Phase I Results and Conclusions (1)

- Statistically significant differences were observed in intra- and inter-laboratory reference standard and control values.
- It was not possible to identify the causes for these differences but some of the contributing factors may be:
  - Lot-to-lot differences in cell culture media and tissue culture supplies (for intra- and inter-lab differences)
  - Differences in luminometers (for inter-lab differences)
- This underscores the importance of developing an historical control database for each individual laboratory.

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Phase I Results and Conclusions (2)

- Factors supporting reliability of the assay:
  - Assay responds robustly to E2 reference estrogen and raloxifene reference anti-estrogen.
  - Assay consistently responds to weak-acting positive controls at concentrations several orders of magnitude higher than the reference estrogen or anti-estrogen
  - Assay plate induction (agonist) or reduction values (antagonist) were consistently greater than three-fold (only 2 of 84 plates tested had values below three-fold)
  - Phase I testing of reference standards and controls established historical databases that produced comparable test plate acceptance criteria for Phase IIb testing

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

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Phase IIa Testing of Reference Standards, Controls and Coded Test Substances

- Range finder and comprehensive testing of coded test substances was conducted to:
  - Demonstrate proficiency with the agonist protocol
  - Provide reference standard, control, and coded test substance data for an evaluation of intra- and inter-laboratory reproducibility
  - Continue to build historical databases that will be used to develop acceptance criteria for tests to be conducted in Phase IIb (reference standard and control data from Phase IIb will be added to historical databases established after Phase I testing)

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Phase IIa Results and Conclusions (1)

- Four coded test substances covering a range of ER agonist and antagonist activities were each tested in at least three independent experiments for both agonist and antagonist protocols at each of the participating laboratories
  - BPA, BPB, and EE were reproducibly classified as estrogenic agonists in all of the participating laboratories
  - DDT tested as negative for estrogenic agonism at Hiyoshi (3/3), and at XDS (2/3), but was positive in all agonist tests conducted at ECVAM (3/3)
  - DBA, PROG, and TAM were reproducibly classified as estrogenic antagonists in all of the participating laboratories
  - NON was reproducibly classified as an estrogenic antagonist at ECVAM (3/3) and Hiyoshi (3/3), but was negative at XDS (3/3)

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Phase IIa Results and Conclusions (2)

- Agonist and Antagonist reference standards and controls were evaluated for intra- and inter-laboratory reproducibility
  - Reference standard and control data were reproducible within laboratories
  - Statistically significant differences were observed in intra- and inter-laboratory reference standard and control values.
  - It was not possible to identify the causes for these differences but some of the contributing factors may be:
    - Lot-to-lot differences in cell culture media and tissue culture supplies (for intra- and inter-lab differences)
    - Differences in luminometers (for inter-lab differences)
  - This underscores the importance of developing and maintaining an historical control database for each individual laboratory

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Evaluation of Phase IIa Acceptance Criteria and Failure Rates

- The failure rates of plates used for the Phase IIa comprehensive testing of coded agonist and antagonist substances were evaluated. The percentages of agonist and antagonist test plates that failed acceptance criteria across the three participating laboratories were 61% (33/54) and 38% (13/34), respectively.
  - At Hiyoshi Corporation, 11% (1/9) of agonist test plates and 0% (0/6) of antagonist test plates failed acceptance criteria
  - At XDS, Inc., 53% (8/15) of agonist plates and 43% (6/14) of antagonist test plates failed acceptance criteria
  - At the ECVAM laboratory, 80% (24/30) of agonist test plates and 50% (7/14) of antagonist test plates failed acceptance criteria

Qualitative and Quantitative Evaluation of Acceptance Criteria

- To determine if changes to the acceptance criteria used for Phase IIa testing could reduce the failure rates of comprehensive test plates without compromising the ability of the assay to detect and quantify test substance agonist or antagonist activity, the qualitative (i.e., classification as an agonist or antagonist) and quantitative (i.e., agonist EC_{50} or antagonist IC_{50} value, if one could be calculated) outcomes for test plates that met all acceptance criteria versus those that failed to meet one or more criterion were compared.
- Results of the comparison indicated that acceptance criteria could be modified to improve failure rates without compromising the ability of the assay to detect and quantify test substance agonist or antagonist activity.

Modifications Made to Test Plate Acceptance Criteria (1)

- Based on the results of the qualitative and quantitative comparisons, the SMT approved of the following changes to be used for agonist and antagonist test plate acceptance criteria for use in Phase IIb testing:
  - Agonist Test Plate Acceptance Criteria:
    - The mean plate dimethyl sulfoxide (DMSO) control relative light unit (RLU) value must be within 2.5 times the standard deviation (SD) of the historical mean RLU value for the DMSO control.
    - Plate induction, as measured by dividing the highest mean estradiol (E2) reference RLU value by the mean DMSO control RLU value, must be greater than three-fold.
    - The E2 reference standard curve should be sigmoidal in shape and have at least three values within the linear portion of the curve.
    - The mean plate methoxychlor control RLU value must be greater than three times the SD of the mean plate RLU value of the DMSO control.

Modifications Made to Test Plate Acceptance Criteria (2)

- Antagonist Test Plate Acceptance Criteria:
  - The mean plate dimethyl sulfoxide (DMSO) control relative light unit (RLU) value must be within 2.5 times the standard deviation (SD) of the historical mean RLU value for the DMSO control.
  - Plate reduction, as measured by dividing the highest mean estradiol (E2) reference RLU value by the mean DMSO control RLU value, must be greater than three-fold.
  - The Raloxifene E2 reference standard curve should be sigmoidal in shape and have at least three values within the linear portion of the curve.
  - The mean E2 control RLU value must be within 2.5 times the standard deviation (SD) of the historical mean RLU value for the E2 control.
  - The mean plate flavone+E2 control RLU value must be greater than three times the SD of the mean plate RLU value of the DMSO control.
- If these revised criteria had been used during Phase IIa, the overall rate of plate acceptance would have increased from 39% (21/54) to 80% (43/54) for agonist test plates, and from 62% (21/34) to 85% (29/34) for antagonist test plates.

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

Overview of Phase IIb of the Validation Study (1)

- Eight coded substances from the ER minimum list were tested for agonism and eight were tested for antagonism in each laboratory.
- The solubility of each coded substance in a 1% v/v solution of DMSO in cell culture media was determined (up to a limit concentration of 1 mg/mL).
- Following a single range finder test, at least three successful independent comprehensive tests were conducted for each coded substance.
- Test results for reference standards, controls, and coded substances were evaluated for intra- and inter-laboratory reproducibility.
- Test results for coded substances were evaluated for accuracy with agonist and antagonist activities vs. the ICCVAM meta-data

Overview of Phase IIb of the Validation Study (2)

- All concentrations of coded substances that were tested in range finder or comprehensive tests were evaluated for cytotoxicity using the visual observation method developed in the LUMI-CELL® ER Protocol Validation study.

