アゴニスト試料は、スポンサーより表１０．の試料を提供された。

表１０．アゴニスト試験で用いる試薬一覧

スポンサーID	状態	保存方法	受け入れ日	受け入れ者	Comments
H0009	Solid	Room Temp	4-Dec-08	Nakamura	
H0010	Solid	Room Temp	4-Dec-08	Nakamura	
H0011	Solid	Room Temp	4-Dec-08	Nakamura	
H0012	Solid	Room Temp	4-Dec-08	Nakamura	
H0013	Solid	Room Temp	4-Dec-08	Nakamura	
H0014	Solid	-4℃	4-Dec-08	Nakamura	
H0015	Solid	Room Temp	4-Dec-08	Nakamura	
H0016	Solid	Room Temp	4-Dec-08	Nakamura	

＊　標準及びコントロール物質は、前回購入したものを使用する。

アンタゴニスト試料は、スポンサーより表１１．の試料を提供された。

表１１．アンタゴニスト試験で用いる試薬一覧

スポンサーID	状態	保存方法	受け入れ日	受け入れ者	Comments
H00017	Liquid	Room Temp	4-Dec-08	Nakamura	
H00018	Solid	Room Temp	4-Dec-08	Nakamura	
H00019	Solid	Room Temp	4-Dec-08	Nakamura	
H00020	Solid	-4℃	4-Dec-08	Nakamura	
H00021	Solid	Room Temp	4-Dec-08	Nakamura	
H00022	Solid	-4℃	4-Dec-08	Nakamura	
H00023	Solid	-4℃	4-Dec-08	Nakamura	
H00024	Solid	Room Temp	4-Dec-08	Nakamura	

＊　標準及びコントロール物質は、前回購入したものを使用する。

3）結果

Lumi-cell ER 細胞の3）－1　Substance solubility、3）－2　Range finding testing、3）－3　Comprehensive testing のアゴニスト活性及びアンタゴニスト活性についての EC_{50} もしくは、IC_{50} 及び Dose response curve の経歴データベースを作成した。

3）－1　Substance solubility の結果は、下記の通りであった。

アゴニスト試料

・　H0009　1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media

- H0010　1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0011　1mg/ml in DMSO, 0.01mg/ml in DMSO/aqueous cell culture media
- H0012　10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0013　10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0014　10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0015　10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media
- H0016　10mg/ml in DMSO, 0.1mg/ml in DMSO/aqueous cell culture media

アンタゴニスト試料

- H0017　2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0018　2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0019　2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0020　2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0021　20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0022　20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media
- H0023　2mg/ml in DMSO, 0.02mg/ml in DMSO/aqueous cell culture media
- H0024　20mg/ml in DMSO, 0.2mg/ml in DMSO/aqueous cell culture media

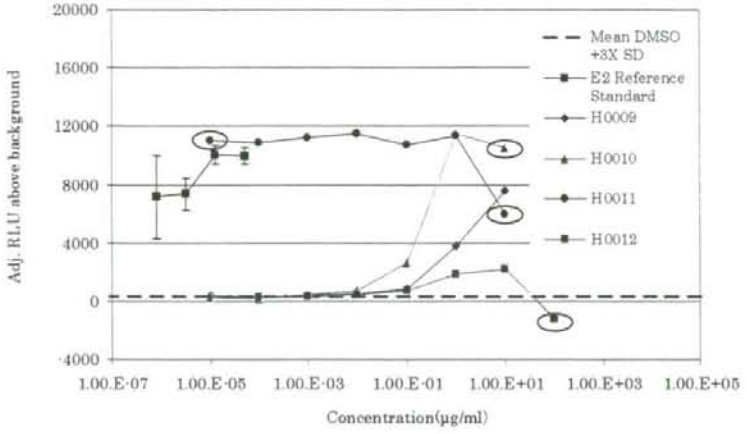
3）－2　Range finding testing の結果は、表１２．及び表１３．図１１．図１２．の通りであった。

表１２．アゴニスト試料における Range finding testing

Experiments: Phase IIb Range Finder Testing					
試験 I.D.	試料 Code	日付	Plate Induction	採用の有無	Rationale for Unacceptability
AgRF 1	H0009	23 Sep. 08	8.3	Used	Acceptable
	H0010	23 Sep. 08		Used	Acceptable
	H0011	23 Sep. 08		Used	Acceptable
	H0012	23 Sep. 08		Used	Acceptable
AgRF 2	H0013	23 Sep. 08	5.9	Used	Acceptable
	H0014	23 Sep. 08		Used	Acceptable
	H0015	23 Sep. 08		Used	Acceptable
	H0016	23 Sep. 08		Used	Acceptable

表１３．アゴニスト試料における Range finding testing

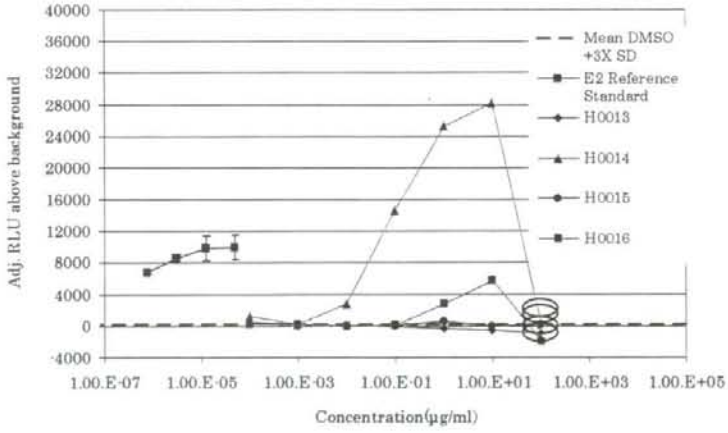
Experiments: Phase IIb Range Finder Testing					
試験 I.D.	試料 Code	日付	Plate Induction	採用の有無	Rationale for Unacceptability
AntRF 1	H0017	23 Sep. 08	8.7	Used	Acceptable
	H0018	23 Sep. 08		Used	Acceptable
	H0019	23 Sep. 08		Used	Acceptable
	H0020	23 Sep. 08		Used	Acceptable
AntRF 2	H0021	23 Sep. 08	8.4	Used	Acceptable
	H0022	23 Sep. 08		Used	Acceptable
	H0023	23 Sep. 08		Used	Acceptable
	H0024	23 Sep. 08		Used	Acceptable



