OECD TEST GUIDELINES PROGRAMME

Standard Project Submission Form

If you require further information please contact the OECD Secretariat
Return completed forms to:
env.tgcontact@oecd.org

PROJECT TITLE

Stably Transfected Transcriptional Activation Assay for the Detection of Estrogen Receptor Agonists and Antagonists

SUBMITTED BY (Country / European Commission / Secretariat)

USA

DATE OF SUBMISSION TO THE SECRETARIAT

September 2007

DETAILS OF LEAD COUNTRY/CONSORTIUM

Country /Organisation:	USA
Agency/ministry/Other:	Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
Mail Address:	National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) National Institute of Environmental Health Sciences NIH, DHHS Bldg. 4401, Room 3129, MD EC-14 79 T.W. Alexander Drive Research Triangle Park, NC 27709
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PROJECT OUTCOMES

☒ New Test Guideline	☐ Guidance document
☐ Revised Test Guideline	☐ Detailed Review Paper
☐ Deletion of an existing Test Guideline	☐ Other, please specify below

PROPOSED WORK PLAN and RESOURCE NEEDS:

1. Draft workplan for development of the proposal, including any need to establish Ad Hoc Expert Group and mode of meetings (face-to-face, teleconference; electronic discussion group). Indicate key milestones, including first and subsequent drafts of documents and timing of meetings.

NICEATM, in conjunction with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM), is currently managing an international validation study of the LUMI-CELL® estrogen receptor (ER) transcriptional activation (TA) assay (LUMI-CELL® ER Assay) to detect substances with ER agonist and antagonist activity. The validation study incorporates essential test method components and 78 reference chemicals based on recommendations from an international expert panel. The validation study is scheduled for completion in April 2008 and a Background Review Document (BRD) summarizing the results of this study and any other relevant data and information will be completed shortly thereafter. An independent, international expert peer review panel meeting will be convened in fall of 2008, with observers from ICCVAM, ECVAM, and JaCVAM. The panel will evaluate the extent to which the BRD supports draft recommendations for (1) test method usefulness and limitations, (2) standardized test method protocols, (3) performance standards, and (4) future studies. ICCVAM will consider the panel report, public comments, and recommendations from ICCVAM's advisory committee, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), when finalizing the ICCVAM test method recommendations. These recommendations will be used to develop a generic Test Guideline based on the validated test method protocol, which will include performance standards for structurally, functionally, and mechanistically similar ER TA assays. A draft Test Guideline and supporting materials are scheduled for submission to the OECD Secretariat in early 2009.

2. Will additional information, including generation or collection of data, be required? If yes, please describe the anticipated process and timelines.

The ongoing validation study for the LUMI-CELL® ER Assay is scheduled for completion in April 2008 and the BRD is scheduled for completion in July 2008. The ICCVAM sponsored peer review of the BRD is scheduled for the fall of 2008. The standardized test method protocol currently being used in the validation study is available on request if there is a need for use by interested parties.

3. Indicate the estimated overall resource need (time/money) for member country / consortium and Secretariat

Funding for the validation study is being provided by NICEATM (U.S. laboratory), ECVAM (European laboratory), and JaCVAM (Japanese laboratory). NICEATM resources will be used for study management and development of the BRD. Funding for travel expenses associated with the international expert peer review meeting will be shared among the organizations participating in the Study Management Team. NICEATM will fund the remainder of the costs associated with this meeting.

4. Is this proposal intended to replace an existing Test Guideline or lead to the deletion of an existing Test Guideline?

No

ESSENTIAL INFORMATION

In this section, please provide the information required by the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. What is the existing or expected regulatory need/data requirement that will be met by the proposed outcome of the project? Please provide details below or as an attachment.

The Test Guideline will be used for a Level 2 *in vitro* test method to detect substances with ER agonist and antagonist activity, as described in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

or as attachment No.__

2. How will the work contribute to further international harmonisation of hazard and risk assessment? Please provide details below or as an attachment.

Development of an OECD Test Guideline for this test method will facilitate harmonization of *in vitro* ER TA agonist/antagonist assays used in Level 2 of the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals.

or as attachment No.__

3. How will the proposed project address issues and /or endpoints which are of major human health or environmental concerns? Please provide details below or as an attachment.

