Substance Name	CASRN	ER TA Activity[1,2]
Oxazepam	604-75-1	PN
Pimozide	2062-78-4	PN
Reserpine	50-55-5	PN
Spironolactone	52-01-7	PN
L-thyroxine	51-48-9	PN

Abbreviations: CASRN = Chemical Abstracts Service Registry Number
[1]+++ Indicates that the substance was strongly active (EC_{50} value was <0.001 M); ++ indicates that the substance was moderately active (EC_{50} value was between 0.001 and 0.1 M); + indicates that the substance was weakly active (EC_{50} value was >0.1 M), or a positive response was reported without an EC50 value. The EC_{50} is the effective concentration that causes half-maximal activation of the receptor.
[2]PP = Presumed Positive; PN – Presumed Negative
[3]Included on the ECVAM Provisional Chemicals Selection List

2. SUBSTANCES FOR EACH PHASE OF THE VALIDATION OF ANTAGONIST PROTOCOLS

2.1 Phase I

- Training and laboratory qualification/protocol refinement by testing reference standards and controls
- Establish historical database for standards and controls by conducting ten independent experiments

Substance Name	CASRN
Raloxifene HCl (Reference Standard)[1]	82640-04-8
Flavone (Weak Positive Control)	525-82-6
17ß estradiol (Negative Control)	50-28-2

Abbreviations: CASRN = Chemical Abstracts Service Registry Number
[1]In the LUMI-CELL antagonist assay the reference standard, positive control and all test substances are run against a fixed concentration of 17ß-estradiol.

2.2 Phase IIa

- Four substances from ER minimum list tested independently three times in each laboratory for antagonism (12 total experiments)
- Substances to be well-characterized for ER TA antagonist activity (one strong positive, one moderately positive, one weakly positive and one negative); having no anticipated difficulties relating to solubility or cytotoxicity

Substance Name	CASRN	ER TA Antagonist Activity[1]
Tamoxifen[2]	10540-29-1	###
Dibenzo[*a.h*]anthracene[2]	53-70-3	##
p-n-nonylphenol[2]	104-40-5	#
Progesterone[2,3]	57-83-0	-

Abbreviations: CASRN = Chemical Abstracts Service Registry Number
[1]### Indicates that the substance was uniformly positive in multiple assays;
indicates that the substance was positive in the majority of assays in which it was tested;
# indicates that the substance was positive in the single assay in which it was tested;
#- indicates the substance was positive in one assay but was also negative in one or more assays;
- indicates that the substance was uniformly negative in multiple assays; PP = Presumed Positive; PN – Presumed Negative
[2]Tested for antagonism during LUMI-CELL protocol standardization.
[3]Included on the ECVAM Provisional Chemicals Selection List

2.3 Phase IIb

- Eight substances from ER minimum list tested independently three times in each laboratory for antagonism (24 total experiments)
- Substances to be well-characterized for ER TA antagonist activity (a mix of strong positive, moderately positive, weakly positive and negative); with some anticipated difficulties relating to solubility or cytotoxicity

Substance Name	CASRN	ER TA Antagonist Activity[1]	Anticipated Difficulty
Flavone[2]	525-82-6	###	
Apigenin	520-36-5	#	
Resveratrol	501-36-0	#	
Atrazine	1912-24-9	-	
Butylbenzyl phthalate[2]	85-68-7	-	
Corticosterone	50-22-6	-	

Substance Name	CASRN	ER TA Antagonist Activity[1]	Anticipated Difficulty
o,p-DDT[2,3]	789-02-6	#	Cytotoxic, can potentially "stick" to plastic tissue cultureware
Genistein[2,3]	446-72-0	#	Relatively insoluble

Abbreviations: CASRN = Chemical Abstracts Service Registry Number
[1]### Indicates that the substance was uniformly positive in multiple assays;
indicates that the substance was positive in the majority of assays in which it was tested;
# indicates that the substance was positive in the single assay in which it was tested;
#- indicates the substance was positive in one assay but was also negative in one or more assays; - indicates that the substance was uniformly negative in multiple assays;
PP = Presumed Positive; PN – Presumed Negative
[2]Tested for antagonism during LUMI-CELL protocol standardization.
[3]Included on the ECVAM Provisional Chemicals Selection List