Viability Score	Description
1	Normal Cell Morphology and Cell Density
2	Altered Cell Morphology and/or Small Gaps between Cells
3	Altered Cell Morphology and/or Large Gaps between Cells
4	Few (or No) Visible Cells
P	Wells containing Precipitation are noted with "P"

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

Solubility and Range Finder Testing of Coded Agonist Substances

Phase IIb Agonist Coded Substances Tested

Substance Name	CASRN	Code (XDS)	Code (ECVAM)	Code (Hiyoshi)
Atrazine (ATZ)	1912-24-9	X0015	V0006	H0015
o,p'-DDT (DDT)	789-02-6	X0011	V0009	H0010
17-α ethinyl estradiol (EE)	57-63-6	X0016	V0012	H0011
Flavone (FLA)	525-82-6	X0013	V0008	H0012
Genistein (GEN)	446-72-0	X0012	V0007	H0014
p-n-nonylphenol (NON)	104-40-5	X0010	V0010	H0016
Butylbenzyl phthalate (BBP)	85-68-7	X0014	V0011	H0009
Vinclozolin (VIN)	50471-44-8	X0009	V0005	H0013

o,p'-DDT = 1,1,1-Trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane

Maximum Solubility of Agonist Test Substances in Culture Media with DMSO (1%)

Test Substance	XDS		ECVAM		Hiyoshi	
	(ug/mL)	(uM)	(ug/mL)	(uM)	(ug/mL)	(uM)
ATZ	100	464	100	464	100	464
BBP	100	320	10	32	10	32
DDT	100	282	100	282	10	28
EE	100	337	100	337	10	34
FLA	100	400	100	400	100	400
GEN	100	370	100	370	100	370
NON	100	454	10	45	100	454
VIN	100	350	10	35	100	350

Agonist Range Finder Testing (1)

- The purpose of range finder testing is to select starting concentrations for comprehensive testing, which is based on the following.
  - If results suggest that the test substance is negative, comprehensive testing is conducted using an 11 point 1:2 serial dilution starting at either the limit concentration or highest soluble concentration
  - If results suggest that the test substance is negative and the higher concentrations in the range finder are cytotoxic, comprehensive testing is conducted using an 11 point 1:2 serial dilution starting at the lowest cytotoxic concentration

Agonist Range Finder Testing (2)

- If results suggest that the test substance is positive, comprehensive testing is conducted using a concentration one log higher than the concentration giving the highest RLU value in the range finder as the starting concentration using either a 1:2 or 1:5 11-point serial dilution scheme based on:
  - An 11-point 1:2 serial dilution is used if the resulting concentration range will encompass the full range of responses based on the concentration response curve generated in the range finder test
  - If the concentration range that would be generated with the 1:2 serial dilution will not encompass the full range of responses based on the concentration response curve in the range finder test, an 11-point 1:5 serial dilution should be used instead
- If a substance exhibits a biphasic concentration response curve both curves are to be resolved using an 11-point 1:5 serial dilution with a top concentration one log higher than the concentration giving the highest RLU of the peak associated with the higher concentration in the range finder.

Agonist Range Finder Concentrations Tested and Cell Viability (ATZ and BBP)

Substance Name	Concentration (µg/mL)	XDS Cell Viability	EDVAM Cell Viability	Hyperion Cell Viability
ATZ	1.0 x 10^{-1}	T	T	T
	7.0 x 10^{-2}	T	T	T
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	7.1 x 10^{-4}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	T	T	T
BBP	1.2 x 10^{-1}	F	NT	NT
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	1.2 x 10^{-3}	T	T	T
	1.2 x 10^{-4}	T	T	T
	2.0 x 10^{-4}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	NT	T	T

NT = not tested

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Agonist Range Finder Concentrations Tested and Cell Viability (DDT and EE)

Substance Name	Concentration (µg/mL)	XDS Cell Viability	EDVAM Cell Viability	Hyperion Cell Viability
DDT	1.0 x 10^{-1}	F	F	NT
	7.0 x 10^{-2}	F	F	T
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	7.0 x 10^{-4}	T	T	T
	7.0 x 10^{-5}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	NT	NT	T
EE	1.0 x 10^{-1}	F	F	NT
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	T	T	T
	1.0 x 10^{-6}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	NT	NT	T

NT = not tested

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Agonist Range Finder Concentrations Tested and Cell Viability (FLA and GEN)

Substance Name	Concentration (µg/mL)	XDS Cell Viability	EDVAM Cell Viability	Hyperion Cell Viability
FLA	1.0 x 10^{-1}	T	F	F
	7.0 x 10^{-2}	T	T	T
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	T	T	T
GEN	1.0 x 10^{-1}	F	T	T
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	1.0 x 10^{-4}	T	T	T
	7.0 x 10^{-5}	T	T	T
	1.0 x 10^{-5}	T	T	T
	1.0 x 10^{-6}	T	T	T

NT = not tested

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Agonist Range Finder Concentrations Tested and Cell Viability (NON and VIN)

Substance Name	Concentration (µg/mL)	XDS Cell Viability	EDVAM Cell Viability	Hyperion Cell Viability
NON	1.0 x 10^{-1}	F	NT	T
	7.0 x 10^{-2}	F	T	T
	1.0 x 10^{-2}	T	T	T
	1.0 x 10^{-3}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	T	T	T
	1.0 x 10^{-6}	T	T	T
	1.0 x 10^{-4}	NT	T	NT
VIN	1.0 x 10^{-1}	F	NT	T
	1.0 x 10^{-2}	F	T	T
	1.0 x 10^{-3}	T	T	T
	1.0 x 10^{-4}	T	T	T
	1.0 x 10^{-5}	T	T	T
	1.0 x 10^{-6}	T	T	T
	1.0 x 10^{-4}	NT	T	NT

NT = not tested

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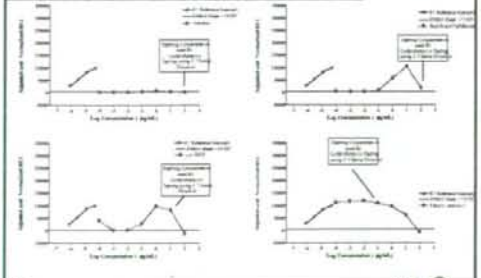
XDS Agonist Range Finder Results (1)



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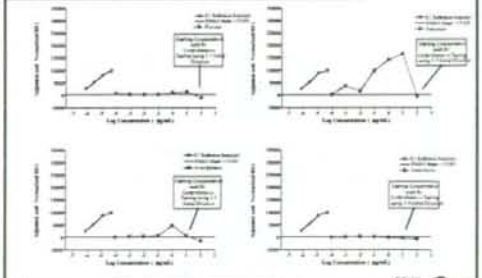
XDS Agonist Range Finder Results (2)



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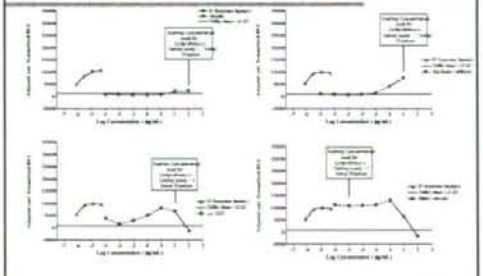
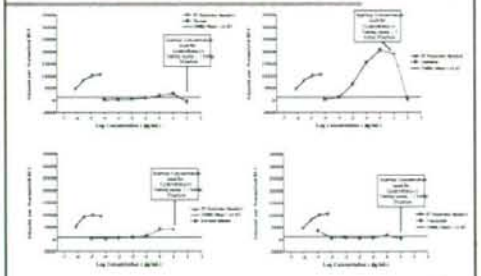
ECVAM Agonist Range Finder Results (1)



37

ECVAM Agonist Range Finder Results (2)



