Regulatory Acceptance of the BG1Luc Estrogen Receptor Transactivation Test Method

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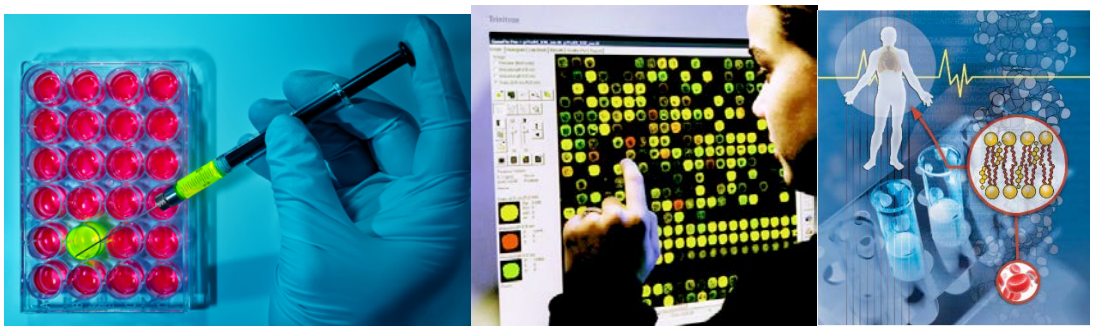
Abstract

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) coordinated an international interlaboratory validation study of the BG1Luc estrogen receptor (ER) transactivation (TA) test method (BG1Luc ER TA) developed by Xenobiotic Detection Systems, Inc. In 2010, the validation study finished evaluating the usefulness and limitations of the BG1Luc ER TA test method to screen for substances with *in vitro* ER agonist or antagonist activity. The international validation study was sponsored by NICEATM, with participation from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. In 2011, NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) released a test method evaluation report on the usefulness and limitations of the BG1Luc ER TA test method. ICCVAM recommended the use of the BG1Luc ER TA as a screening test to identify substances with *in vitro* ER agonist and antagonist activity and recommended that the BG1Luc ER TA test method could be considered as an alternative to the existing ER TA test guideline (EPA OPPTS 890.1300/OECD TG 455). All 15 ICCVAM member agencies, including the U.S. Environmental Protection Agency, concurred with the ICCVAM recommendations. NICEATM sponsored the new method for evaluation by the Organisation for Economic Co-operation and Development (OECD), which approved the BG1Luc ER TA test method and added the BG1 agonist protocol to the existing Test Guideline 455. The BG1 antagonist method has been adopted as OECD Test Guideline 457. Acceptance of the BG1Luc ER TA test method by U.S. and international agencies is an example of increased cooperation and collaboration to support the international adoption of scientifically valid test methods that will protect people, animals, and the environment while reducing, refining, and replacing animal use. (ILS staff supported by NIEHS contract N01-ES 35504.)



Introduction

- Endocrine-active compounds (EACs) are both naturally occurring and synthetic substances that may interfere with the normal function of hormones in the endocrine system.
- Public health concerns have resulted largely from studies indicating that animal populations exposed to high concentrations of EACs have increased incidence of reproductive and developmental abnormalities (EPA 1997; NRC 1999).
- In 2004, Xenobiotic Detection Systems, Inc. (XDS), nominated the LUMI-CELL® BG1Luc4E2 ER TA test method (BG1Luc ER TA test method) for an interlaboratory validation study.
- ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) recommended that, in light of the lack of adequately validated test methods and the regulatory and public health need for such test methods, the BG1Luc ER TA test method should have high priority for evaluation in an interlaboratory study.
- NICEATM subsequently led and coordinated an international validation study with its counterparts in Japan (the Japanese Center for the Validation of Alternative Methods) and Europe (the European Centre for the Validation of Alternative Methods, now known as EURL ECVAM), using laboratories sponsored by each validation organization.
- The previous OECD test guideline for a luciferase-based, stably transfected estrogen receptor agonist detection method was Test Guideline 455 (TG 455), the Stably Transfected Human Estrogen Receptor-α Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals (OECD 2009).
  - Developed by the Chemicals Evaluation and Research Institute, Japan
  - Utilizes the hERα-HeLa-9903 cell line to measure the ability of a test chemical to induce hERα-mediated transactivation of luciferase gene expression. The hERα-HeLa-9903 cell line is derived from a human cervical tumor, with two stably inserted constructs: (1) the hERα expression construct (encoding the full-length human