The proposed test method will serve as a screening assay for substances (agonists and antagonists) that may modulate human and/or wildlife endocrine systems via ER mediated pathways.

or as attachment No.__

4. Will the project have general support from OECD member countries or is the outcome relevant for just one or a few member countries / stakeholders? Provide details of the countries and the rationale for this view below.

☒ Many countries ☐ A few countries ☐ Only for the submitting country

Given the global interest in identifying test methods that are useful in screening chemicals for estrogen agonist/antagonist activity, it is anticipated that the proposed Test Guideline will be supported by all member countries.

5. If the Test Guideline is not intended for general use, indicate if the Test Guideline would be intended for:

☐ Specific (limited) applications such as pesticide usage, or

☐ for specific classes of chemicals (e.g. surfactants) rather than for chemicals in general.

6. If the expected outcome of this proposal is a Test Guideline or a Guidance Document, provide information on the intended use, applicability and limitations of the test method.

The test method is applicable for use as a Level 2 *in vitro* screening assay as described in the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals. Applicability and limitations will be determined taking into account the results of the validation study and the independent peer review, as well as SACATM and public comments.

7. Provide supporting information on the validation status (i.e. relevance and reliability) of the method. Principles for validation of test methods for OECD Test Guidelines are described in Guidance Document 34.

Provide justification and rationale for the test, including data.

If there are no or limited data available to support the reliability and relevance of the proposed test, indicate if validation work is included in the project.

If there is no need for validation provide a detailed justification.

Standardized LUMI-CELL® ER Assay protocols to detect ER agonists and antagonists were developed and evaluated for intra-(within)laboratory reproducibility using 16 ICCVAM reference substances (8 for agonist activity, 8 for antagonist activity) in standardization studies completed in July 2006. NICEATM, in conjunction with ECVAM and JaCVAM, is currently conducting an international validation study of the test method to further evaluate intralaboratory reproducibility and to evaluate interlaboratory reproducibility and comparative performance against the ICCVAM database of ER TA assays (http://iccvam.niehs.nih.gov/methods/endocrine/endocrine.htm). The validation study is scheduled for completion by April 2008 and a draft Test Guideline and supporting materials are scheduled for submission to the OECD Secretariat in early 2009.

ADDITIONAL INFORMATION

In this section please provide further information to allow the Working Group of National Coordinators of the Test Guidelines Programme to assess the suitability of the project for the workplan of the Test Guidelines Programme

1. If the expected outcome of the project proposal is a Test Guideline and is based on existing, regional or international documents such as guidelines, protocols or guidance material, please provide that information here or as an attachment.

The Test Guideline will be based on detailed test method protocols for which adequate validation studies have been conducted, and the recommendations of an independent expert panel after evaluation of available data and information of *in vitro* ER binding and transcriptional activation test methods for detecting substances with potential endocrine disrupting activity. This information was summarized in Background Review Documents (ICCVAM 2002a, b); the Panel Report was published in 2002 (both the BRDs and the Panel Report are available at http://iccvam.niehs.nih.gov/methods/endocrine/end_bckgnd.htm). ICCVAM considered the conclusions and recommendations from the Panel as well as SACATM, along with public comments received, when developing final test method recommendations that included essential test method components and a list of 78 reference substances that should be included in future validation studies for *in vitro* endocrine test methods for the detection of potential endocrine disruptors. These recommendations were published in the report, "ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor (ER) and Androgen Receptor Binding and Transcriptional Activation Assays," (ICCVAM 2003; available at http://iccvam.niehs.nih.gov/methods/endocrine/end_TMER.htm). In 2006, a revised list of reference substances was published (ICCVAM 2006; available at http://iccvam.niehs.nih.gov/docs/endo_docs/EDAddendFinal.pdf). A brief description of the background for the LUMI-CELL® ER Assay for the detection of ER agonists and antagonists is provided in Attachment 1.

or as attachment No.__

2. If Animal Welfare considerations are addressed in the project proposal, provide details below or as an attachment. Explain if the project is aimed at refining, reducing and/or replacing the use of animals.

If the project is not specifically developed for animal welfare purposes, indicate if the animal welfare considerations have been a component of the project proposal.

Indicate if animal welfare considerations are irrelevant to the project, for example for physico-chemical properties.

