.1.4 *mutation*—any heritable change in the genetic material, not caused by genetic segregation or genetic recombination, and that is transmitted to daughter cells.

2.2 Definitions of Terms Specific to This Standard:

2.2.1 *chromosome mutation*—a mutation resulting from a structural change to a chromosome involving the gain, loss, or relocation of chromosome segments. Chromosome mutations can be either intrachromosomal or interchromosomal.

2.2.2 *relative suspension growth (RSG)*—used to measure the cytotoxicity of a given treatment based on the growth of cells in suspension culture relative to the untreated or solvent control(s). RSG is calculated according to the method of Clive and Spector **(1)**.²

2.2.3 *relative total growth (RTG)*—used as a means to measure the relative toxicity to cells (survival) following treatment in the mouse lymphoma assay. RTG is calculated according to the method of Clive and Spector **(1)** and includes RSG as well as the ability to form colonies in the clonal phase of the assay.

2.3 Symbols:

2.3.1 *BrUdR*—5-bromo-2'-deoxyuridine.

2.3.2 *BUdR*—bromouracil deoxyriboside.

2.3.3 *CAS*—chemical abstract service.

2.3.4 *DMSO*—dimethylsulfoxide.

2.3.5 *MLA*—mouse lymphoma assay.

¹ This guide is under the jurisdiction of Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

Current edition approved Sept. 10, 2003. Published September 2003. Originally approved in 1989. Last previous edition approved in 1997 as E 1280 – 97.

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