Results of a Protocol Standardization Study for the LUMI-CELL® Estrogen Receptor (ER) Transcriptional Activation (TA) Bioassay.

FH Deal[1], P Ceger[1], GC Clark[3], JD Gordon[3], JM Charles[1], RR Tice[2], WS Stokes[2].
[1]ILS, Inc., RTP, NC; [2]NICEATM/NIEHS/NIH/DHHS, RTP, NC; [3]Xenobiotic Detection Systems, Inc. (XDS), Durham, NC.

In 2003, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) concluded that none of the ER TA assays considered for inclusion in the EPA Endocrine Disruptor Screening Programs Tier 1 battery had been adequately validated. NICEATM subsequently conducted a study to standardize protocols to be used in formal validation studies for the XDS LUMI-CELL® ER TA test method, a proposed Tier 1 *in vitro* screening assay for the detection of ER agonists and antagonists. The objective of the study was to develop standardized protocols that can be easily transferred to other laboratories and be used to obtain reproducible results for the detection of ER agonists and antagonists. The study included optimization of the use of reference standards and controls, and compared quantitative and qualitative methods for evaluating cytotoxicity. The intralaboratory reproducibility and accuracy of the standardized protocols was demonstrated using eight coded substances covering a range of ER agonist activities, including negatives (atrazine [ATZ], bisphenol A [BPA], bisphenol B [BPB], corticosterone [COR], *o,p'*-DDT [DDT], diethylstilbestrol [DES], 17α-ethinyl estradiol [EE], and flavone [FLA]) and eight coded substances covering a range of ER antagonist activities, including negatives (butylbenzyl phthalate [BBP], dibenzo[*a,h*]anthracene [DBA], flavone [FLA], genistein [GEN], nonylphenol [NON], progesterone [PRO], *o,p'*-DDT [DDT], and tamoxifen [TAM]). EE, DES, BPA, BPB, DDT, and FLA were classified as estrogenic agonists, while ATZ and COR did not induce a significant ER TA response. TAM, DBA, FLA, and GEN, were classified as antagonists, while BBP, PRO, NON, and DDT did not affect ER TA response. This study demonstrated the intralaboratory reproducibility and accuracy of this test method and indicated its readiness for a multi-laboratory validation study. Supported by NIEHS Contract N01-ES-85424.

Character Count: 2253/2300
Keywords: prevalidation, estrogen, bioassay
Categories: Alternatives to Mammalian Models (2nd choice - Reproductive System)