Testing of Coded Substances for a Multi-phased International Validation Study of an Estrogen Receptor (ER) Transcriptional Activation (TA) Assay

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The LUMI-CELL® ER assay is an ER TA test method developed to detect ER agonists and antagonists. NICEATM, ECVAM, and JaCVAM are conducting an international multi-laboratory validation study to evaluate the reproducibility and accuracy of this assay. This four-phased study will evaluate all 78 of the reference substances recommended by ICCVAM for validation of *in vitro* ER test methods. Phase I results demonstrated acceptable intralaboratory reproducibility and established quality controls for testing of coded reference substances in subsequent phases. In Phase II, repeat testing of coded reference substances covering a range of estrogenic activities was conducted in two stages (four substances in Phase IIa and eight substances in Phase IIb) to optimize agonist and antagonist protocols to be used for the testing of the remaining reference substances in Phases III and IV. A large number of tests failed one or more study acceptance criteria during Phase IIa (52% [46/88]). Therefore, the Study Management Team (SMT) recommended protocol modifications in order to increase plate acceptance without compromising the ability of the assay to detect and quantify agonist or antagonist activity. Phase IIb results indicated interlaboratory differences in the maximum concentration selected for evaluation, based on differences in perceived solubility and/or cytotoxicity. For some substances, this resulted in interlaboratory discordance in calls. The SMT subsequently recommended protocol modifications to better standardize these steps in the assay. These results underscore the importance of a phased study design to allow for necessary protocol refinements. ILS staff supported by NIEHS contract N01-ES-35504.