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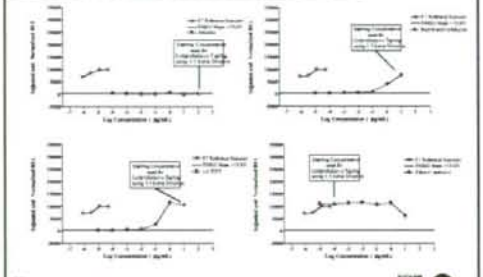
Hiyoshi Agonist Range Finder Results (1)



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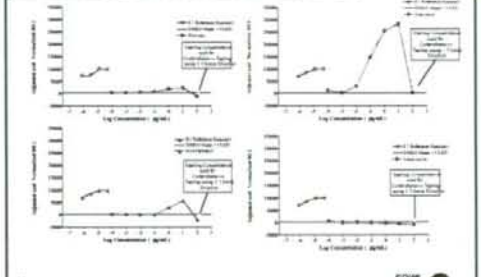
Hiyoshi Agonist Range Finder Results (2)



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

Comprehensive Testing of Coded Agonist Substances

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Overview of Phase IIb Comprehensive Agonist Testing

- Based on starting concentrations determined by range finder testing, at least three successful independent comprehensive agonist tests were conducted for ATZ, BBP, DDT, EE, FLA, GEN, NON, and VIN.
- Results from the successful testing of reference standard, controls, and the eight agonist substances were evaluated for intra- and inter-laboratory reproducibility.
- Test results for coded substances were evaluated for accuracy vs. agonist activities from the ICCVAM meta-data.
- Test plate failure rates were determined.

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Acceptance Criteria for Phase IIb Agonist Comprehensive Testing

- Acceptance or rejection of comprehensive agonist tests conducted in Phase IIb was based on evaluation of test plate reference standards and controls. Results were evaluated against acceptance criteria derived from the historical databases established from Phase I and Phase IIb testing at each laboratory. Agonist test plate acceptance criteria used for Phase IIb are summarized as follows:
  - Plate induction, the averaged highest E2 reference standard RLU value divided by the averaged DMSO control value, must be greater than three-fold
  - DMSO control RLU values must be within 2.5 times the SD of the historical DMSO control value
  - The E2 reference standard concentration-response curve should be sigmoidal in shape and have at least three values within the linear portion of the concentration-response curve
  - Methoxychlor control RLU values must be above the line representing the DMSO mean plus three times the standard deviation from the DMSO mean

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

Intra- and Inter-laboratory Reproducibility of Agonist Reference Standard and Controls

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Intralaboratory Reproducibility of Agonist Reference Standards and Controls

- Intralaboratory reproducibility of the RLU values associated with the DMSO control wells, and the fold-induction of E2 at its maximum response from test plates that met acceptance criteria was analyzed.
  - Means and standard deviations of reference standard and control values from each laboratory were evaluated and graphed
  - Linear regression analyses were conducted to assess intralaboratory reproducibility of reference standard and control results over time for each laboratory

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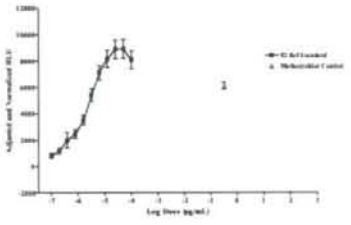
Reference Standard and Control Results from Agonist Plates Tested at XDS



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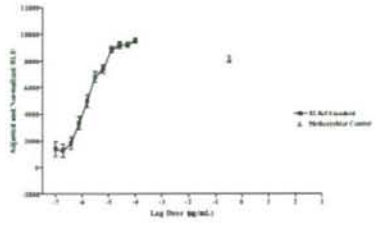
Reference Standard and Control Results from Agonist Plates Tested at ECVAM



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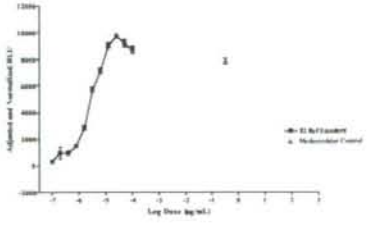
Reference Standard and Control Results from Agonist Plates Tested at Hiyoshi



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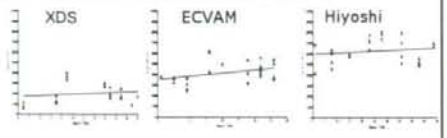
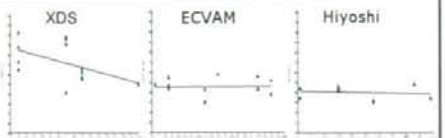
DMSO Linear Regression Analysis



	N*	Intercept†	Slope	P-value (slope)‡
XDS	26	1700	22	0.47
ECVAM	26	3500	30	0.07
HIYOSHI	27	6000	12	0.42

*Number of plates tested (combined agonist and antagonist plates)
†Intercept values are reported as unadjusted relative light units
‡Statistically significant from zero at p<0.05

Agonist E2 Fold-Induction Linear Regression Analysis



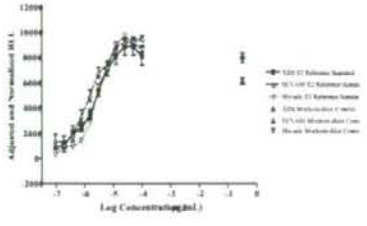
	N*	Intercept†	Slope	P-value (slope)‡
XDS	13	8.7	-0.22	0.089
ECVAM	12	4.5	0.01	0.950
HIYOSHI	13	4.2	-0.01	0.887

*Number of plates tested.
†Intercept values are reported as fold-induction.
‡Statistically significant from zero at p<0.05.

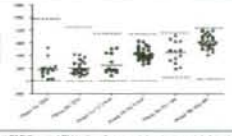
Interlaboratory Reproducibility of Reference Standard and Controls

- Interlaboratory reproducibility of the RLU values associated with the DMSO control wells and the fold-induction of E2 at its maximum response from test plates that meet acceptance criteria was evaluated:
  - Means, standard deviations and coefficients of variation (CV) of reference standard and control values were compared
  - Variability of reference standard and control values across laboratories was evaluated by conducting an analysis of variance (ANOVA)
- If a significant p-value was obtained for the ANOVA, a Newman-Keuls post-test was used to test for significant differences in reference standard and control values between pairs of laboratories.

Interlaboratory Comparison of Reference Standard and Controls



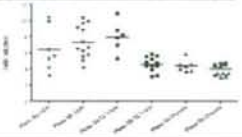
Interlaboratory Comparison of DMSO Control



Data points represent DMSO control RLU values from agonist and antagonist plates tested in Phase IIb. Solid horizontal lines represent the mean DMSO control RLU value for each data set. Dashed lines indicate the mean DMSO control RLU historical database value plus and minus 2.5 times the standard deviation from the mean. DMSO control RLU values cannot be less than zero.

	Plates Tested		Mean (RLU)		SD (RLU)		CV	
	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb
XDS	12	25	2261	2000	1649	1025	73%	51%
ECVAM	10	26	2929	4197	1835	1007	63%	24%
Hiyoshi	13	27	4440	6272	1788	1065	40%	17%

Interlaboratory Comparison of E2 Fold-Induction



Data points represent E2 maximum fold-induction values from plates tested in Phase IIb. Solid horizontal lines represent the E2 fold-induction value for each data set. Plates are rejected if the fold-induction for the maximum E2 response is less than three.