2.4 Phase III

- Remaining 41 substances from ER minimum list tested once in each laboratory for antagonism (41 total experiments)

Substance Name	CASRN	ER TA Antagonist Activity[1]
4-hydroxytamoxifen[2]	68047-06-3	###
Raloxifene HCl[2]	82640-04-8	###
Bisphenol A	80-05-7	-
Coumestrol	479-13-0	-
Daidzein	486-66-8	-
p,p'-DDE	72-55-9	-
Dicofol	115-32-2	-
Diethylstilbestrol	56-53-1	-
17α- ethinyl estradiol[2]	57-63-6	-
Estrone	53-16-7	-
Fluoranthene	206-44-0	-
Kaempferol	520-18-3	-
p,p'-methoxychlor	72-43-5	-
Di - *n* -butyl phthalate[2]	84-74-2	-
Vinclozolin[2]	50471-44-8	PP
17α-Estradiol	57-91-0	PN
Actinomycin D	57-76-0	PN
4-androstenedione	63-05-8	PN
Bisphenol B	77-40-7	PN
2-*sec*-butylphenol	89-72-5	PN
Clomiphene citrate	50-41-9	PN
4-cumylphenol	599-64-4	PN

Substance Name	CASRN	ER TA Antagonist Activity[1]
Dexamethasone[2]	50-02-2	PN
5α–dihydrotestosterone	521-18-6	PN
17ß-estradiol[2]	50-28-2	PN
meso-hexestrol[2]	84-16-2	PN
Hydroxyflutamide	52806-53-8	PN
Kepone	143-50-0	PN
Morin	480-16-0	PN
Norethynodrel[2]	68-23-5	PN
4-*tert*-octylphenol[2]	140-66-9	PN
Ethyl paraben	120-47-8	PN
Phenobarbital	50-06-6	PN
Phenolphtalin	81-90-3	PN
Diethylhexyl phthalate	117-81-7	PN
Propylthiouracil	51-52-5	PN
Sodium azide	26628-22-8	PN
Testosterone[2]	58-22-0	PN
Methyl testosterone	28-18-4	PN
12 - *O* - Tetradecanoylphorbol-13-acetate	16561-29-8	PN
2,4,5-Trichlorophenoxyacetic acid	93-76-5	PN

Abbreviations: CASRN = Chemical Abstracts Service Registry Number
[1]### Indicates that the substance was uniformly positive in multiple assays;
indicates that the substance was positive in the majority of assays in which it was tested;
# indicates that the substance was positive in the single assay in which it was tested;
#- indicates the substance was positive in one assay but was also negative in one or more assays;
- indicates that the substance was uniformly negative in multiple assays; PP = Presumed Positive;
PN – Presumed Negative
[3]Included on the ECVAM Provisional Chemicals Selection List

2.5 Phase IV

- Testing of remaining 25 substances from ER list for antagonism tested once in one laboratory (may be divided between participating laboratories) (25 total experiments)

Substance Name	CASRN	ER TA Antagonist Activity[1]
Fenarimol	60168-88-9	#
Fluoxymestrone	76-43-7	-
Ammonium perchlorate	7790-98-9	PN
Apomorphine	58-00-4	PN

Substance Name	CASRN	ER TA Antagonist Activity[1]
Bicalutamide	90357-06-5	PN
Chrysin	480-40-0	PN
Cycloheximide	66-81-9	PN
Cyproterone acetate	427-51-0	PN
Finasteride	98319-26-7	PN
Haloperidol	52-86-8	PN
4-hydroxy androstenedione[2]	566-48-3	PN
Ketoconazole	65277-42-1	PN
Linuron[2]	330-55-2	PN
Medroxyprogesterone acetate	71-58-9	PN
Mifepristone[2]	84371-65-3	PN
Nilutamide	63612-50-0	PN
19-nortestosterone	434-22-0	PN
Oxazepam	604-75-1	PN
Pimozide	2062-78-4	PN
Procymidone	32809-16-8	PN
Reserpine	50-55-5	PN
Spironolactone	52-01-7	PN
L-thyroxine	51-48-9	PN
17ß-trenbolone	10161-33-8	PN
Flutamide[2]	13311-84-7	PP

