ICCVAM Performance Standards for the BG1Luc ER TA Test Method

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Abstract

Performance standards can be used to evaluate the accuracy and reliability of proposed test methods that are functionally and mechanistically similar to an accepted test method. ICCVAM recently recommended performance standards for the BG1Luc estrogen receptor (ER) transactivation (TA) test method. The performance standards were based on results from an international interlaboratory validation study, and include essential test method components, reference substances, and standards for accuracy and reliability. Essential components include: a cell line that endogenously expresses human ERs and is stably transfected with a reporter gene, use of a solvent miscible with cell culture media, a defined concentration limit for agonist (1 mM) or antagonist (10 µM) testing, evaluation of cytotoxicity, a reference estrogen, anti-estrogen, and positive and solvent controls. The reference substances should cover the range of ER responses, both positive and negative. ICCVAM selected 34 agonist and 10 antagonist reference substances. The evaluation of these reference substances yielded the following results for agonists: accuracy of 100% (34/34), sensitivity of 100% (27/27), specificity of 100% (7/7), a false positive rate of 0% (0/7), and a false negative rate of 0% (0/27). For antagonists, results were: accuracy of 100% (10/10), sensitivity of 100% (3/3), specificity of 100% (7/7), a false positive rate of 0% (0/7), and a false negative rate of 0% (0/3). Evaluation of reference substances by a newly proposed method should yield similar results. Although it is not realistic to expect test methods to perform identically, the basis for any discordant results should be discussed along with the impact on the proposed use. These ICCVAM performance standards are expected to facilitate the efficient evaluation of new test methods proposed for evaluation of ER agonist and/or antagonist activity.

Introduction

- The BG1Luc estrogen receptor (ER) transactivation (TA) test method:
  - Is a transactivation method that uses an ER-responsive reporter gene to assess substances with *in vitro* ER agonist or antagonist activity
  - Shows excellent concordance with other internationally accepted test methods
  - Was considered scientifically valid based on results from an international multilaboratory validation study and subsequent independent peer review. This comprehensive evaluation (ICCVAM 2011) served as the basis for ER TA performance standards.
- Performance standards (see **Figures 1** and **2**):
  - Are based on a validated reference test considered adequate for regulatory testing purposes
  - Provide criteria upon which new test methods can be developed that are functionally and mechanistically similar to the reference test method
  - Can also be used by naive laboratories to demonstrate technical proficiency

Figure 1. Components of the ICCVAM Performance Standards

Essential test method components	• Essential test method elements including unique characteristics, critical procedural details, and quality control measures. See **Figure 2**.
Minimum list of reference substances	• A representative subset of substances used to evaluate accuracy and reliability of the validated test method.
Accuracy and reliability standards	• Standards that should be met or exceeded when evaluating the minimum list of reference substances.

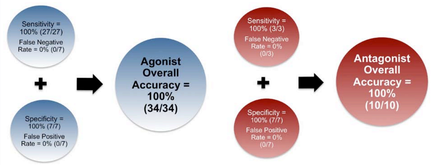
Figure 2. Essential Components of the BG1Luc ER TA Agonist and Antagonist Test Method Performance Standards

Cell Line	• Must express human ERs • Must be stably transfected with reporter gene system
Solvent	• Must be miscible with cell culture media at noncytotoxic concentrations • Must not interfere with test system
Limit Concentrations and Cytotoxicity	• Maximum concentration is 1 mM ER TA for agonist testing, 10 µM for antagonist testing unless limited by solubility, cytoxicity, or interference with test method. • Seven concentrations at log10 intervals should be tested. • Cytotoxicity should be evaluated; viability must be greater than 80%.
Reference Standards	• A reference estrogen and anti-estrogen should be tested in a full dose-response curve to demonstrate adequacy of method. • Estrogen and anti-estrogen reference standards should have 3-fold induction and reduction, respectively. • The substances should cover a range of ER responses, both positive and negative.
Controls	• Agonist test methods should include a vehicle control and a weak agonist. • Antagonist test methods should include a vehicle control, a weak antagonist, and a reference estrogen.

Accuracy Standards

- *Accuracy* is the closeness of agreement between a test method result and an accepted reference value. Accuracy for the BG1Luc ER TA test method, based on test results with the agonist and antagonist performance standards substances (listed in **Tables 1 and 2**), is shown in **Figure 3**. A functionally and mechanistically similar test method should have equivalent accuracy when testing these same performance standards substances.

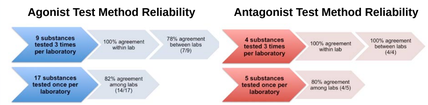
Figure 3. Accuracy of BG1Luc ER TA Agonist and Antagonist Test Methods Based on Reference Standards



Reliability Standards

- *Reliability* is the extent to which a test method can be performed reproducibly within and among laboratories over time. BG1Luc ER TA test method reliability, based on test results with the agonist and antagonist performance standards substances, is shown in **Figure 4**. A functionally and mechanistically similar test method should be at least as reliable when testing these same performance standards substances.

