Performance of the Tox21 BG1Luc and ER β-Lactamase Estrogen Receptor Transactivation Assays

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Two estrogen receptor (ER) transactivation assays (BG1Luc and HEK293 ER β-lactamase [ER-Bla]) were adapted for the Tox21 high-throughput screening program. Both assays detect substances with ER agonist (Ag) or antagonist (Ant) activity. BG1Luc endogenously expresses full-length ER and is stably transfected with a luciferase reporter gene. ER-Bla is stably transfected with the human ER-α ligand-binding domain and a β-lactamase reporter gene. Approximately 10,000 chemicals in the Tox21 compound library were tested three times in each assay in Ag and Ant modes. To differentiate antagonism from cytotoxicity, cell viability was determined. Concentration–response data (N=15) were analyzed to evaluate assay performance. Data quality was high in all assays with acceptable signal to background ratio (2.5 to 8), CV (<10.5%), reproducibility (outcomes across 3 runs ≥87%), and Z' factor (≥0.4). Results were compared to ICCVAM ER transactivation performance standards. Ag accuracy, sensitivity, and specificity were, respectively, 97%, 96%, and 100% for BG1 versus 90%, 87%, and 100% for ER-Bla. Ant accuracy, sensitivity, and specificity were all 100% for both assays. Ag EC_{50} reference standard values for estradiol were 30 pM (BG1) and 275 pM (ER-Bla), while Ant IC_{50} reference values for hydroxytamoxifen were 71 nM (BG1) and 6 nM (ER-Bla). Understanding the factors contributing to differences in performance of these assays is critical to their regulatory acceptance and utilization.

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