	Plates Tested		Mean (RLU)		SD (RLU)		CV	
	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb
XDS	7	13	4.7	7.3	2.7	2.0	43%	28
ECVAM	6	12	8.1	4.6	1.9	0.8	24%	13
Hiyoshi	8	13	4.5	4.0	0.7	0.7	15%	18

Agonist ANOVA - Interlaboratory Comparison of Reference Standard & Control Values

	p-value[1,2,3]	F value[3]
DMSO	<0.001	41.8
Fold-Induction	<0.001	21.6

[1]Variability is statistically significant at p<0.05.
[2]ANOVA analyzed values from the three participating laboratories.
[3]Values in italics have p values that are less than 0.05.
[4]F=ratio of between-laboratory variability to within-laboratory variability - a ratio of 1.0 indicates that the within-laboratory variability is equal and a ratio of zero indicates that all means are equal.

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Newman-Keuls - Interlaboratory Comparison of Agonist Reference Standard & Controls

	DMSO[1]		Fold-Induction	
	Mean Difference	p value[2,3]	Mean Difference	p value[2,3]
XDS vs ECVAM	2228	<0.001	2.7	<0.001
XDS vs Hiyoshi	4780	<0.001	3.3	<0.001
ECVAM vs Hiyoshi	1961	<0.001	0.6	>0.05

[1]Presented in relative light units.
[2]Variability is statistically significant at p<0.05.
[3]Values in italics have p values that are less than 0.05.

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

Intra- and Inter-laboratory Reproducibility of Testing Results for Agonist Substances

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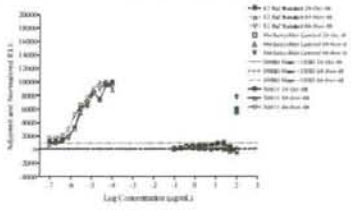
Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for ATZ

	XDS		ECVAM		Hiyoshi	
Substance Name	Concentration (log10 M)	Cell Viability[1]	Concentration (log10 M)	Cell Viability[1]	Concentration (log10 M)	Cell Viability[1]
ATZ	-5.00 x 10^-1	1.2.2	-5.00 x 10^-1	1	-5.00 x 10^-1	1
	-5.00 x 10^-2	1	-5.00 x 10^-2	1	-5.00 x 10^-2	1
	-5.00 x 10^-3	1	-5.00 x 10^-3	1	-5.00 x 10^-3	1
	-7.25 x 10^-4	1	-7.25 x 10^-4	1	-7.25 x 10^-4	1
	-8.25 x 10^-5	1	-8.25 x 10^-5	1	-8.25 x 10^-5	1
	-3.13 x 10^-5	1	-3.13 x 10^-5	1	-3.13 x 10^-5	1
	-1.04 x 10^-7	1	-1.04 x 10^-7	1	-1.04 x 10^-7	1
	-7.81 x 10^-7	1	-7.81 x 10^-7	1	-7.81 x 10^-7	1
	-2.91 x 10^-7	1	-2.91 x 10^-7	1	-1.91 x 10^-7	1
	-1.56 x 10^-7	1	-1.56 x 10^-7	1	-1.56 x 10^-7	1
	-5.77 x 10^-7	1	-5.77 x 10^-7	1	-5.77 x 10^-7	1

[1]The cell viability score indicates the score that was given for that concentration on all plates tested.

58

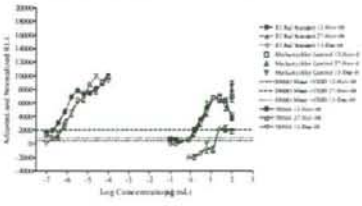
Phase IIb Agonist Results for ATZ at XDS



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

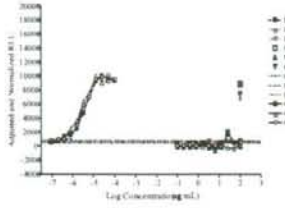
59

Phase IIb Agonist Results for ATZ at ECVAM



60

Phase IIb Agonist Results for ATZ at Hiyoshi



61

Interlaboratory Comparison of ATZ Agonist Activity

	Plates Tested	Plates Testing Positive for Agonism	Plates Testing Negative for Agonism	ICCVAM Meta-data
XDS	3	1	2	Negative
ECVAM	3	3	0	
Hiyoshi	3	1	2	

If mean adjusted RLU values for a given concentration(s) of test substance is greater than the mean plus three times the standard deviation of the plate DMSO control values, the test substance is considered positive for agonist activity - any response below this threshold is considered negative for agonist activity.

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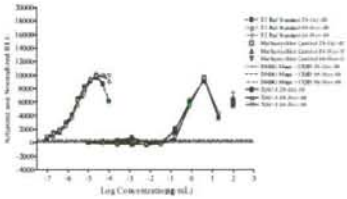
Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for BBP

Substance Name	XDS		ECVAM		Hiyoshi	
	Concentration (log/mL)	Cell Viability*	Concentration (log/mL)	Cell Viability*	Concentration (log/mL)	Cell Viability*
BBP	1.00 x 10^{-1}	3.0.2	1.00 x 10^{-1}	1	1.00 x 10^{-1}	1
	3.20 x 10^{-2}	1.2.1	5.00 x 10^{-2}	1	2.00 x 10^{-2}	1
	4.00 x 10^{-2}	1	2.00 x 10^{-2}	1	4.00 x 10^{-2}	1
	8.00 x 10^{-3}	1	1.26 x 10^{-2}	1	8.00 x 10^{-3}	1
	1.60 x 10^{-3}	1	6.30 x 10^{-3}	1	1.60 x 10^{-3}	1
	3.20 x 10^{-4}	1	2.73 x 10^{-3}	1	3.20 x 10^{-4}	1
	6.40 x 10^{-4}	1	1.58 x 10^{-3}	1	6.40 x 10^{-4}	1
	1.28 x 10^{-4}	1	7.81 x 10^{-4}	1	1.28 x 10^{-4}	1
	2.56 x 10^{-5}	1	3.97 x 10^{-4}	1	2.56 x 10^{-5}	1
	5.12 x 10^{-5}	1	1.95 x 10^{-4}	1	5.12 x 10^{-5}	1
	1.02 x 10^{-5}	1	9.77 x 10^{-5}	1	1.02 x 10^{-5}	1

*The cell viability score indicates the score that was given for that concentration on all plates tested.

63

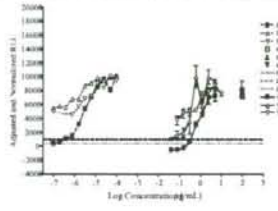
Phase IIb Agonist Results for BBP at XDS



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

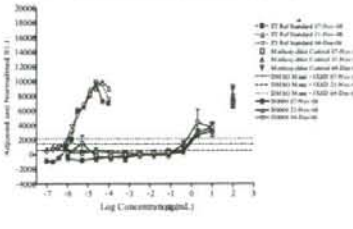
64

Phase IIb Agonist Results for BBP at ECVAM



65

Phase IIb Agonist Results for BBP at Hiyoshi



66

Interlaboratory Comparison of BBP Agonist Activity

	Plates Tested	Plates Testing Positive for Agonism[1]	Plates Testing Negative for Agonism[1]	ICCVAM Meta-data[2]
XDS	3	3	0	++
ECVAM	3	3	0	
Hiyoshi	3	3	0	

[1] If mean adjusted RLU values for a given concentration(s) of test substance is greater than the mean plus three times the standard deviation of the plate DMSO control values, the test substance is considered positive for agonist activity - any response below this threshold is considered negative for agonist activity.