Abbreviations: CASRN = Chemical Abstracts Service Registry Number

[1]### Indicates that the substance was uniformly positive in multiple assays;
indicates that the substance was positive in the majority of assays in which it was tested;
# indicates that the substance was positive in the single assay in which it was tested;
#- indicates the substance was positive in one assay but was also negative in one or more assays;
- indicates that the substance was uniformly negative in multiple assays; PP = Presumed Positive;
PN – Presumed Negative

[2]Included on the ECVAM Provisional Chemicals Selection List

DRAFT STATEMENT OF WORK

VALIDATION OF THE LUMI-CELL® ESTROGEN RECEPTOR TRANSCRIPTIONAL ACTIVATION ASSAYS FOR THE DETECTION OF ESTROGEN RECEPTOR AGONISTS AND ANTAGONISTS

Prepared by

Integrated Laboratory Systems, Inc.

for Contract (TBA) Supporting

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

1 November 2006

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STATEMENT OF WORK

Validation of the LUMI-CELL® Estrogen Receptor (ER) Transcriptional Activation (TA) Assay for the Detection of ER Agonists and Antagonists

1.0 PROJECT OBJECTIVES AND GENERAL REQUIREMENTS

1.1 Project Objectives

This Statement of Work specifies the procedures that Xenobiotic Detection Systems, Inc. (XDS) will use as a participating laboratory in the conduct of an international validation study of the LUMI-CELL® estrogen receptor (ER) transcriptional activation (TA) assay (LUMI-CELL® ER assay) for the detection of ER agonists and antagonists. The list of 78 ICCVAM recommended substances, which possess varying degrees of ER agonist and/or antagonist activity (ICCVAM 2002; ICCVAM 2003; Federal Register, Vol. 71, No. 51, pp. 13597-13598, March 16, 2006;), will be used in this validation study to characterize the reliability and relevance of the LUMI-CELL® ER assay.

1.2 General Capabilities

XDS will adhere to this Statement of Work throughout the validation study and is capable of the following:

1. Providing Standard Operating Procedures (SOPs, see **Section 1.4**) for the performance of the LUMI-CELL® ER TA agonist and antagonist assays
2. Conducting all aspects of the study in accordance with Good Laboratory Practices (GLP)
3. Providing study reports and all associated data from studies outlined in this document (e.g.,) to the Study Management Team (SMT) through the designated contacts listed in **Section 2.2**.

1.3 Guidelines

The Project Officer and designated members of the SMT may inspect the XDS testing facilities and audit any procedures. XDS should notify the SMT of any changes in Key Personnel (see **Section 3.1.1**)

1.4 Definitions

Good Laboratory Practices (GLPs): Regulations governing the conduct, procedures, and operations of toxicology laboratories developed to assure the quality and integrity of the data and to address such matters as organization and personnel, facilities, equipment, facility operations, and study conduct (OECD, 1998).

Standard Operating Procedures (SOPs): Written documents that describe in sufficient detail the routine procedures to be followed for a specific operation, analysis, or action. Consistent use of an approved SOP ensures conformance with organizational practices; reduced work effort; reduction in error occurrences; and improved data comparability, credibility, and defensibility, SOPs also serve as resources for training and for ready reference and documentation of proper procedures.

Statement of Work: A description of all phases of the validation study and the purpose of the procedures; also provides guidance for the preparation of reports.

Test Method Protocols: Specific and detailed guides for performing the LUMI-CELL® ER assay for the detection of ER agonists and antagonists.

Test Substances: Chemicals supplied to XDS that are coded and distributed such that only the Project Officer, the SMT, and the Substance Inventory and Distribution Management (identified in **Section 2.2.2**) have knowledge of the identity of each test substance. The test substances will be purchased, aliquoted, coded, and distributed by the Substance Inventory and Distribution Management, under the guidance of the Project Officer and the SMT.