◯ ; Starting Concentration used for Comprehensive testing

H0009, H0010,H0011,H0012 is Half-Log serial dilution

図１１－１．Range finder testing（アゴニスト試験）のグラフ

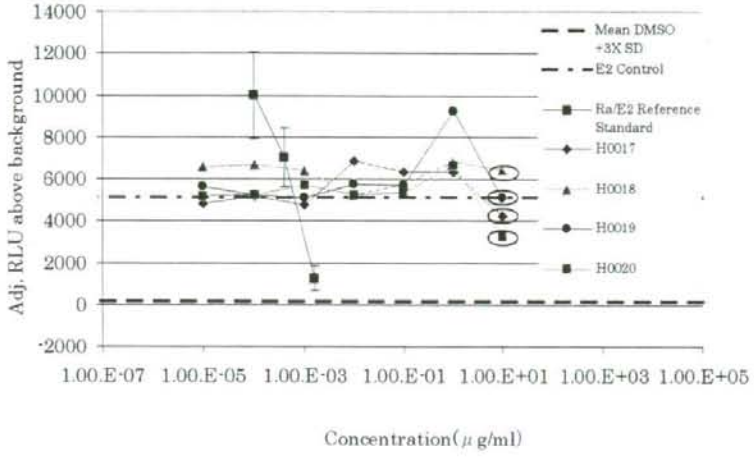


◯ ; Starting Concentration used for Comprehensive testing

H0013,H0015,H0016 is Double serial dilution

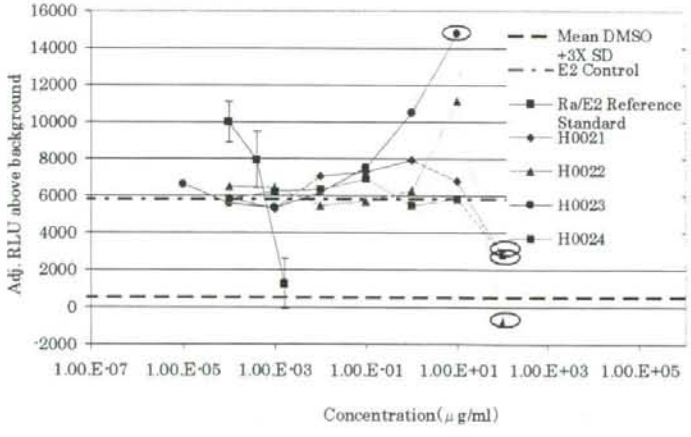
H0014 is Half-Log serial dilution

図１１－２．Range finder testing（アゴニスト試験）のグラフ



◯ ; Starting Concentration used for Comprehensive testing
H0017～H0020 is Double serial dilution

図１２－１．Range finding testing（アンタゴニスト試験）のグラフ



◯ ; Starting Concentration used for Comprehensive testing
H0021～H0024 is Double serial dilution

図１２－２．Range finding testing（アンタゴニスト試験）のグラフ

3－3　Comprehensive testing の結果は、表１４．及び表１５．図１３．図１４．の通りであった。

表１４．Comprehensive testing（アンタゴニスト試験）の結果一覧

Experiments: Phase IIb Comprehensive Testing;Agonist						
試験 I.D.	試料 Code	日付	Plate Reduction	IC_{50} (μg/mL)	採用の有無	不採用の理由
CT 1-1	H0009	07-Nov-08	3.5	0.83	Used	Acceptable
	H0010			0.27	Used	Acceptable
CT 2-1	H0011	07-Nov-08	1.6	$1.7x10^{-6}$	Repeated	Fail[1]
	H0012			NA	Repeated	Fail[1]
CT 3-1	H0013	07-Nov-08	4.1	$1.1x10^{+1}$	Used	Acceptable
	H0014			0.051[2]	Used	Acceptable
CT 4-1	H0015	07-Nov-08	2.2	21	Repeated	Fail[2]
	H0016			0.80	Repeated	Fail[2]
CT 1-2	H0009	21-Nov-08	4.3	0.99	Used	Acceptable
	H0010			0.26	Used	Acceptable
CT 2-2	H0011	21-Nov-08	4.9	$3.0x10^{-6}$	Used	Acceptable
	H0012			0.49[2]	Used	Acceptable
CT 3-2	H0013	21-Nov-08	4.5	$1.9x10^{+1}$	Repeated	Fail[3]
	H0014			0.12	Repeated	Fail[3]
CT 4-2	H0015	21-Nov-08	4.2	Positive[4]	Used	Acceptable
	H0016			1.4[2]	Used	Acceptable
CT 1-3	H0009	04-Dec-08	3.1	0.43	Used	Acceptable
	H0010			0.21	Used	Acceptable
CT 2-3	H0011	04-Dec-08	3.1	$1.9x10^{-6}$	Used	Acceptable
	H0012			0.79	Used	Acceptable
CT 3-3	H0013	04-Dec-08	3.0	1.5	Repeated	Fail[5]
	H0014			0.0050	Repeated	Fail[5]
CT 4-3	H0015	04-Dec-08	3.0	Negative	Used	Acceptable
	H0016			1.3	Used	Acceptable
CT 2-4	H0011	19-Dec-08	4.7	$2.6x10^{-6}$	Used	Acceptable
	H0012			0.41	Used	Acceptable
CT 3-4	H0013	19-Dec-08	4.8	$1.9x10^{+1}$	Used	Acceptable
	H0014			0.16	Used	Acceptable

Experiments: Phase IIb Comprehensive Testing;Agonist						
試験 I.D.	試料 Code	日付	Plate Reduction	IC_{50} (μg/mL)	採用の有無	不採用の理由
CT 4-4	H0015	19-Dec-08	3.3	Negative	Used	Acceptable
	H0016			8.2[2]	Used	Acceptable
CT 3-5	H0013	25-Dec-08	3.3	$2.5x10^{+1}$	Used	Acceptable
	H0014			0.14[2]	Used	Acceptable

NA = Not applicable, EC_{50} values are not calculated for substances tested on plates not meeting acceptance criteria.