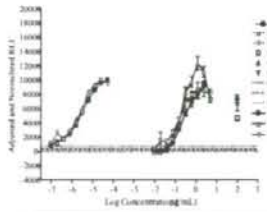
[2] ++ indicates that the substance is classified as being moderately active in the ICCVAM meta-data (EC_{50} value was between 0.0001 and 0.1 µM

Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for DDT

Substance Name	XDS Concentration (µg/mL)	XDS Cell Viability[1]	ECVAM Concentration (µg/mL)	ECVAM Cell Viability[1]	Hiyoshi Concentration (µg/mL)	Hiyoshi Cell Viability[1]
DDT	1.00 x 10^{-1}	1	1.00 x 10^{-1}	1	1.00 x 10^{-1}	1
	3.00 x 10^{-2}	2 1 1	3.00 x 10^{-2}	1	3.00 x 10^{-2}	1
	2.01 x 10^{-2}	1	4.50 x 10^{-3}	1	4.50 x 10^{-3}	1
	1.35 x 10^{-3}	1	9.00 x 10^{-4}	1	9.00 x 10^{-4}	1
	6.30 x 10^{-4}	1	1.80 x 10^{-4}	1	1.80 x 10^{-4}	1
	2.13 x 10^{-4}	1	3.61 x 10^{-5}	1	3.61 x 10^{-5}	1
	1.00 x 10^{-4}	1	3.40 x 10^{-5}	1	3.40 x 10^{-5}	1
	7.61 x 10^{-5}	1	1.28 x 10^{-5}	1	1.28 x 10^{-5}	1
	3.81 x 10^{-5}	1	7.68 x 10^{-6}	1	7.68 x 10^{-6}	1
	1.90 x 10^{-5}	1	2.72 x 10^{-6}	1	2.72 x 10^{-6}	1
	9.71 x 10^{-6}	1	1.02 x 10^{-6}	1	1.02 x 10^{-6}	1

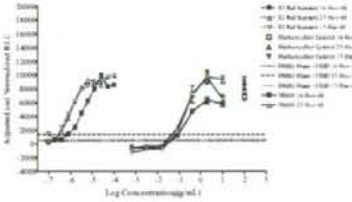
[1] Multiple scores indicate the cell viability scores for concentrations tested in each plate when scores differed from plate to plate. A single cell viability score indicates the score that was given for that concentration on all plates tested.

Phase IIb Agonist Results for DDT at XDS

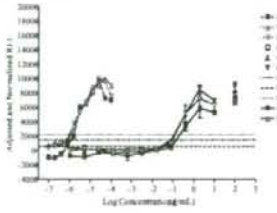


Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

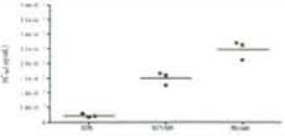
Phase IIb Agonist Results for DDT at ECVAM



Phase IIb Agonist Results for DDT at Hiyoshi



Interlaboratory Comparison of DDT EC_{50} Values



Data points represent DDT EC_{50} values from plates tested in Phase IIb.

	Plates Tested	Mean (µg/mL)	SD (µg/mL)	CV (%)	ICCVAM Meta-data[1]
XDS	3	2.2 x 10^{-1}	6.6 x 10^{-2}	30	+
ECVAM	3	15.0 x 10^{-1}	24.7 x 10^{-1}	15	
Hiyoshi	3	24.7 x 10^{-1}	32.1 x 10^{-1}	13	

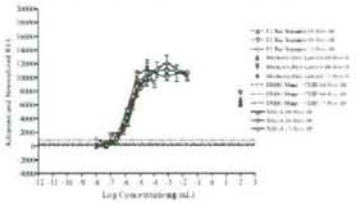
[1] + indicates that the substance is classified as being weakly active in the ICCVAM meta-data (EC_{50} value was > 0.1µM)

Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for EE

Substance Name	XDS		ECVAM		Hiyoshi	
	Concentration (ng/mL)	Cell Viability*	Concentration (ng/mL)	Cell Viability*	Concentration (ng/mL)	Cell Viability*
EE	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

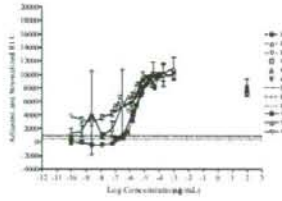
*The cell viability score indicates the score that was given for that concentration on all plates tested.

Phase IIb Agonist Results for EE at XDS

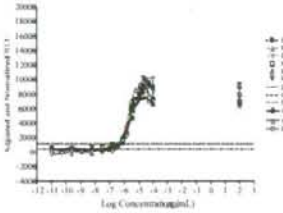


Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

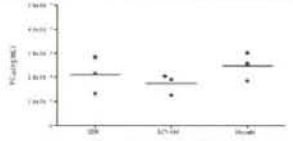
Phase IIb Agonist Results for EE at ECVAM



Phase IIb Agonist Results for EE at Hiyoshi



Interlaboratory Comparison of EE E2 EC_{50} Values



Data points represent EE EC_{50} values from plates tested in Phase IIb

	Plates Tested	Mean (pg/mL)	SD (pg/mL)	CV (%)	ICCVAM Meta-data[1]
XDS	3	2.1×10^{-6}	7.8×10^{-7}	36	
ECVAM[2]	3	1.7×10^{-6}	4.3×10^{-7}	25	***
Hiyoshi	3	2.5×10^{-6}	5.9×10^{-7}	24	

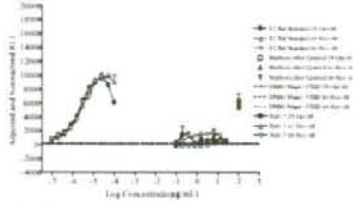
*** Indicates that the substance is classified as being strongly active in the ICCVAM meta-data (EC_{50} value was < 0.0001 uM)

Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for FLA

Substance Name	XDS		ECVAM		Hiyoshi	
	Concentration (ng/mL)	Cell Viability*	Concentration (ng/mL)	Cell Viability*	Concentration (ng/mL)	Cell Viability*
FLA	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*The cell viability score indicates the score that was given for that concentration on all plates tested.

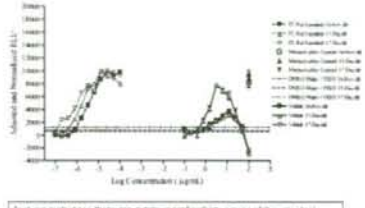
Phase IIb Agonist Results for FLA at XDS



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

79

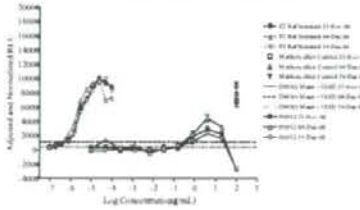
Phase IIb Agonist Results for FLA at ECVAM



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

80

Phase IIb Agonist Results for FLA at Hiyoshi



81

Interlaboratory Comparison of FLA Agonist Activity

	Plates Tested	Plates Testing Positive for Agonism[1]	Plates Testing Negative for Agonism[1]	ICCVAM Meta-data[2]
XDS	3	3	0	+
ECVAM	3	3	0	
Hiyoshi	3	3	0	

[1] If mean adjusted RLU values for a given concentration(s) of test substance is greater than the mean plus three times the standard deviation of the plate DMSO control values, the test substance is considered positive for agonist activity - any responses below this threshold is considered negative for agonist activity.

