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40 C.F.R. § 798.5500

Differential growth inhibition of repair proficient and repair deficient bacteria: "Bacterial DNA damage or repair tests."

(a) *Purpose.* Bacterial DNA damage or repair tests measure DNA damage which is expressed as differential cell killing or growth inhibition of repair deficient bacteria in a set of repair proficient and deficient strains. These tests do not measure mutagenic events *per se.* They are used as an indication of the interaction of a chemical with genetic material implying the potential for genotoxicity.

(b) *Definition.* Test for differential growth inhibition of repair proficient and repair deficient bacteria measure differences in chemically induced cell killing between wild-type strains with full repair capacity and mutant strains deficient in one or more of the enzymes which govern repair of damaged DNA.

(c) *Reference substances.* These may include, but need not be limited to, chloramphenicol or methyl methanesulfonate.

(d) *Test method*—(1) *Principle.* The tests detect agents that interact with cellular DNA to produce growth inhibition or killing. This interaction is recognized by specific cellular repair systems. The assays are based upon the use of paired bacterial strains that differ by the presence of absence of specific DNA repair genes. The response is expressed in the preferential inhibition of growth or the preferential killing of the DNA repair deficient strain since it is incapable of removing certain chemical lesions from its DNA.

(2) *Description.* Several methods for performing the test have been described. Those described here are:

(i) Tests performed on solid medium (diffusion tests).

(ii) Tests performed in liquid culture (suspension tests).

(3) *Strain selection*—(i) *Designation.* At the present time, *Escherichia coli polA* (W3110/p3478) or *Bacillus subtilis rec* (H17/M45) pairs are recommended. Other pairs may be utilized when appropriate.

(ii) *Preparation and storage.* Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(4) *Bacterial growth.* Good microbiological techniques should be used to grow fresh cultures of bacteria. The phase of growth and cell density should be documented and should be adequate for the experimental design.