[1]The reason for the failure is because 1) DMSO Control / E2Reference Standard EC50/ Methoxchlor is higher than the criteria and 2) Induction is lower than the criteria.

[2] The reason for the failure is because 1) DMSO Control / Methoxchlor is higher than the criteria and 2) Induction is lower than the criteria

[3] The reason for the failure is because E2 Reference Standard curve did not show sigmoid shape.

[4] Positive for agonism at 25×10^{0} μg/mL

[5] The reason for the failure is because Methoxchlor Control is higher than the criteria.

表１５．Comprehensive testing（アンタゴニスト試験）の結果一覧

Experiments: Phase IIb Comprehensive Testing;Antagonist						
試験 I.D.	試料 Code	日付	Plate Reduction	IC_{50} (μg/mL)	採用の有無	不採用の理由
CT 1-1	H0017	14-Nov-08	9.8	positive[1]	Used	Acceptable
	H0018			positive[2]	Used	Acceptable
CT 2-1	H0019	14-Nov-08	8.8	negative	Used	Acceptable
	H0020			negative	Used	Acceptable
CT 3-1	H0021	14-Nov-08	13	550	Used	Acceptable
	H0022			25	Used	Acceptable
CT 4-1	H0023	14-Nov-08	13	negative	Used	Acceptable
	H0024			positive[3]	Used	Acceptable

Experiments: Phase IIb Comprehensive Testing:Antagonist						
試験 I.D.	試料 Code	日付	Plate Reduction	IC_{50} (μg/mL)	採用の有無	不採用の理由
CT 1-2	H0017	29-Nov-08	8.2	negative	Repeated	Fail[4]
	H0018			negative	Repeated	Fail[4]
CT 2-2	H0019	29-Nov-08	10	negative	Repeated	Fail[4]
	H0020			positive[5]	Repeated	Fail[4]
CT 3-2	H0021	29-Nov-08	9.7	28	Used	Acceptable
	H0022			26	Used	Acceptable
CT 4-2	H0023	29-Nov-08	12	negative	Used	Acceptable
	H0024			positive[2]	Used	Acceptable
CT 1-3	H0017	12-Dec-08	10	negative	Used	Acceptable
	H0018			negative	Used	Acceptable
CT 2-3	H0019	12-Dec-08	11	negative	Used	Acceptable
	H0020			positive[5]	Used	Acceptable
CT 3-3	H0021	12-Dec-08	9.6	18	Used	Acceptable
	H0022			27	Used	Acceptable
CT 4-3	H0023	12-Dec-08	12	negative	Used	Acceptable
	H0024			positive[1]	Used	Acceptable
CT 1-4	H0017	19-Dec-08	8.4	positive[1]	Used	Acceptable
	H0018			positive[6]	Used	Acceptable
CT 2-4	H0019	19-Dec-08	8.8	negative	Used	Acceptable
	H0020			positive[5]	Used	Acceptable

[1] positive for antagonism at 1.0*10+1μg/mL & 5.0*10+0μg/mL

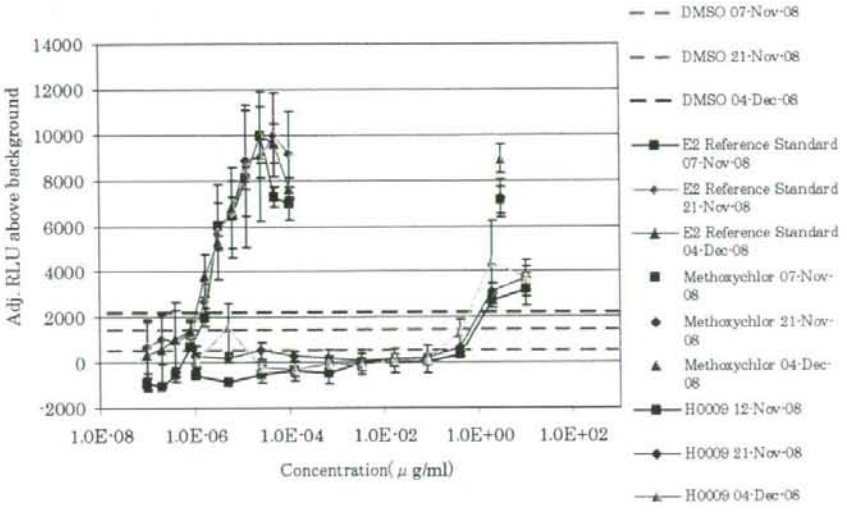
[2] positive for antagonism at 1.0*10+1μg/mL & 5.0*10+0μg/mL & 2.5*10+0μg/mL

[3] positive for antagonism at 1.0*10+1μg/mL & 5.0*10+0μg/mL & 2.5*10+0μg/mL & 1.25*10+0μg/ml & 6.3*10-1μg/ml

[4] for the failure is because Raloxifene/E2 Reference Standard IC50 is lower than the criteria.

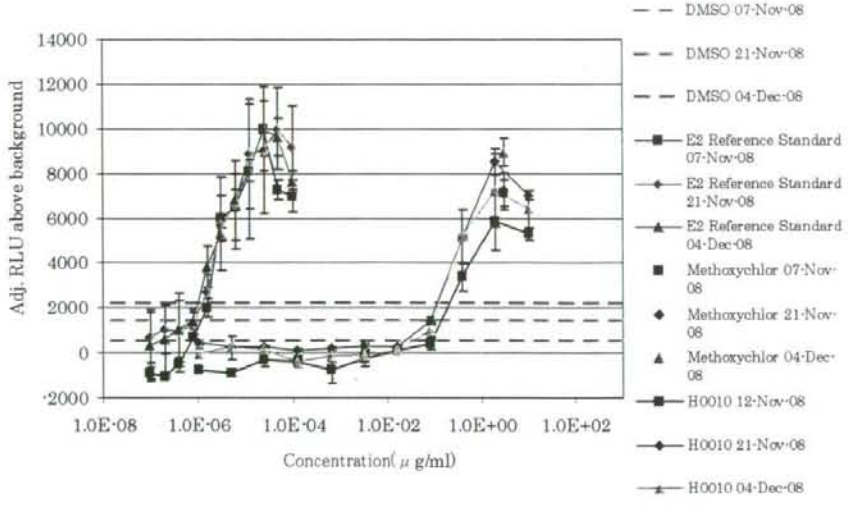
[5] positive for antagonism at 1.0*10+1μg/mL