[2] + Indicates that the substance is classified as being weakly active in the ICCVAM meta-data ($EC_{1.5}$ value was < 0.1 µM)

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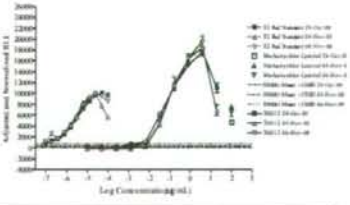
Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for GEN

Substance Name	XDS Concentration (log(M))	XDS Cell Viability[1]	ECVAM Concentration (log(M))	ECVAM Cell Viability[1]	Hiyoshi Concentration (log(M))	Hiyoshi Cell Viability[1]
GEN	-1.00 x 10^-1	5.0.0	-1.00 x 10^-1	1	-1.00 x 10^-1	1
	-2.00 x 10^-1	1.1.0	-2.00 x 10^-1	1	-2.00 x 10^-1	1
	-4.00 x 10^-1	1	-4.00 x 10^-1	1	-4.00 x 10^-1	1
	-8.00 x 10^-1	1	-8.00 x 10^-1	1	-8.00 x 10^-1	1
	-1.60 x 10^0	1	-1.60 x 10^0	1	-1.60 x 10^0	1
	-3.20 x 10^0	1	-3.20 x 10^0	1	-3.20 x 10^0	1
	-6.40 x 10^0	1	-6.40 x 10^0	1	-6.40 x 10^0	1
	-1.28 x 10^1	1	-1.28 x 10^1	1	-1.28 x 10^1	1
	-2.56 x 10^1	1	-2.56 x 10^1	1	-2.56 x 10^1	1
	-5.12 x 10^1	1	-5.12 x 10^1	1	-5.12 x 10^1	1
	-1.02 x 10^2	1	-1.02 x 10^2	1	-1.02 x 10^2	1

[1] The cell viability score indicates the score that was given for that concentration on all plates tested.

83

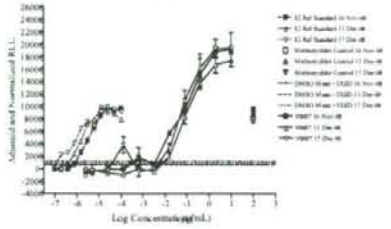
Phase IIb Agonist Results for GEN at XDS



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

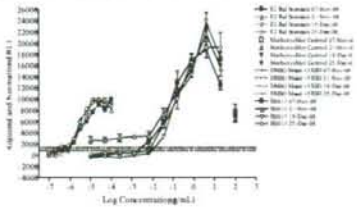
84

Phase IIb Agonist Results for GEN at ECVAM



85

Phase IIb Agonist Results for GEN at Hiyoshi



86

Interlaboratory Comparison of GEN EC_{50} Values

Data points represent GEN EC_{50} values from plates tested in Phase IIb

Plates Tested	Mean (µg/mL)	SD (µg/mL)	CV (%)	ICCVAM Meta-data[1]
XDS	3	5.7×10^{-1}	1.8×10^{-1}	28	+
ECVAM	3	81.1×10^{-1}	8.8×10^{-1}	11	
Hiyoshi	4	118.4×10^{-1}	47.8×10^{-1}	40	

[1] + Indicates that the substance is classified as being weakly active in the ICCVAM meta-data (EC_{50} value was > 0.1µM)

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Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for NON

Substance Name	XDS Concentration (µg/mL)	XDS Cell Viability[1]	ECVAM Concentration (µg/mL)	ECVAM Cell Viability[1]	Hiyoshi Concentration (µg/mL)	Hiyoshi Cell Viability[1]
NON	1.00×10^{-1}	5	1.00×10^{-1}	2F / 2P	1.00×10^{-1}	4
	3.00×10^{0}	2,2	3.00×10^{0}	1	3.00×10^{0}	4
	9.00×10^{0}	1	9.00×10^{0}	1	9.00×10^{0}	4
	2.70×10^{1}	1	2.70×10^{1}	1	2.70×10^{1}	2
	8.10×10^{1}	1	8.10×10^{1}	1	8.10×10^{1}	1
	2.43×10^{2}	1	2.43×10^{2}	1	2.43×10^{2}	1
	7.29×10^{2}	1	7.29×10^{2}	1	7.29×10^{2}	1
	2.19×10^{3}	1	2.19×10^{3}	1	2.19×10^{3}	1
	6.56×10^{3}	1	6.56×10^{3}	1	6.56×10^{3}	1
	1.97×10^{4}	1	1.97×10^{4}	1	1.97×10^{4}	1
	5.90×10^{4}	1	5.90×10^{4}	1	5.90×10^{4}	1

[1] Multiple scores indicate the cell viability scores for concentrations tested in each plate when scores differed from plate to plate. A single cell viability score indicates the score that was given for that concentration on all plates tested.

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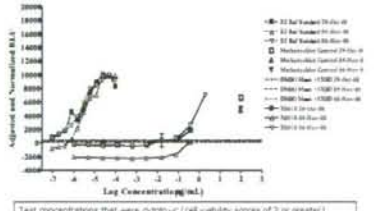
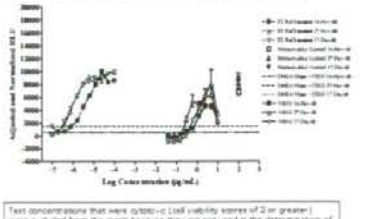
Phase IIb Agonist Results for NON at XDS



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of agonist activity.

89

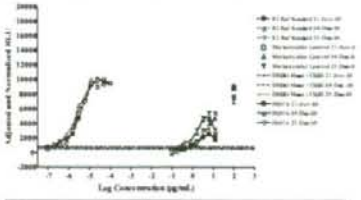
Phase IIb Agonist Results for NON at ECVAM



Test concentrations that were cytotoxic (cell viability scores of 2 or greater) were excluded from the graph because they are not used in the determination of antagonist activity.

90

Phase IIb Agonist Results for NON at Hiyoshi



Test concentrations that were cytoto-xic (cell viability scores of 2 or greater) were e-cluded from the graph because they are not used in the determination of agonist activity.

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Interlaboratory Comparison of NON Agonist Activity

	Plates Tested	Plates Testing Positive for Agonism[1]	Plates Testing Negative for Agonism[1]	ICCVAM Meta-data[2]
XDS	3	3	0	++
ECVAM	3	3	0	
Hiyoshi	3	3	0	

[1] If mean adjusted RLU values for a given concentration(s) of test substance is greater than the mean plus three times the standard deviation of the plate DMSO control values, the test substance is considered positive for agonist activity - any response below this threshold is considered negative for agonist activity.