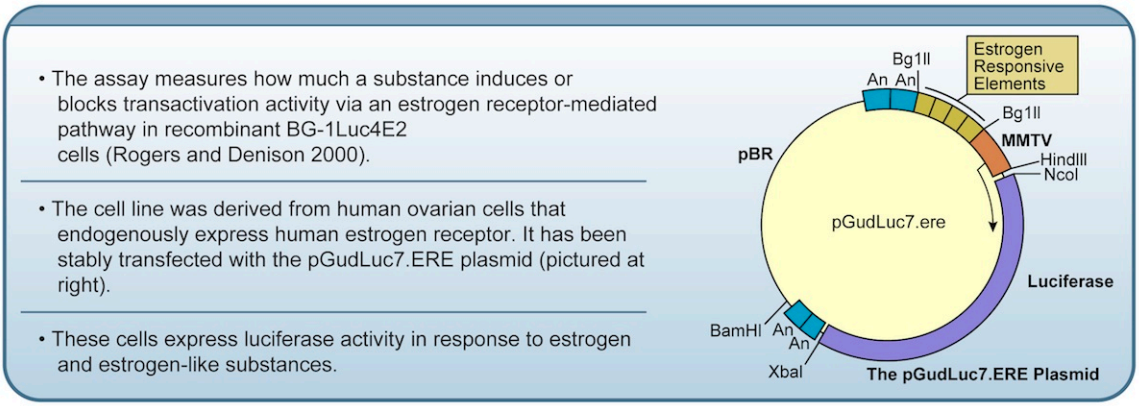
receptor) and (2) a firefly luciferase reporter construct coupled to an estrogen response element.

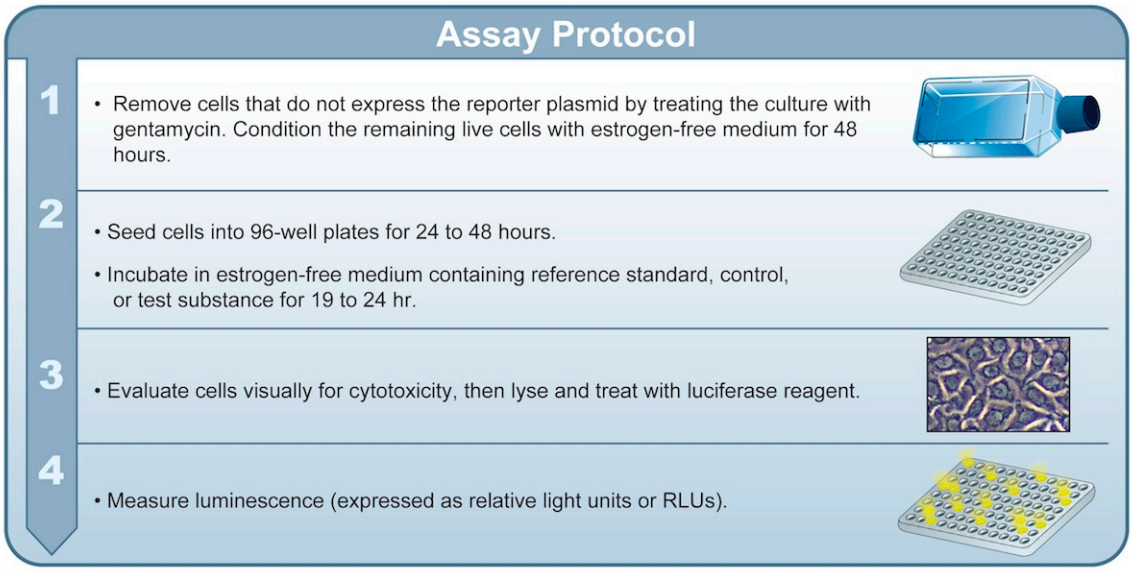
— Was adopted by the U.S. Environmental Protection Agency (EPA) as OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line [HeLa-9903]) (EPA 2009)

Overview of Method

- The BG1Luc ER TA test method is an ER transactivation test method that screens substances that may induce (agonism) or inhibit (antagonism) estrogenic activity *in vitro* (**Figure 1**).