[6] positive for antagonism at 5.0*10+0μg/mL



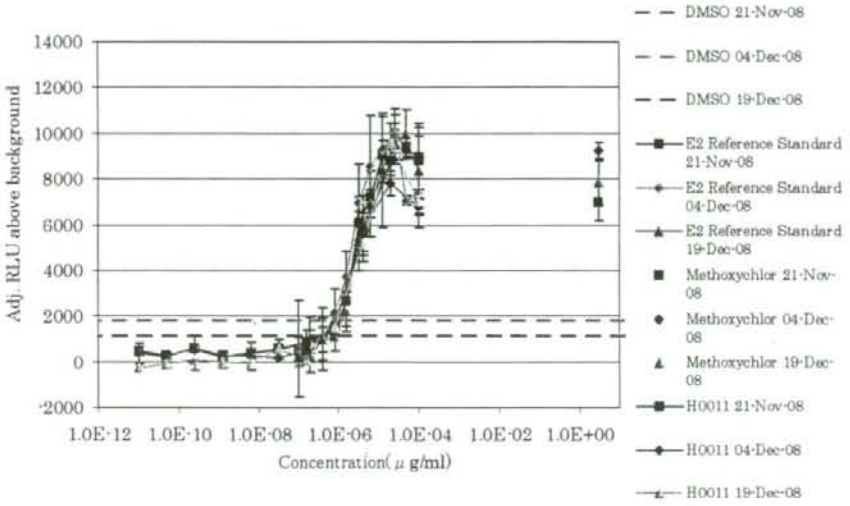
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１３－１．Comprehensive testing（アゴニスト試験）H0009 グラフ



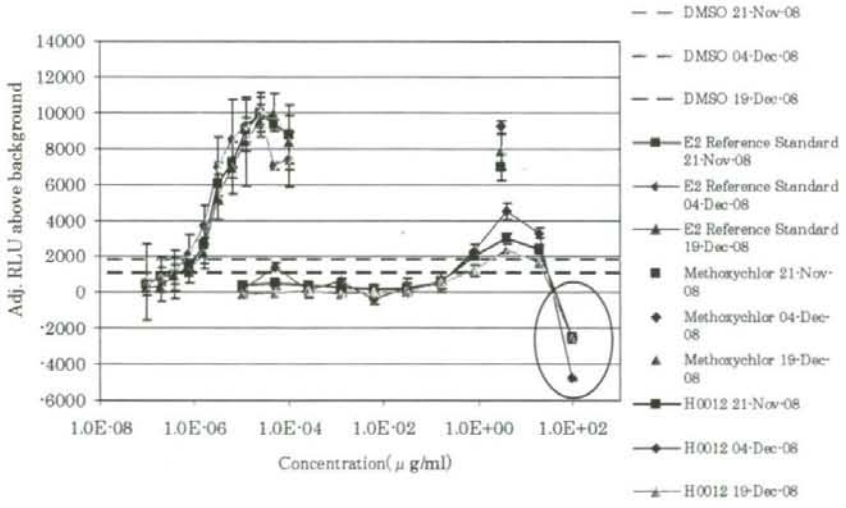
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１３－２．Comprehensive testing（アゴニスト試験）H0010 グラフ



* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

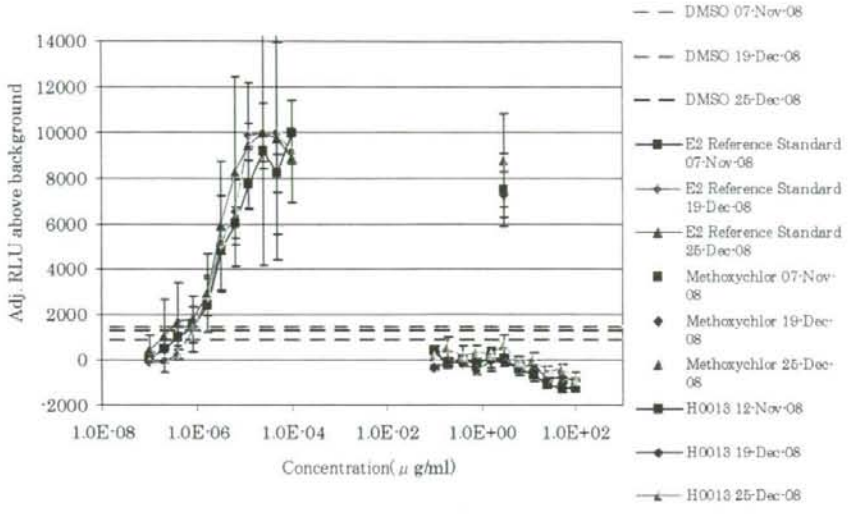
図１３－３．Comprehensive Testing（アゴニスト試験）H0011 グラフ



○ : Deleted 1 high concentration points to calculate EC_{50} of H0012.

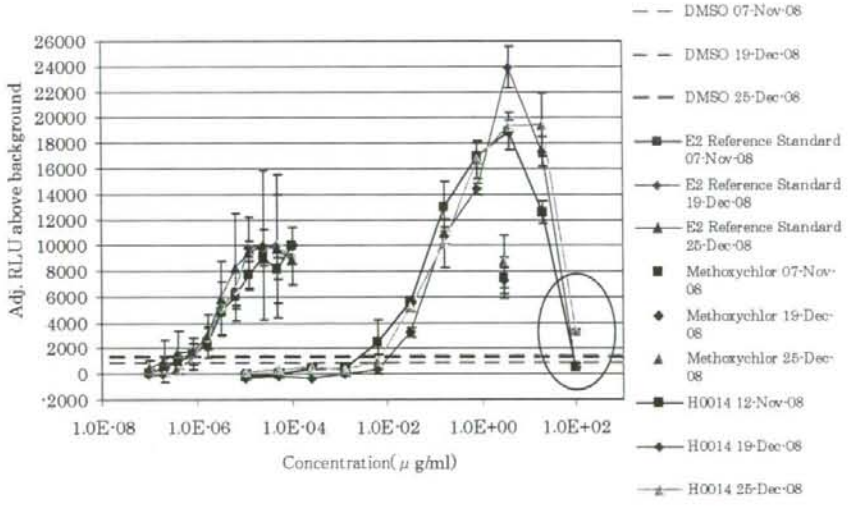
[1] Line represents the mean of three E2 replicates plus three times the standarddeviation of the E2 mean