[2] ++ indicates that the substance is classified as being moderately active in the ICCVAM meta-data (EC_{150} value was between 0.0001 and 0.1 µM

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Agonist Comprehensive Testing - Concentrations Tested & Cell Viability for VIN

Substance Name	XDS		ECVAM		Hiyoshi	
	Concentration (µg/mL)	Cell Viability[1]	Concentration (µg/mL)	Cell Viability[1]	Concentration (µg/mL)	Cell Viability[1]
VIN	1.00 x 10^{-1}	3.3.2	1.00 x 10^{-1}	1	1.00 x 10^{-1}	1
	2.00 x 10^{-2}	3.2.1	1.00 x 10^{-2}	1	5.00 x 10^{-2}	1
	4.00 x 10^{-2}	1	2.50 x 10^{-2}	1	2.50 x 10^{-2}	1
	8.00 x 10^{-3}	1	1.25 x 10^{-2}	1	1.25 x 10^{-2}	1
	1.60 x 10^{-3}	1	6.25 x 10^{-3}	1	6.25 x 10^{-3}	1
	3.20 x 10^{-3}	1	3.13 x 10^{-3}	1	3.13 x 10^{-3}	1
	6.40 x 10^{-4}	1	1.56 x 10^{-3}	1	1.56 x 10^{-3}	1
	1.28 x 10^{-4}	1	7.81 x 10^{-4}	1	7.81 x 10^{-4}	1
	2.56 x 10^{-5}	1	3.91 x 10^{-4}	1	2.91 x 10^{-4}	1
	5.12 x 10^{-5}	1	1.95 x 10^{-4}	1	1.95 x 10^{-4}	1
	1.02 x 10^{-5}	1	9.77 x 10^{-5}	1	9.77 x 10^{-5}	1

[1] The cell viability score indicates the score that was given for that concentration on all plates tested.

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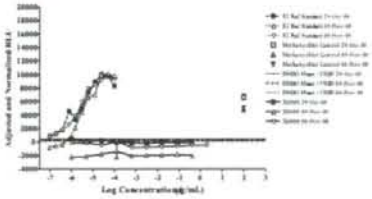
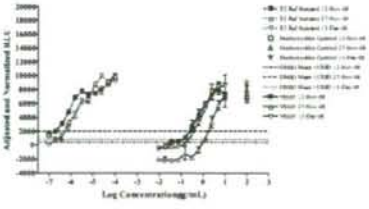
Phase IIb Agonist Results for VIN at XDS



Test concentrations that were cytoto-xic (cell viability scores of 2 or greater) were e-cluded from the graph because they are not used in the determination of agonist activity.

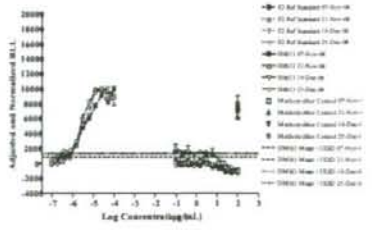
94

Phase IIb Agonist Results for VIN at ECVAM



95

Phase IIb Agonist Results for VIN at Hiyoshi



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Interlaboratory Comparison of VIN Agonist Activity

	Plates Tested	Plates Testing Positive for Agonism	Plates Testing Negative for Agonism	ICCVAM Meta-data
KDS	3	0	3	Negative
ECVAM	3	3	0	
Hyasynth	4	1	3	

*If mean adjusted RLU values for a given concentration(s) of test substance is greater than the mean plus three times the standard deviation of the plate DMSO control values, the test substance is considered positive for agonist activity - any response below this threshold is considered negative for agonist activity

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

Solubility and Range Finder Testing of Coded Antagonist Substances

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Phase IIb Antagonist Coded Substances Tested

Substance Name	CASRN	Code (XDS)	Code (ECVAM)	Code (Hayashi)
Apigenin (API)	520-36-5	X0024	V0089	0020
Atrazine (ATZ)	1912-24-9	X0023	V0087	H0024
Corticosterone (CORT)	50-22-6	X0021	V0085	H0021
o,p'-DDT[1] (DDT)	789-02-6	X0018	V0084	H0018
Flavone (FLA)	525-82-6	X0020	V0090	H0019
Genistein (GEN)	446-72-0	X0019	V0083	H0023
Butylbenzyl phthalate (BBP)	85-68-7	X0022	V0086	H0017
Resveratrol (RES)	501-36-0	X0017	V0088	H0022

[1] o,p'-DDT = 1,1,1-Trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane

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Maximum Solubility of Antagonist Test Substances in Culture Media with DMSO (1%)

Test Substance	XDS		ECVAM		Hayashi	
	(µg/mL)	(µM)	(µg/mL)	(µM)	(µg/mL)	(µM)
API	100	370	10	37	10	37
ATZ	100	464	100	464	100	464
BBP	100	320	10	32	10	32
CORT	1000	2886	1000	2886	100	289
DDT	100	282	10	28	10	28
FLA	100	460	100	460	10	46
GEN	100	370	100	370	10	37
RES	100	438	100	438	100	438

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Antagonist Range Finder Testing (1)

- The purpose of range finder testing is to select starting concentrations for comprehensive testing, which is based on the following.
  - If results suggest that the test substance is negative, comprehensive testing is conducted using an 11 point 1:2 serial dilution with the limit dose as the starting concentration
  - If results suggest that the test substance is negative and the higher concentrations in the range finder are cytotoxic, comprehensive testing is conducted using an 11 point 1:2 serial dilution with the lowest cytotoxic concentration as the starting concentration

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Antagonist Range Finder Testing (2)

- If results suggest that the test substance is positive, comprehensive testing is conducted using a top concentration one log higher than the concentration giving the highest RLU value in the range finder as the starting concentration using either a 1:2 or 1:5 11-point serial dilution scheme based on:
  - An 11-point 1:2 serial dilution is used if the resulting concentration range will encompass the full range of responses based on the concentration response curve generated in the range finder test
  - If the concentration range that would be generated with the 1:2 serial dilution will not encompass the full range of responses based on the concentration response curve in the range finder test, an 11-point 1:5 serial dilution should be used instead
- If a substance exhibits a biphasic concentration response curve both curves are to be resolved using an 11-point 1:5 serial dilution with a top concentration one log higher than the concentration giving the highest RLU of the peak associated with the higher concentration in the range finder.

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Antagonist Range Finder Concentrations Tested and Cell Viability (API and ATZ)

Substance Name	Concentration (µg/mL)	XDS Cell Viability	ECVAM Cell Viability	Hayashi Cell Viability
API	1.0 x 10^-1	X	NT	NT
	1.0 x 10^-2	X	1	1
	1.0 x 10^-3	1	1	1
	1.0 x 10^-4	1	1	1
	1.0 x 10^-5	1	1	1
	1.0 x 10^-6	1	1	1
	1.0 x 10^-7	1	1	1
	1.0 x 10^-8	NT	1	1
ATZ	1.0 x 10^-1	1	1	1
	1.0 x 10^-2	1	1	1
	1.0 x 10^-3	1	1	1
	1.0 x 10^-4	1	1	1
	1.0 x 10^-5	1	1	1
	1.0 x 10^-6	1	1	1
	1.0 x 10^-7	1	1	1

NT = not tested

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Antagonist Range Finder Concentrations Tested and Cell Viability (BBP and CORT)

Substance Name	Concentration (log[M/L])	XDS Cell Viability	ECVAM Cell Viability	Hyperion Cell Viability
BBP	1.0×10^{-3}	1	NT	NT
	2.0×10^{-3}	1	1	1
	4.0×10^{-3}	1	1	1
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	NT	1	1
DERT	1.0×10^{-3}	4	4	NT
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	1	1	1
	1.0×10^{-9}	1	1	1
	1.0×10^{-10}	NT	NT	1

NT = not tested

Antagonist Range Finder Concentrations Tested and Cell Viability (DDT and FLA)

Substance Name	Concentration (log[M/L])	XDS Cell Viability	ECVAM Cell Viability	Hyperion Cell Viability
DDT	1.0×10^{-3}	4	NT	NT
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	1	1	1
	1.0×10^{-9}	1	1	1
	1.0×10^{-10}	NT	1	1
FLA	1.0×10^{-3}	4	3	NT
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	1	1	1
	1.0×10^{-9}	1	1	1
	1.0×10^{-10}	NT	NT	1