Availability of a test guideline based on an adequately validated and standardized test method protocols for this test method will avoid the need for animals to be killed as a source of ER for some commonly used ER binding assays. Most importantly, this test guideline will be able to be used to detect both the agonist and antagonist activity of substances. If substances are negative in the agonist assay, then either an antagonist assay or receptor binding assay would need to be conducted to determine if the substance binds to the receptor but does not result in transcription., When used in a tiered approach with weight-of-evidence decisions prior to conducting extensive *in vivo* testing, it may have the potential to reduce the overall number of animals required for testing.

or as attachment No.__

3. Provide information on expected or possible resource savings in member countries as a result of this project.

The proposed test method is amenable to efficiently screening large numbers of chemicals for possible ER agonist or antagonist activity. In addition, results from this test method may be used in a tiered testing, weight-of-evidence approach to reduce the number of *in vivo* tests required, which would also result in resource savings.

4. If the expected outcome of the proposed project is a Guidance Document or Detailed Review Paper, will it be directly linked to the development of a particular Test Guideline or a series of Test Guidelines?

☐ Yes, it is the initial step in the development of a new or revision of existing Guidelines.

☐ Yes, additional guidance is needed for the most appropriate selection of the Guidelines on the subject.

☐ No, the guidance is on issues related to testing or the development of Test Guidelines in general.

There is 1 attachment added to this form.

ASSESSMENT OF PROJECT PROPOSAL

(To be completed by all member countries /stakeholders except the submitter)

Country / Organisation:	

Representative: **(Preferably NC):**	

Taking into account the project information, requested above, does this project meet the needs of the member countries for addition to the workplan of the Test Guidelines Programme

☐ Yes ☐ No ☐ Further information needed

If the response is "No" or "Further information needed", please provide justification:

Remarks as appropriate, including further information needs, if any:

ATTACHMENT 1

BACKGROUND INFORMATION for the LUMI-CELL® ER ASSAY

In May of 2002, in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the ICCVAM Endocrine Disruptor Working Group (EDWG), the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) organized an independent evaluation of available data and information of *in vitro* test methods for detecting substances with potential endocrine disrupting activity. This information was summarized in Background Review Documents (ICCVAM 2002a, b, c, d), which were in turn reviewed by an international scientific expert panel (Panel). The Panel concluded that there were no adequately validated *in vitro* test methods for the detection of potential endocrine disruptors, but they did provide recommendations on the following:

- Specific test methods that should undergo further evaluation in validation studies and their relative priority for evaluation
- The adequacy of proposed minimum procedural standards (now referred to as *essential test method components*)
- The adequacy of protocols for specific test methods recommended for validation
- The adequacy and appropriateness of reference substances proposed for validation studies

ICCVAM considered the conclusions and recommendations from the Panel as well as the U.S. Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), along with public comments received, when developing final test method recommendations that included essential test methods components and a list of 78 reference substances that should be included in future validation studies for *in vitro* endocrine test methods for the detection of potential endocrine disruptors. These recommendations were published in the report, "ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor (ER)

and Androgen Receptor Binding and Transcriptional Activation Assays," (ICCVAM 2003). ICCVAM also requested the nomination of in *vitro test* methods for use in the U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP) as part of a tiered screening battery of *in vitro* and *in vivo* test assays that will be used to reach weight-of-evidence decisions on whether to conduct large multi-generational *in vivo* studies. In 2005, NICEATM re-assessed the commercial availability and cost for the 78 reference substances. During this assessment, NICEATM identified substances that had limited or no commercially availability and substances that were considered relatively expensive. ICCVAM considered this information and subsequently made revisions to the original list. The revisions are described in detail in the Report Addendum to the ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays (ICCVAM 2006).

In January 2004, NICEATM received a nomination from Xenobiotic Detection Systems, Inc. (XDS) for validation of the LUMI-CELL® ER assay. In April 2004, NICEATM received a submission for the LUMI-CELL® ER assay containing the historical development and rationale for the assay, assay protocols, and other supporting materials. In accordance with the ICCVAM nomination process, NICEATM conducted a pre-screen evaluation of the submission to determine the extent that it addressed the ICCVAM prioritization criteria, submission guidelines, and recommendations for the standardization and validation of *in vitro* endocrine disruptor test methods (ICCVAM 2003). Based on the NICEATM pre-screen evaluation, ICCVAM recommended that:

- The LUMI-CELL® ER assay should be considered as a high priority for validation studies as an *in vitro* test method for the detection of test substances with ER agonist and antagonist activity.
- To facilitate independent and timely standardization and validation studies, NICEATM should manage the needed studies by exercising a validation coordination option in its support contract.
- Validation studies should include coordination and collaboration with the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) to include one laboratory in each of the

three respective geographic regions supported by these three Centers (i.e., United States, European Union, Japan).