図１３－４．Comprehensive Testing（アゴニスト試験）H0012 グラフ



* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

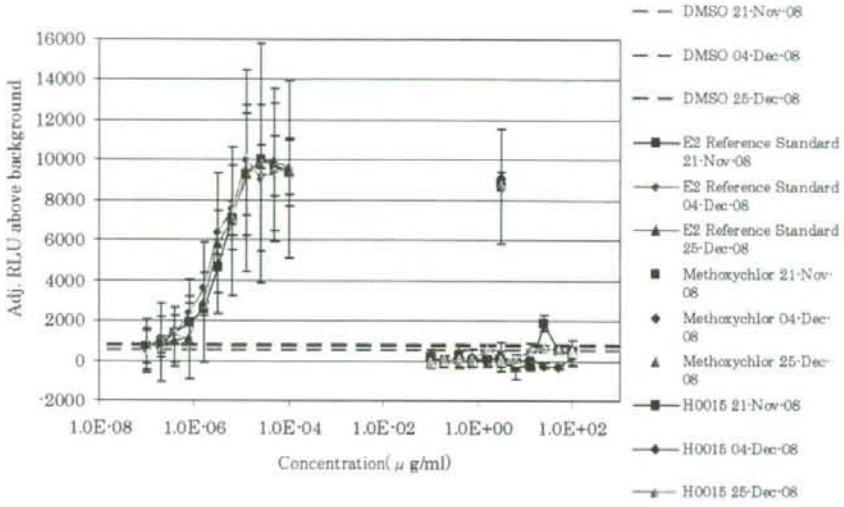
図１３－５．Comprehensive testing（アゴニスト試験）H0013 グラフ



○: Deleted 1 high concentration points to calculate EC_{50} of H0014.

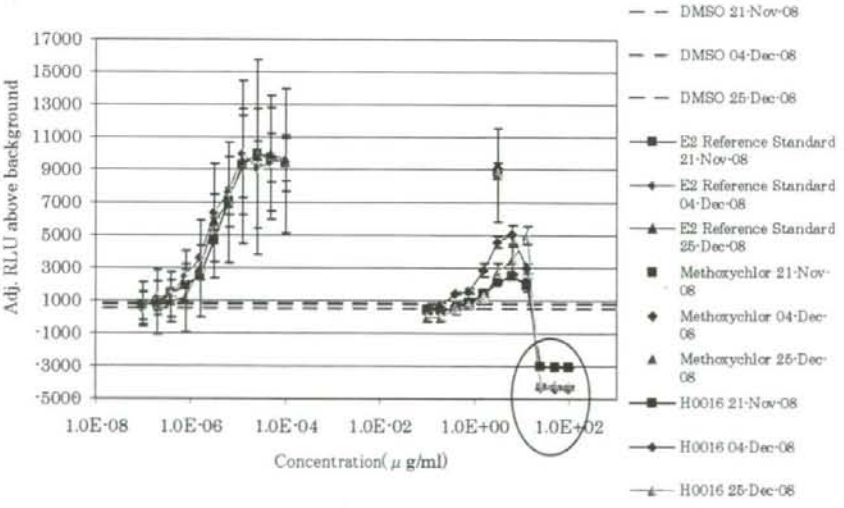
[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the mean of E2.

図１３－６．Comprehensive testing（アゴニスト試験）H0014 グラフ



* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

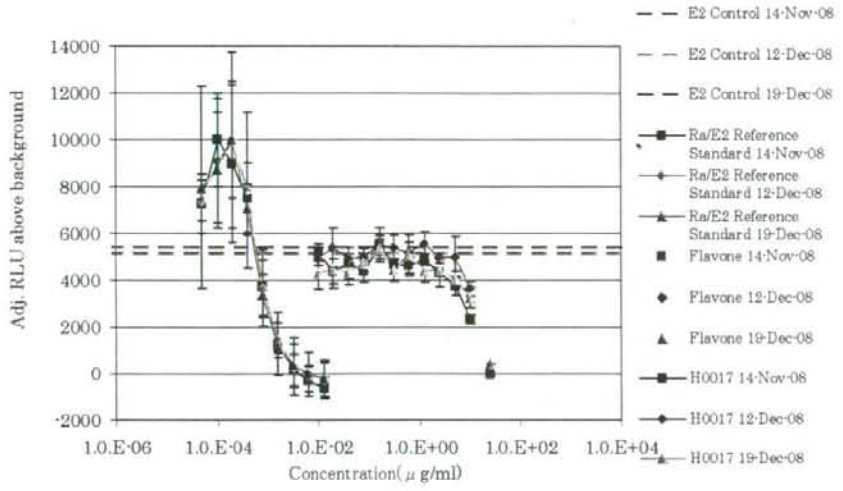
図１３－７．Comprehensive testing（アゴニスト試験）H0015 グラフ



⬭ : Deleted 3 high concentration points to calculate EC_{50} of H0016

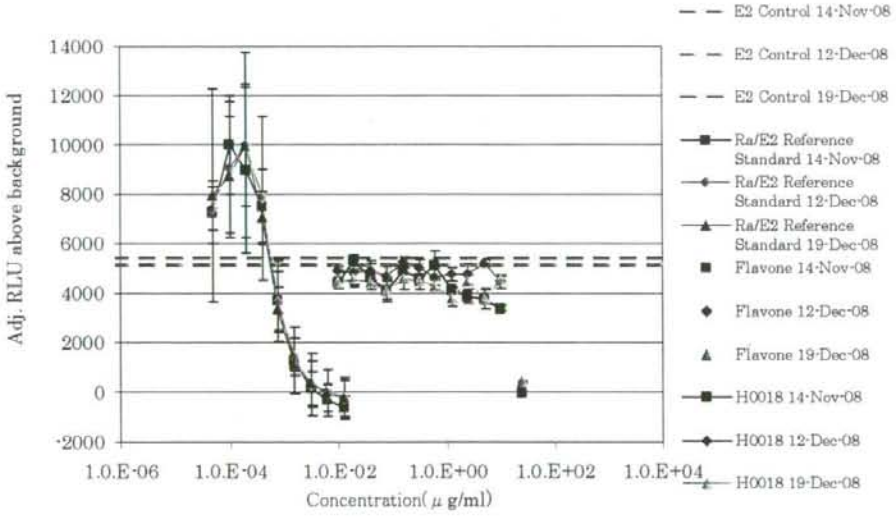
[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１３－８．Comprehensive testing（アゴニスト試験）H0016 グラフ



