



Standard Guide for Determining DNA Single-Strand Damage in Eukaryotic Cells Using the Comet Assay¹

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1. Scope

1.1 This guide covers the recommended criteria for performing a single-cell gel electrophoresis assay (SCG) or Comet assay for the measurement of DNA single-strand breaks in eukaryotic cells. The Comet assay is a very sensitive method for detecting strand breaks in the DNA of individual cells. The majority of studies utilizing the Comet assay have focused on medical applications and have therefore examined DNA damage in mammalian cells in vitro and in vivo **(1-4)**.² There is increasing interest in applying this assay to DNA damage in freshwater and marine organisms to explore the environmental implications of DNA damage.

1.1.1 The Comet assay has been used to screen the genotoxicity of a variety of compounds on cells in vitro and in vivo **(5-7)**, as well as to evaluate the dose-dependent anti-oxidant (protective) properties of various compounds **(3, 8-11)**. Using this method, significantly elevated levels of DNA damage have been reported in cells collected from organisms at polluted sites compared to reference sites **(12-15)**. Studies have also found that increases in cellular DNA damage correspond with higher order effects such as decreased growth, survival, and development, and correlate with significant increases in contaminant body burdens **(13, 16)**.

1.2 This guide presents protocols that facilitate the expression of DNA alkaline labile single-strand breaks and the determination of their abundance relative to control or reference cells. The guide is a general one meant to familiarize lab personnel with the basic requirements and considerations necessary to perform the Comet assay. It does not contain procedures for available variants of this assay, which allow the determination of non-alkaline labile single-strand breaks or double-stranded DNA strand breaks **(8)**, distinction between different cell types **(13)**, identification of cells undergoing apoptosis (programmed cell death, **(1, 17)**), measurement of cellular DNA repair rates **(10)**, detection of the presence of

photoactive DNA damaging compounds **(14)**, or detection of specific DNA lesions **(3, 18)**.

1.3 *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 requirements prior to use.*

1.4 This guide is arranged as follows:

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2. Referenced Documents

2.1 ASTM Standards:

E 1706 Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates³

E 1847 Practice for Statistical Analysis of Toxicity Tests Conducted Under ASTM Guidelines⁴

3. Terminology

3.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express the strongest possible recommendation, just short of an absolute requirement. “Must” is only used in connection with factors that relate directly to the acceptability of the test. “Should” is used to state that the specific condition is recommended and ought to be met if possible. Although violation of on “should” is rarely a serious matter, the violation of several will often render the results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used

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

³ *Annual Book of ASTM Standards*, Vol 11.01.

⁴ *Annual Book of ASTM Standards*, Vol 11.05.