2.0 ORGANIZATION

2.1 Validation Study Sponsors

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

The European Centre for the Validation of Alternative Methods (ECVAM)

The Japanese Center for the Validation of Alternative Methods (JaCVAM)

2.2 Study Management

2.2.1 International Study Management Team

2.2.1.1 *NICEATM*

Dr. William Stokes (NICEATM/NIEHS) – Co-Chair/Project Officer

Dr. Raymond Tice (NICEATM/NIEHS) – Co-Chair

Dr. David Allen (NICEATM/ILS) – NICEATM Principal Investigator

Mr. Frank Deal (NICEATM/ILS) – Project Coordinator

Ms. Patricia Ceger (NICEATM/ILS) – Assistant Project Coordinator

Mailing Address:

79 T.W. Alexander Drive

Bldg. 4401, MD-EC-17

3rd Floor, Room 3126

P.O. Box 12233

Research Triangle Park, NC 27709

2.2.1.2 *ECVAM*

Dr. Susanne Bremer

Dr. Miriam Jacobs

Mailing Address:

Joint Research Center – European Commission

21020 Ispra (VA), Italy

2.2.1.3 *JaCVAM*

Dr. Hajime Kojima

Dr. Jun Kanno

Mailing Address:

National Institute of Health Sciences

Kamiyouga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan

2.2.2 Substance Inventory and Distribution Management

Dr. Cynthia Smith

Chemistry Resources Group Leader

Mailing Address:

NIEHS

111 Alexander Dr.

Research Triangle Park, NC 27709

2.2.3 Contract Management

Mr. Doug Winters (NICEATM/ILS) – NICEATM Project Manager

Ms. Kelly Inman (ILS) – Contract Specialist

Mailing Address:

ILS, Inc.

P.O. Box 13501

Research Triangle Park, NC 27709

3.0 TESTING FACILITY AND KEY PERSONNEL

3.1 Competence and Capabilities

XDS should be competent in the conduct of the LUMI-CELL® ER assay and will provide competent personnel, adequate facilities, equipment, supplies, proper health and safety guidelines, and quality assurance procedures.

3.1.1 Personnel

3.1.1.1 *Facility Management*

XDS facility management is responsible for establishing scientific guidelines and procedures, training and supervision of technical staff, and evaluation of results. The facility manager must maintain training files that include qualifications, experience, and a job description for each individual involved in the LUMI-CELL® ER assay validation study.

3.1.1.2 *Study Director*

The Study Director has the overall responsibility for the LUMI-CELL® ER assay validation study conducted at XDS. The Study Director should be responsible for providing GLP compliant Standard Operating Procedures (OECD 1998) for use during the validation study.

3.1.1.3 *Director of Quality Assurance (QA)*

The Director of QA should monitor the validation study to assure compliance with GLP requirements for all aspects of the validation study.

3.1.1.4 *Consultant(s)*

Consultants are scientists or other professionals of appropriate education, training, and experience with the LUMI-CELL® ER assay who provide scientific guidance to XDS.

3.1.1.5 *Laboratory Technician(s)*

Each individual engaged in the conduct of or responsible for the supervision of the assay should have education, training, and experience, or combination thereof, to enable that individual to perform the assigned duties. Technical ability must be documented as per GLP requirements.

3.1.1.6 *Safety Officer*

A designated Safety Officer (someone not involved in the actual conduct of the validation study) will receive the blinded (coded) test substances from Substance Inventory and Distribution Management and transfer the substances to the Study Director. A sealed health and safety information package will accompany the coded test substances and the Safety Officer should retain the package until the completion of the validation study. The Safety Officer will promptly notify the SMT Project Coordinator if this is opened at any time during the validation study.

245 3.1.2 Facilities, Equipment, and Supplies

246 3.1.2.1 *Cell Culture Laboratory*

247 A designated cell culture laboratory should be available to ensure that the LUMI-CELL® ER
248 assay can be performed using good cell culture practice (Coecke et al. 2005). Access to the
249 validation study assays and reference substances should be restricted to appropriate personnel as
250 determined by XDS management.

251 3.1.2.2 *Equipment*

252 **Appendices B** and **C** detail the types of equipment that are required for conducting the LUMI-
253 CELL® ER agonist and antagonist assays. All equipment maintenance and calibration should be
254 routinely performed and documented as per GLP guidelines (OECD. 1998).

