International Validation Study of an *In Vitro* Cell Proliferation Test Method for Screening Potential Estrogenic Agonists and Antagonists in MCF-7 cells

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The MCF-7 Cell Proliferation test method developed by CertiChem Inc. (CCi) uses a human cell line that endogenously expresses estrogen receptor (hER-alpha and hER-beta) to screen for substances that may induce or inhibit cell proliferation via ER-mediated pathways. NICEATM, in collaboration with JaCVAM and KoCVAM, coordinated an international validation study to evaluate test method accuracy and reliability. Three laboratories (one each in the U.S., Japan, and Korea) tested ICCVAM recommended reference substances with well-characterized *in vitro* ER data. An initial study using a subset of these substances was conducted at CCi to refine protocols and demonstrate intralaboratory reproducibility and accuracy of the test method. Multi-phased interlaboratory studies were subsequently initiated at laboratories in Japan and Korea to demonstrate transferability and further evaluate test method accuracy and reliability. Phases 1 and 2 of the interlaboratory study were used to demonstrate proficiency, establish historical databases, and to evaluate intra- and interlaboratory reproducibility by testing 12 substances in three independent experiments at both laboratories. Phase 3 testing of 14 additional ICCVAM reference substances (tested once at each laboratory) provided data for a more comprehensive evaluation of interlaboratory reproducibility and accuracy. Phase 4 testing, conducted only at CCi, completed testing for the entire list of 78 ICCVAM reference substances. Results from this validation study will be used as the basis for ICCVAM recommendations on usefulness and limitations of *in vitro* Endocrine Disruptor test methods, and to develop performance standards for the expedited validation of functionally and mechanistically similar test methods. Supported by NIEHS Contract N01-ES-35504 and SBIR44ES014806.

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Poster Session: Alternatives to Mammalian Models for Testing