Testing of Coded Substances in the NICEATM/ECVAM/JaCVAM LUMI-CELL® STTA Multiphase International Validation Study.

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The LUMI-CELL® ER transcriptional activation (TA) assay uses the BG-1 cell line, a human cell line with an endogenous estrogen receptor (ER) and a stably transfected reporter gene to screen for substances that may induce or inhibit TA activity via the ER. Based on an ICCVAM recommendation, NICEATM, ECVAM, and JaCVAM initiated a four-phase validation study to evaluate the LUMI-CELL® ER assay. Three laboratories (one each in the United States, Europe, and Japan) tested the minimum list of 53 reference substances recommended by ICCVAM for validation of *in vitro* ER test methods. Phase 1 was the laboratory evaluation phase where each laboratory tested reference standards and controls 10 times to demonstrate initial proficiency, and to establish laboratory-specific acceptance criteria for subsequent phases. In Phase 2, the protocol refinement phase, 12 agonist and antagonist substances from the ICCVAM minimum list covering the range of activities (i.e., strong, moderate, weak, negative agonists and/or antagonists) were tested in two stages (4 in Phase 2a, 8 in Phase 2b). Protocol refinements made during Phase 2 were incorporated into the final optimized protocols used for all subsequent testing. Phase 3 provided the data necessary to evaluate inter-laboratory reproducibility and accuracy of the optimized protocols by testing the remaining 41 substances from the minimum list at least once at each laboratory. Phase 4 was an additional testing phase where the lead laboratory tested the remaining 25 substances on the complete ICCVAM list of 78 recommended substances for agonist and antagonist activity. The validated assay will be used to support the EPA EDSP Tier 1 screening program and the development of an OECD performance based test guideline for similar ER TA test methods. Supported by ECVAM, JaCVAM, and NIEHS Contract N01-ES-35504.

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