255 3.1.3 Health and Safety

256 XDS should conform to all local, state, and federal statutes in effect at the time of this validation
257 study. The designated Safety Officer should be the point of contact for health and safety issues.

258 3.1.4 Quality Assurance

259 XDS should conduct this validation study in compliance with Good Laboratory Practice (GLP)
260 Standards (OECD 1998). The QA unit (as per GLPs) should review the protocol and audit the in-
261 life phase, study workbook, and final report data.
262 The final reports for all phases of the validation study should be audited by the XDS QA unit for
263 GLP compliance and a QA Statement should be provided with each final report. Each final report
264 should identify: 1) the phases and data inspected, 2) the dates of inspection, and 3) the dates
265 findings were reported to the Study Director and XDS management. The QA Statement should
266 identify whether the methods and results described in the final report accurately reflect the raw
267 data produced during the validation study.
268

4.0 TEST PHASES AND SCHEDULE

4.1 Study Timeline and Deliverables

4.1.1 Study Timeline

TASK	ACTIVITIES	TIMELINE
Phase I	• Development of automated testing procedures (XDS) • Qualification/protocol refinement by testing reference standards and controls • Establish historical database for standards and controls by conducting independent experiments (10 each for the agonist and antagonist protocols) • Submission of draft report and review by SMT	Nov. 06 – Mar. 07
Phase IIa	• Four substances from ER minimum list tested independently three times for agonism and antagonism (24 total experiments to include the quantitative assessment of cell viability in parallel plates in the agonist and antagonist assays) • Submission of draft report and review by SMT	Apr. 07 – May 07
Phase IIb	• Eight substances from ER minimum list tested independently three times (48 total experiments) • Submission of draft report and review by SMT	Jun. 07 – Aug. 07
Phase III	• Remaining 41 substances from ER minimum list tested once for agonism and antagonism (82 total experiments) • Submission of draft report and review by SMT	Sep. 07 – Oct. 07
Phase IV	• Testing of remaining 25 substances from ER list for agonism and antagonism (XDS only), (50 total experiments) • Submission of draft report and review by SMT	Nov. 07

4.1.2 Study Deliverables

4.1.2.1 *Test Results (Phases I-IV)*

XDS will provide raw and quality control data in electronic format (i.e., email with attachments) to the SMT Project Coordinator on a weekly basis during *in-life* (i.e., during those weeks when LUMI-CELL® ER bioassay data is being collected and/or analyzed) portions of the study.

4.1.2.2 *Study Status Reports (Phases I-IV)*

XDS will provide study status reports during each phase of the study to the SMT Project Coordinator on a biweekly basis. These reports will be provided in electronic format (i.e., email with attachments) and will include raw and quality control data as the study progresses. Reports should contain the information outlined in **Appendix A**.

4.1.2.3 *Draft Reports (Phases I-IV)*

At the conclusion of each phase of the study, a draft report will be provided by the Study Director to the SMT Project Coordinator. The draft report will be provided electronically in Word®. Reports should contain the information outlined in **Appendix A** and should follow the recommended formats and styles provided in the "Style Guide for LUMI-CELL® ER Validation Study Laboratory Reports and Documents" (**Appendix D**).

4.1.2.4 *Final Reports (Phases I-IV)*

Each draft report that is approved by the SMT will be followed by a final report, which has been reviewed by the QA Officer for GLP compliance, for each phase of the study. The final report will be provided electronically in Word® by the Study Director to the SMT Project Coordinator. Copies of the audited Study Workbook pages should submitted in electronic format (i.e., pdf files) as an attachment to the report. However, completion of the final report is not required prior to initiation of the next phase of the validation study.

4.1.3 Estimated Due Dates for Reports

ESTIMATED DUE DATES					
REPORTS	**PHASE I**	**PHASE IIa**	**PHASE IIb**	**PHASE III**	**PHASE IV**
Study Status	*	*	*	*	*
Draft	Mar., 2007	May, 2007	Aug., 2007	Oct., 2007	Nov., 2007
Final	Apr., 2007	Jun., 2007	Sep., 2007	Nov., 2007	Dec., 2007

*Study status reports will be provided biweekly during each phase of the study.