- In preparation for the interlaboratory validation study, XDS should conduct additional protocol standardization studies with an emphasis on conducting additional antagonist studies to more comprehensively demonstrate the suitability of the LUMI-CELL® ER assay for the detection of substances with ER antagonist activity.

In October 2005, NICEATM initiated a standardization study for the LUMI-CELL® ER protocols and to conduct additional antagonist testing. The primary goal of the study was to develop standardized protocols for detecting ER agonists and antagonists that can be easily transferred to other laboratories and be used to obtain reproducible results. Reference standards, controls, and methods for assessing cell viability were selected and standardized for both LUMI-CELL® ER assay agonist and antagonist protocols, and a historical database was generated for use in establishing quality control criteria. The adequacy of the standardized agonist and antagonist protocols was evaluated using a subset of the substances recommended by ICCVAM for the development, optimization, and/or validation of *in vitro* ER assays. The study was completed in July 2006 and results indicated that intralaboratory reproducibility and accuracy of the standardized protocols were adequate for moving forward to a multi-laboratory validation study. Results from the protocol standardization study were presented at the Society of Toxicology Annual Meeting (Deal et al. 2007a), and the 6th World Congress on Alternatives and Animal Use in the Life Sciences (Deal et al. 2007b).

NICEATM, ECVAM, and JaCVAM initiated the multi-laboratory, international validation study in March 2007, which is scheduled for completion in April 2008. The study design and timelines for the validation study were presented at the Society of Toxicology Annual Meeting (Stokes et al. 2007a), and the 6th World Congress on Alternatives and Animal Use in the Life Sciences (Stokes et al. 2007b).

References:

Deal F, Ceger P, Clark G, Gordon J, Charles J, Tice R, et al. 2007a. Results of a Protocol Standardization Study for the LUMI-CELL Estrogen Receptor (ER) Transcriptional Activation (TA) Bioassay. Society of Toxicology Annual Meeting, 2007.

Deal F, Ceger P, Clark G, Gordon J, Tice R, Stokes W. 2007b. Standardization of Protocols for the Validation of an In Vitro Estrogen Receptor Transcriptional Activation Assay. 6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007.

ICCVAM. 2002a. Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Binding Assays. Background Review Document. NIH Pub. No. 03-4504. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/end_bckgnd.htm [accessed 4 September 2007].

ICCVAM. 2002b. Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Binding Assays. Background Review Document. NIH Pub. No. 03-4506. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/end_bckgnd.htm [accessed 4 September 2007].

ICCVAM. 2002c. Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Estrogen Receptor Transcriptional Activation Assays. Background Review Document. NIH Pub. No. 03-4505. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/end_bckgnd.htm [accessed 4 September 2007].

ICCVAM. 2002d. Current Status of Test Methods for Detecting Endocrine Disruptors: *In Vitro* Androgen Receptor Transcriptional Activation Assays. Background Review Document. NIH Pub. No. 03-4507. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/end_bckgnd.htm [accessed 4 September 2007].

ICCVAM. 2003. ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503. Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/endocrine/end_TMER.htm [accessed 4 September 2007].

ICCVAM. 2006. Addendum to ICCVAM Evaluation of *In Vitro* Test Methods for Detecting Potential Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcriptional Activation Assays. NIH Pub. No. 03-4503 Research Triangle Park, NC: National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/endo_docs/EDAdendFinal.pdf [accessed 4 September 2007].

Stokes W, Bremer S, Jacobs M, Kojima H, Kanno J, Ceger P, et al. 2007a. NICEATM/ECVAM/JaCVAM Multi-phased International Validation Study of a Stably-Transfected Estrogen Receptor (ER) Transcriptional Activation (TA) Test Method. Society of Toxicology Annual Meeting, 2007.

Stokes W, Bremer S, Jacobs M, Ono A, Kojima H, Ceger P, et al. 2007b. NICEATM/ECVAM/JaCVAM Multi-phased International Validation Study of an In Vitro Estrogen Receptor Transcriptional Activation Assay to Detect Agonist and Antagonist Activity. 6th World Congress on Alternatives & Animal Use in the Life Sciences, 2007.