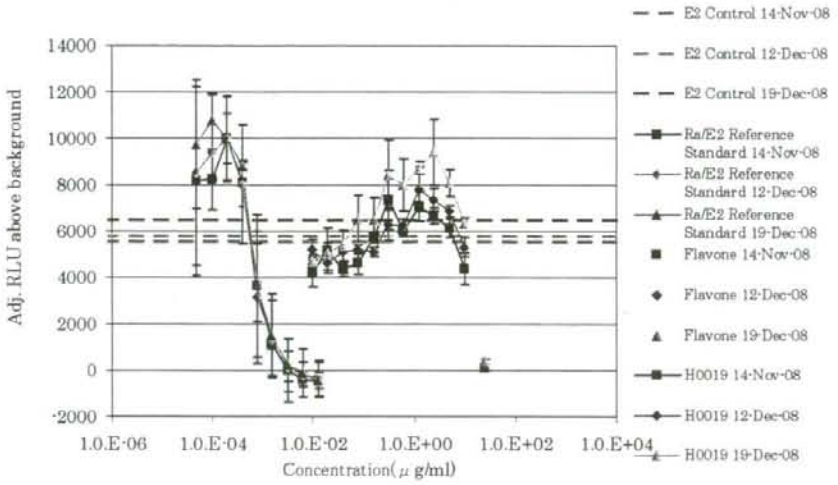
* Lire resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－１．Comprehensive testing（アンタゴニスト試験）H0017 グラフ



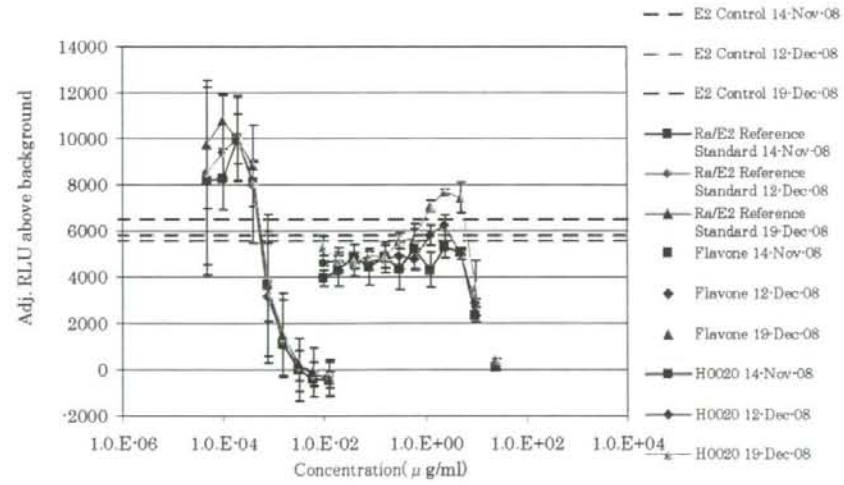
[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－２．Comprehensive testing（アンタゴニスト試験）H0018 グラフ



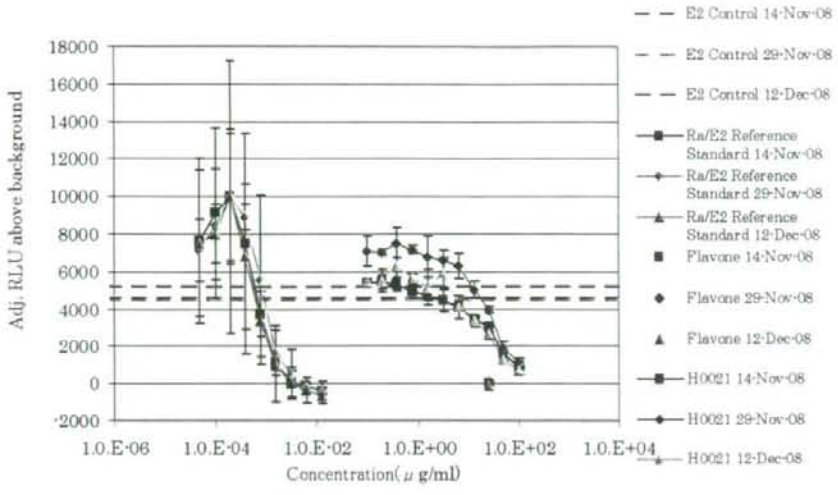
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－３．Comprehensive testing（アンタゴニスト試験）H0019 グラフ



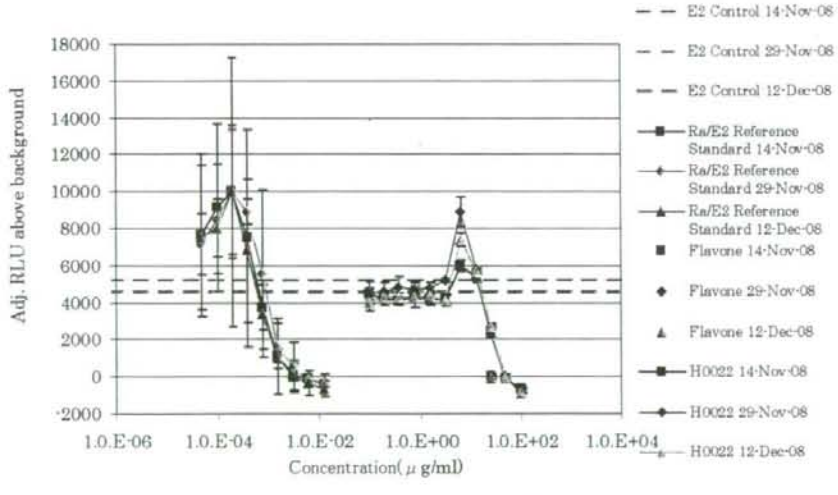
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－４．Comprehensive testing（アンタゴニスト試験）H0020 グラフ



