Standardization of Protocols for the Validation of an *In Vitro* Estrogen Receptor Transcriptional Activation Assay

FH Deal[1], P Ceger[1], GC Clark[3], JD Gordon[3], RR Tice[2], WS Stokes[2]

[1]ILS, Inc., Contractor Supporting NICEATM, RTP, NC, US; [2]National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)/NIEHS/NIH/DHHS, RTP, NC, US; [3]Xenobiotic Detection Systems, Inc., Durham, NC, US

Abstract

NICEATM conducted a study to standardize protocols to be used in validation studies of an *in vitro* transcriptional activation (TA) test method for the detection of estrogen receptor (ER) agonists and antagonists (LUMI-CELL® ER, Xenobiotic Detection Systems, Inc.). The objective of this study was to develop reproducible protocols in a lead laboratory that can be easily transferred to other laboratories. The study included optimization of reference standards and controls, and compared quantitative and qualitative methods for evaluating cytotoxicity. Protocol reproducibility and accuracy was demonstrated using 16 coded substances covering a range of ER agonist and antagonist activities, including negatives. ER agonist activity was evaluated with atrazine, bisphenol A, bisphenol B, corticosterone, *o,p'*-DDT, diethylstilbestrol, 17α-ethinyl estradiol, and flavone. ER antagonist activity was evaluated with butylbenzyl phthalate, dibenzo[*a,h*]anthracene, flavone, genistein, nonylphenol, *o,p'*-DDT, progesterone, and tamoxifen. Bisphenol A, bisphenol B, *o,p'*-DDT, diethylstilbestrol, 17α-ethinyl estradiol, and flavone tested positive for agonism, while atrazine and corticosterone were negative. Dibenzo[*a,h*]anthracene, flavone, genistein and tamoxifen tested positive for antagonism, while butylbenzyl phthalate, progesterone, nonylphenol, and *o,p'*-DDT were negative. This study demonstrated the reproducibility and accuracy of this test method using the standardized protocol and indicated its readiness for entering a multi-laboratory validation study. Supported by NIEHS Contract N01-ES-35504.