Figure 1 Overview of the BG1Luc ER TA Test Method





Reference Substances

	Vehicle Control	Reference Standard	Reference Estrogen	Positive Control
Agonist Protocol	1% DMSO	17β-estradiol	(none used)	*p,p'*-methoxychlor (weak positive)
Antagonist Protocol	1% DMSO	17β-estradiol + raloxifene HCl	17β-estradiol	17β-estradiol + tamoxifen

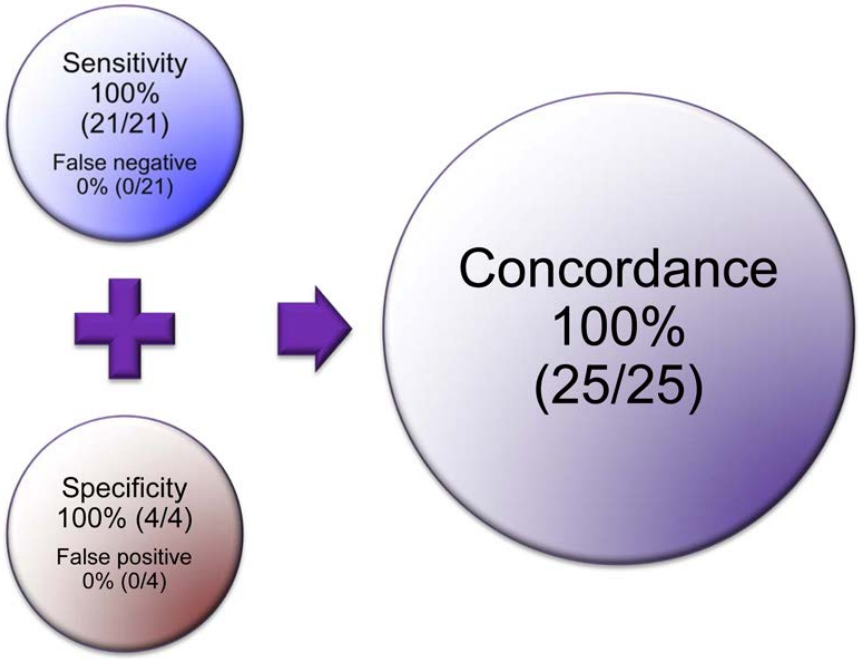
- The BG1Luc ER TA test method is conducted in cells that endogenously express both the estrogen receptor and its associated transcriptional machinery. The HeLa-9903 assay uses a cell line into which the ER and the estrogen response element have been transfected. Both cell lines contain a stably transfected reporter gene.

- Unlike the HeLa-9903 assay, the BG1Luc ER TA test method now has a validated and accepted antagonist protocol.
- The BG1Luc ER TA test method has been tested to higher concentrations and for a wider range of chemicals compared to the HeLa-9903 assay.

Validation Status of the BG1Luc ER TA Test Method

- In 2010, validation of the BG1Luc ER TA test method was completed.
- NICEATM evaluated the usefulness and limitations of the BG1Luc ER TA test method to screen for substances with *in vitro* ER agonist or antagonist activity.
- Based on the results of the international multilaboratory validation study and subsequent peer review, ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) determined that the BG1Luc ER TA test method was sufficiently reproducible and accurate to support its use for identifying ER agonist and antagonist activity of chemicals and other substances
- ICCVAM developed recommendations for use of the BG1Luc ER TA assay after considering feedback from the peer review panel, the public, and SACATM.
    - The BG1Luc ER TA test method may be used as screening tests to identify substances with *in vitro* ER agonist or antagonist activity.
    - The accuracy of the BG1Luc ER TA agonist assay is at least equivalent to the only ER TA test method currently in a U.S. regulatory test guideline, the Environmental Protection Agency's OPPTS 890.1300: Estrogen Receptor Transcriptional Activation (Human Cell Line [HeLa-9903]) (EPA 2009) (**Figure 2**).