4.2 Phase I

This phase will be used for initial laboratory qualification/protocol refinement by all participating laboratories and is limited to the testing of reference standards, positive controls, and the solvent control. The results will be used to establish an historical database in each laboratory for reference standards and controls.

4.2.1 Initial Laboratory Qualification/Protocol Refinement

Repetitive testing of agonist and antagonist reference standards and positive/solvent controls will be used to demonstrate proficiency with the LUMI-CELL® ER assay, demonstrate intralaboratory repeatability and intra- and inter-laboratory reproducibility, and establish an historical database. Results will be compared to historical control data established during the LUMI-CELL® ER Protocol Standardization Study. If there is excessive variation of reference standard and control data within or among the participating laboratories, the SMT (through the designated contacts) will work with the laboratories to determine cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.2.2 Criteria for Advancing to Phase II

The SMT will decide when all laboratories will advance to Phase II of the validation study, based on the following criteria:

- Data, reviewed by the QA Officer (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated by the participating laboratories
- A suitable historical negative and positive control database has been established

4.3 Phase II

Phase II provides for initial laboratory qualification using procedures that have been refined in Phase I, but is also the initial phase for testing substances from the ICCVAM list of 78 reference substances recommended for validation of ER TA assays. In this phase, four coded test substances (Phase IIa) and then eight coded test substances (Phase IIb) will be tested in all three participating laboratories. Acceptance criteria for experimental data for Phase IIa will be based on the historical database established in Phase I for reference standards and controls. Reference

standard and control data collected during Phase IIa will also be included in the historical database, which will then be used to establish acceptance criteria for Phase IIb.

4.3.1 Phase IIa Limited Testing of Protocol and Protocol Refinement

After a range-finding assay is completed for each of the four coded test substances in Phase IIa, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each test substance should not begin until the starting concentrations have been approved, and they should not be modified without approval from the SMT. The comprehensive concentration-response experiment should be performed three times, once on each of three different days. Laboratories will calculate EC_{50} values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation within or among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.3.2 Criteria for Advancing to Phase IIb

The SMT will decide when all laboratories will advance to the Phase IIb of the validation study, based on the following criteria:

- Data, reviewed by the QA Officer (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated by the participating laboratories

4.3.3 Phase IIb Testing of Protocol and Protocol Refinement

Phase IIb includes the testing of eight coded substances and is the last phase for evaluating any protocol refinements from Phase I or IIA.

After a range-finding assay is completed for each of the eight coded test substances in Phase IIb, recommended starting concentrations for the comprehensive concentration-response experiment and the rationale for their selection are to be sent to the SMT for review and approval. The comprehensive concentration-response experiment for each test substance should not begin until the starting concentrations have been approved and should not be modified without approval of the SMT. The comprehensive concentration-response experiment should be performed three times, once on each of three different days. Laboratories will calculate EC_{50} values for the agonist reference standard or IC_{50} values for the antagonist reference standard (in µg/mL). Laboratories will also calculate EC_{50} or IC_{50} values (in µg/mL), when possible, for coded test substances. These data, along with all quality control, raw, derived and supporting data, will be reported to the SMT through the designated contacts. If there is excessive variation within or among participating laboratories, the SMT will work with the laboratories to determine the cause and recommend appropriate actions needed to reduce variation. Statements of Work, Test Method Protocols, and SOPs will be revised, if necessary, and testing repeated until acceptable proficiency is demonstrated (i.e., acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility). The SMT may convene a teleconference with appropriate participants of the validation study to discuss information concerning the progression of the validation study.

4.3.4 Criteria for Advancing to Phase III

The SMT will decide when all laboratories will advance to the Phase III of the validation study, based on the following criteria:

- Data, reviewed by the QA Officer (or independent reviewer), has been received by the SMT
- All participating laboratories have submitted acceptable draft reports as outlined in **Section 4.1.2.2**.
- Acceptable intralaboratory repeatability and intra- and inter-laboratory reproducibility has been demonstrated within and among the participating laboratories