Figure 4. Reliability of BG1Luc ER TA Agonist and Antagonist Test Methods Based on Reference Standards



Reference Substances for BG1Luc ER TA Performance Standards

- ICCVAM previously recommended a list of 78 substances for use in validation studies of ER TA test methods (ICCVAM 2003, 2006).
- Performance standards substances were selected from this list of 78 based on:
  - A well-defined chemical structure
  - Comparatively low systemic toxicity
  - Commercial availability and minimal disposal cost
  - A concentration–response range that is measurable by the test method
  - Definitive positive or negative classification
- Thirty-four agonist and 10 antagonist performance standards reference substances were selected for use in this validation study.
- These reference substance lists may be updated as additional substances matching these criteria are identified (see the NICEATM-ICCVAM website at http://iccvam.niehs.nih.gov/).

Table 1. Reference Substances for Assessing Agonist Test Methods for Accuracy and Reliability

Substance	CASRN	ICCVAM Consensus	BG1Luc ER TA Consensus
17ß-Estradiol	50-28-2	POS	POS
17+-Estradiol	57-91-0	POS	POS
17+-Ethinyl estradiol	57-63-6	POS	POS
19-Nortestosterone	434-22-0	POS	POS
4-Cumylphenol	599-64-4	POS	POS
4-tert-Octylphenol	140-66-9	POS	POS
Apigenin	520-36-5	POS	POS
Bisphenol A	80-05-7	POS	POS
Bisphenol B	77-40-7	POS	POS
Butylbenzyl phthalate	85-68-7	POS	POS
Chrysin	480-40-0	POS	POS
Coumestrol	479-13-0	POS	POS
Daidzein	486-66-8	POS	POS
Diorstrol	115-32-2	POS	POS
Diethylstilbestrol	56-53-1	POS	POS
Estrone	53-16-7	POS	POS
Ethyl paraben	120-47-8	POS	POS
Fenarimol	60168-88-9	POS	POS
Genistein	446-72-0	POS	POS
Kaempferol	520-18-3	POS	POS
Kepone	143-50-0	POS	POS
meso-Hexestrol	84-16-2	POS	POS
Methyl testosterone	58-18-4	POS	POS
Norethynodrel	68-23-5	POS	POS
o,p'-DDT	789-02-6	POS	POS
p-n-Nonylphenol	104-40-5	POS	POS
p,p'-Methoxychlor	72-43-5	POS	POS
Atrazine	1912-24-9	NEG	NEG
Bicalutamide	90357-06-5	NEG	NEG
Corticosterone	50-22-6	NEG	NEG
Hydroxyflutamide	52806-53-8	NEG	NEG
Linuron	330-55-2	NEG	NEG
Phenobarbital	50-06-6	NEG	NEG
Spironolactone	52-01-7	NEG	NEG

Abbreviations: CASRN = CAS Registry Number® (American Chemical Society)

Table 2. Reference Substances for Assessing Antagonist Test Methods for Accuracy and Reliability

Substance	CASRN	ICCVAM Consensus	BG1Luc ER TA Consensus
4-Hydroxytamoxifen	68047-06-3	POS	POS
Raloxifene HCl	82640-04-8	POS	POS
Tamoxifen	10540-29-1	POS	POS
17+-Ethinyl estradiol	57-63-6	NEG	NEG
Apigenin	520-36-5	NEG	NEG
Chrysin	480-40-0	NEG	NEG
Coumestrol	479-13-0	NEG	NEG
Genistein	446-72-0	NEG	NEG
Kaempferol	520-18-3	NEG	NEG
Resveratrol	501-36-0	NEG	NEG

Abbreviations: CASRN = CAS Registry Number® (American Chemical Society)

BG1Luc ER TA Peel Panel Review of the Performance Standards

- Members of the Peer Panel (see roster below) were asked to assess the adequacy of the performance standards for evaluating accuracy and reliability of a novel test method with scientific principles similar to those of the BG1Luc ER TA test method.
  - The Panel agreed the ICCVAM performance standards are useful to evaluate test methods that are functionally and mechanistically similar to the BG1Luc ER TA test method.
  - The Panel found the list of reference substances adequate.
  - The Panel supported quantification of agonist and antagonist activities in addition to the positive/negative classification.
  - The Panel concluded that there should be some tolerance for discordance in the classification of weakly active reference substances.
  - The Panel agreed that discordant results need to be discussed in regard to the ability of the test method to detect potency ranges and intrinsic activities similar to those of currently validated test methods.

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Conclusions

- The BG1Luc ER TA performance standards can be used by developers of novel ER TA test methods to efficiently determine validation status. They can also be used by naïve laboratories to demonstrate technical proficiency.
- The use of reference standards allows for assessment of test method accuracy and reliability based on substances with consistent activities.
- The accuracy and reliability of a test method should be similar to or better than a currently validated ER TA test method.
- Discordant results and the impact on the proposed use of the test method should be discussed.
- ICCVAM encourages developers of novel test methods to consult with ICCVAM prior to use of performance standards in a validation study.
- Validation study results can be submitted to ICCVAM to evaluate the usefulness and limitations of the test method.

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