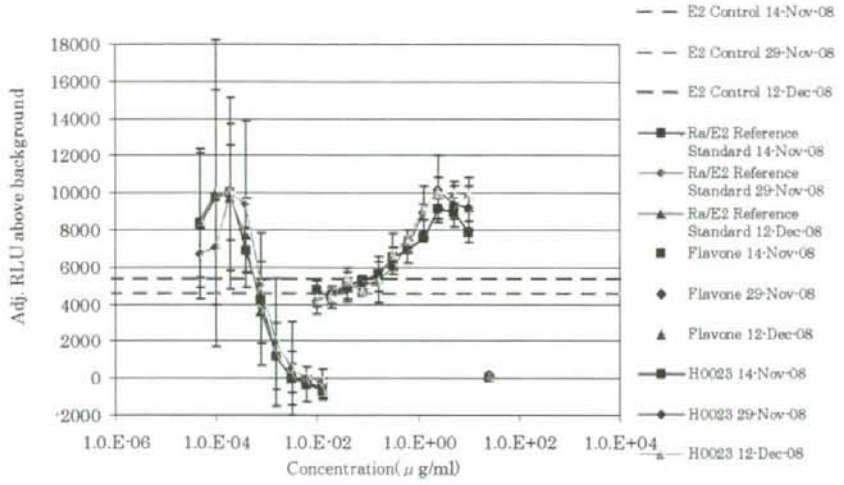
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－５．Comprehensive testing（アンタゴニスト試験）H0021 グラフ



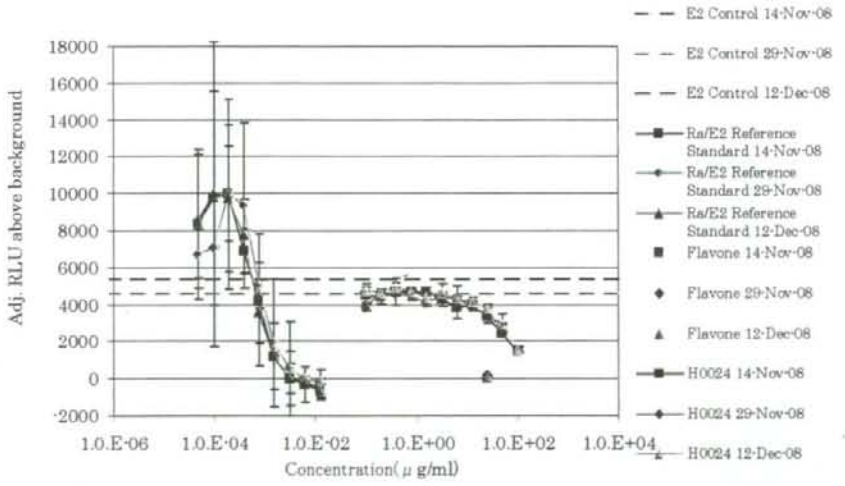
* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－６．Comprehensive testing（アンタゴニスト試験）H0022 グラフ



* Line resents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－７．Comprehensive testing（アンタゴニスト試験）H0023 グラフ



[1] Line represents the mean of three E2 replicates plus three times the standard deviation of the E2 mean

図１４－８．Comprehensive testing（アンタゴニスト試験）H00242 グラフ

4）考察

アゴニスト試験において、16 試験中 4 試験が Criteria に入らなかったため、Fail 判定となった。アンタゴニスト試験において、14 試験中 2 試験が Criteria に入らなかったため、Fail 判定となった。表９．に Phase Ⅱb の Criteria を示している。主な原因は、DMSO Control 及び一部の well における過剰な活性を引き起こした為の Criteria 判定以上の数値を示すことに原因があり、Agonist 試験であれば、DMSO Control 過剰に伴い、E_2Reference Standard EC50 / Induction の Fail 判定や Methoxchlor Control 過剰の Fail 判定、アンタゴニスト試験であれば、Ral/E2 Reference Standard IC_{50} の Fail 判定が起こっている。培養細胞の継代数が 50 代に至っていないもののなるべく早期の細胞を使用することが必要かもしれない。Criteria は、通常、Phase 毎に設定されるが、今後、一定の Criteria を可能な限り複数機関で共通の数値で運用することを検討することが必要であると思われる。

検量線及び被検液の Comprehensive testing の際、一部 S.D.の高いものが見受けられたが、どの程度までであれば、採用してよいか判定が必要かもしれない。

被検液の EC_{50}（3 回繰返し）の結果は、下記の通りであった。

表１０．被検液の EC_{50}（3 回繰返し）結果一覧

Code	1st.	2nd.	3rd.	Ave.	S.D.	C.V.
H0009	0.83	0.99	0.43	0.75	0.29	38
H0010	0.27	0.26	0.21	0.25	0.032	13
H0011	3.0E-06	1.9E-06	2.6E-06	2.5E-06	5.9E-07	24
H0012	0.49	0.79	0.41	0.56	0.20	36
H0013	11	18	25	18	7.3	40
H0014	0.051	0.16	0.14	0.12	0.058	49
H0015	positive	negative	negative	-	-	-
H0016	1.4	1.3	8.1	3.6	3.95	110
H0017	positive	negative	positive	-	-	-
H0018	positive	negative	positive	-	-	-
H0019	negative	negative	negative	-	-	-
H0020	negative	positive	positive	-	-	-
H0021	550	28	18	200	300	150
H0022	25	26	27	26	0.9	3.5
H0023	negative	negative	negative	-	-	-
H0024	positive	positive	positive	-	-	-

We need to decide up to which level of variability (C.V.) is good and acceptable. I also felt that we need protocols to confirm that the result from Graph pad prism is appropriate, and the determination of the data deleted.