Figure 2 Agonist Accuracy – Comparison of the BG1Luc ER TA Test Method to EPA OPPTS 890.1300/OECD TG 455



Acceptance by U.S. Regulatory Agencies

- In 2011, ICCVAM published the ICCVAM Test Method Evaluation Report: The LUMI-CELL® ER (BG1Luc ER TA) Test Method: An *In Vitro* Assay for Identifying Human Estrogen Receptor Agonist and Antagonist Activity of Chemicals (ICCVAM 2011).
- All ICCVAM member agencies concurred with the ICCVAM recommendations for the BG1Luc ER TA test method.
- Comments of particular interest include:
  - **Department of the Interior**: "This new method provides a major improvement over the only method that has been validated and currently approved by the U.S. EPA for testing estrogen receptor activation."
  - **Consumer Product Safety Commission**: "Information from the LUMI-CELL assay may be invaluable when determining whether a compound is a chronic hazard in a weight-of-evidence approach."
  - **Environmental Protection Agency**: "The EPA regards the BG 1 Luc assay as an alternative to the OPPTS 890.1300 test guideline for transcriptional activation currently used in the EPA's Endocrine Disruptor Screening Program.... Adequately

performed studies using the BG 1 Luc assay will satisfy the Estrogen Receptor Transcriptional Activation requirement of the EDSP."

Adoption by the Organisation for Economic Co-operation and Development

- The Organisation for Economic Co-operation and Development (OECD), which was founded in 1961, includes 34 member countries committed to democratic government and the market economy.
- The OECD Guidelines for the Testing of Chemicals were developed to harmonize testing methods used for risk assessment.
- OECD Test Guidelines give detailed descriptions of how to perform test methods, as well as requirements for reporting data generated with these test methods.
- In 1981, Test Guidelines were incorporated into a legally binding Council Decision (the Mutual Acceptance of Data [MAD] Decision).
- MAD states that data generated in an OECD member state in accordance with OECD Test Guidelines and Principles of Good Laboratory Practice (GLP) will be accepted in other member countries for assessment purposes and other uses relating to the protection of health and the environment (OECD 2011).
- NICEATM and ICCVAM sponsored the BG1Luc ER TA agonist and antagonist methods as an OECD test guideline.
- In September 2012, the OECD adopted:
  - TG 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists (OECD 2012a)
  - TG 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists (OECD 2012b)

Advantages of the BG1Luc ER TA Test Method

- The BG1Luc ER TA test method has several advantages over EPA OPPTS 890.1300/original OECD TG 455:
  - Has more detailed and complete test method protocols than those provided in EPA OPPTS 890.1300/original OECD TG 455
  - Has a validated antagonist protocol
  - Is validated for testing up to 1 mM per EPA requirements. EPA OPPTS 890.1300/original OECD TG 455 is only validated up to a limit dose of 10 μM.
  - Has more restrictive classification criteria for determination of a positive response, which will reduce the number of false positive results, resulting in fewer follow-up tests conducted in animal studies

— Endogenously expresses both hERα and hERβ, whereas the HeLa-9903 cell line used in EPA OPPTS 890.1300/original OECD TG 455 was transfected only with hERα

Conclusions

- The BG1Luc ER TA test method is a scientifically validated, transferable test method for the detection of substances with *in vitro* ER agonist or antagonist activity.
- Acceptance of the BG1Luc ER TA test method by U.S. Federal and international agencies is an example of increased cooperation and collaboration to support the international adoption of scientifically valid test methods that will protect people, animals, and the environment while replacing, reducing, and refining animal use.

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Acknowledgements

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