5）その他特記事項

・Luminometer は、Centro LB 960, BERTHOLD TECHNOLOGIES, Germany を使用する。

・細胞は、#37～#41 継代数で行った。

4．今後

問題となった点として、1)細胞の継代数 50 代程度までの使用期限をさらに 25 代程度までの継代数の少ない細胞使用を行うことで、突発的な活性などを極力軽減させてばらつきを無くすことが必要である。2)アッセイデータでの Criteria が個別ラボでの指標であり、測定をするたびに変動をしていくため、今後複数ラボでの共通化を図ることが必要である。

今後、上記の件を検証しながら PhaseⅢ試料全 80 物質（アゴニスト試験 40 物質、アンタゴニスト試験 40 物質）の試験を繰返し 1 回の試験を行う予定である。

参考文献

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2) LUMI-CELLÒ ER ASSAY ANTAGONIST PROTOCOL, National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) Developed by:Xenobiotic Detection Systems, Inc.1601 E. Geer St., Suite S Durham, NC 27704, 17 October 2008
3) QA/QC の手引き(概略版)
4) LUMI-CELL® ER ASSAY Visual Observation Cell Viability Manual, 26 September 2007

NICEATM **ICCVAM**

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

Interagency Coordinating Committee on the Validation of Alternative Methods

Results of Phase IIb of the LUMI-CELL® ER Assay International Validation Study

Overview (1)

Overview (2)

List of Acronyms and Abbreviations (1)

ANOVA	Analysis of Variance
API	Apigenin
ATZ	Atrazine
BBP	Butylbenzyl phthalate
CASRN	Chemical Abstracts Service Registry Number
CORT	Corticosterone
CV	Coefficient of Variation
DDT	o,p'-DDT
DMSO	Dimethyl sulfoxide
E2	17ß-Estradiol
EC_{50}	Half-maximal effective concentration
EE	Ethinyl estradiol
ER	Estrogen receptor
ECVAM	European Centre for the Validation of Alternative Methods

List of Acronyms and Abbreviations (2)

FLA	Flavone
GEN	Genistein
Hiyoshi	Hiyoshi Corporation
IC_{50}	Concentration of substance that inhibits the reference estrogen response by 50%
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
JaCVAM	Japanese Center for the Validation of Alternative Methods
NICEATM	National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Methods
NON	*p*-n-nonylphenol
o,p'-DDT	1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
Ral	Raloxifene HCl

List of Acronyms and Abbreviations (3)

RES	Resveratrol
RLU	Relative Light Units
SD	Standard Deviation
TAM	Tamoxifen
TA	Transcriptional Activation
VIN	Vinclozolin
XDS	Xenobiotic Detection Systems, Inc.

The LUMI-CELL® ER Assay International Validation Study

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The International Validation Study Design

- A four phase international validation study to evaluate the reproducibility and accuracy of the LUMI-CELL® ER assay was organized by NICEATM, ECVAM, and JaCVAM.
- The study uses three laboratories, one each in the United States, Europe, and Japan.
- The study includes:
  - An evaluation of the ability of the standardized LUMI-CELL® ER assay (agonist and antagonist) protocols developed at XDS to be transferred to other laboratories.
  - An opportunity between phases of the validation study for protocol refinement.
  - Testing of 78 coded ICCVAM-recommended test substances
  - Evaluation of assay performance (comparison of results against the published literature and intra- and inter-laboratory reproducibility).

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LUMICELL® ER Assay Validation Study: Study Management Team and Participating Labs

Study Management Team:

- NICEATM
  - William S. Stokes, D.V.M., D.A.C.L.A.M. (NIEHS/NTP) Chair
  - Raymond Tice, Ph.D. (NIEHS/NTP) Co-Chair
  - Frank Deal (ILS, Inc.) Project Coordinator
  - Patricia Ceger (ILS, Inc.) Asst. Project Coordinator
  - David Allen, Ph.D. (ILS, Inc.) Principal Investigator NICEATM Support Contract
- ECVAM
  - Susanne Bremer, Ph.D.
- JaCVAM
  - Hajime Kojima, Ph.D.
  - Atsushi Ono, Ph.D.

Participating Laboratories:

- Xenobiotic Detection Systems, Inc. (Lead Laboratory), Durham, North Carolina, U.S.
  - John Gordon, Ph.D. (Study Director)
- ECVAM Internal Laboratory, Ispra, Italy
  - Pathize Paiss, Ph.D. (Study Director)
  - Jan de Lange
- Hiyoshi Corporation, Omi Hachiman, Japan
  - Masafumi Nakamura (Study Director)
  - Hiroshi Honda

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International Validation Study Testing Phases and Timelines

STUDY PHASE	ACTIVITY	ORIGINAL TIMELINE	CURRENT TIMELINE
Phase I	Each laboratory conducts multiple testing of reference standards and controls to demonstrate proficiency with agonist and antagonist protocols, establish historical databases to be used to develop acceptability criteria for tests conducted in Phase IIb, and to provide measures for calculated reference standard and control data for an evaluation of intra- and inter- laboratory reproducibility	Jan. 07 - May 07	Nov. 07 - Feb. 08
Phase IIa	Four substances each from the ICCVAM-recommended ER minimum list tested independently by each laboratory three times for agonist and antagonist activity	Jun. 07 - Jul. 07	May 08 - Sep. 08
Phase IIb	Eight substances each from the ICCVAM-recommended ER minimum list tested independently by each laboratory three times for agonist and antagonist activity	Aug. 07 - Oct. 07	Sep. 08 - Jan. 09
Phase III	Remaining 41 substances from ICCVAM-recommended ER minimum list tested once by each laboratory for agonist and antagonist activity	Nov. 07 - Dec. 07	Jan. 09 - Mar. 09
Phase IV	Remaining 23 substances from ICCVAM-recommended ER list tested once each by the lead laboratory only for agonist and antagonist activity	Jan. 07 - Feb. 07	Apr. 09

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The LUMI-CELL® ER Assay Validation Study - Phase I

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Overview of Phase I Activities

- XDS evaluated new range finder and comprehensive test plate designs that were modified to improve testing efficiency.
- XDS, ECVAM, and Hiyoshi conducted multiple testing of agonist and antagonist protocol reference standards and controls using standardized protocols to:
  - Demonstrate proficiency with agonist and antagonist protocols
  - Demonstrate intra- and inter-laboratory reproducibility
  - Develop quality control criteria for Phase IIb testing from a historical database established from the Phase I and IIa testing of reference standards and controls

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