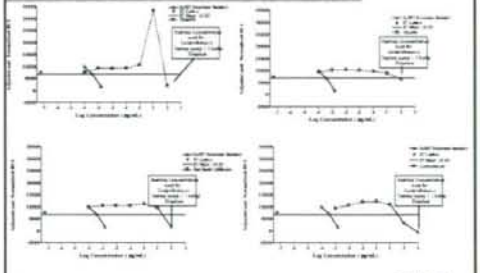
NT = not tested

Antagonist Range Finder Concentrations Tested and Cell Viability (GEN and RES)

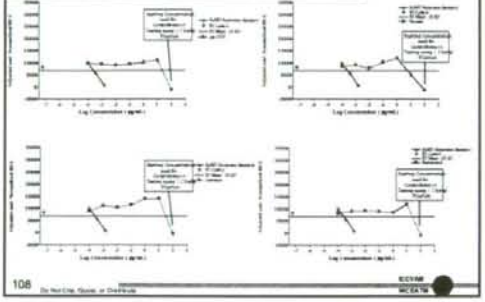
Substance Name	Concentration (log[M/L])	XDS Cell Viability	ECVAM Cell Viability	Hyperion Cell Viability
GEN	1.0×10^{-3}	3	1	NT
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	1	1	1
	1.0×10^{-9}	1	1	1
	1.0×10^{-10}	NT	NT	1
RES	1.0×10^{-3}	4	1	1
	1.0×10^{-4}	1	1	1
	1.0×10^{-5}	1	1	1
	1.0×10^{-6}	1	1	1
	1.0×10^{-7}	1	1	1
	1.0×10^{-8}	1	1	1
	1.0×10^{-9}	NT	1	1

NT = not tested

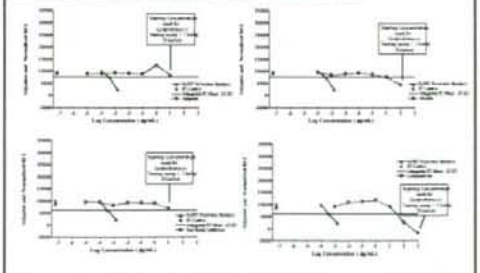
XDS Antagonist Range Finder Results (1)



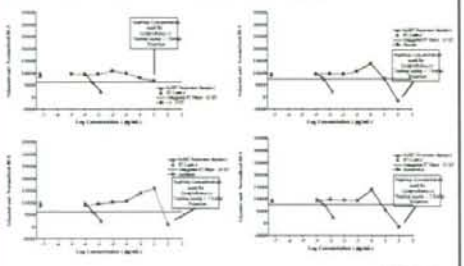
XDS Antagonist Range Finder Results (2)



ECVAM Antagonist Range Finder Results (1)



ECVAM Antagonist Range Finder Results (2)



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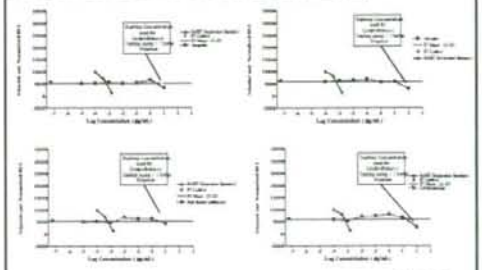
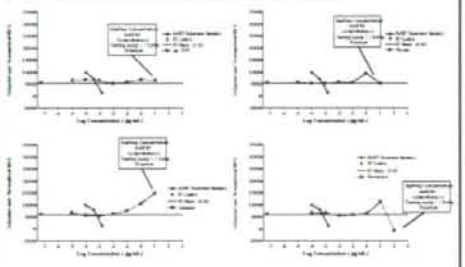
Hiyoshi Antagonist Range Finder Results (1)



111

Hiyoshi Antagonist Range Finder Results (2)



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

Antagonist Comprehensive Testing

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Overview of Phase IIb Comprehensive Antagonist Testing

- Based on starting concentrations determined by range finder testing, at least three successful independent comprehensive agonist tests were conducted for API, ATZ, BBP, CORT, DDT, FLA, GEN, and RES.
- Results from the successful testing of reference standard, controls, and the eight antagonist substances were evaluated for intra- and inter-laboratory reproducibility.
- Test results for coded substances were evaluated for concordance with antagonist activities from the ICCVAM meta-data
- Test plate failure rates were determined

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Acceptance Criteria for Phase IIb Antagonist Testing

- Acceptance or rejection of antagonist tests conducted in Phase IIb was based on evaluation of test plate reference standard and control results. Results were compared to acceptance criteria derived from the historical databases established from Phase I and IIa testing at each laboratory. Antagonist test plate acceptance criteria used in Phase IIb are summarized as follows:
  - Plate reduction, the averaged highest Ral/E2 reference standard RLU value divided by the averaged lowest Ral/E2 reference standard value (comprehensive testing only) must be greater than three-fold.
  - DMSO control RLU values must be within 2.5 times the of the historical DMSO control value range finder and comprehensive testing)
  - E2 control RLU values must be within 2.5 times of the historical E2 control value (comprehensive testing only).
  - The Ral/E2 reference standard concentration-response curve should be sigmoidal in shape and have at least three values within the linear portion of the concentration-response curve.
  - Flavone/E2 control RLU values must less than the E2 control mean minus three times the standard deviation from the E2 control mean.

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

Intra- and Inter-laboratory Reproducibility of Antagonist Reference Standard and Controls

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Intralaboratory Reproducibility of Antagonist Reference Standards and Controls

- Intralaboratory reproducibility of the RLU values associated with the DMSO control wells, the fold-reduction of Ral/E2 at its maximum response, and the adjusted and normalized RLU values associated with the E2 control wells were statistically analyzed.
  - Means and standard deviations of reference standard and control values from each laboratory were evaluated and graphed
  - Linear regression analyses were conducted to assess intralaboratory reproducibility of reference standard and control results over time for each laboratory
- If a significant p-value was obtained for the ANOVA, a Newman-Keuls post-test was used to test for significant differences in reference standard and control values between pairs of laboratories.

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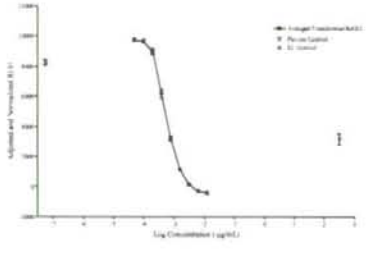
Test Plate Results for Reference Standard from Antagonist Testing at XDS



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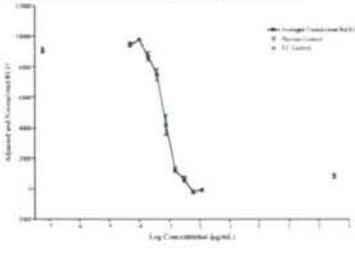
Test Plate Results for Reference Standard from Antagonist Testing at ECVAM



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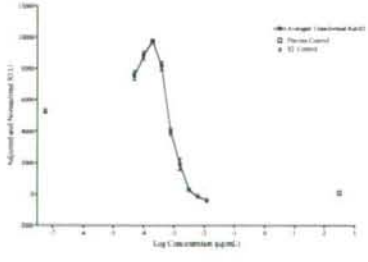
Test Plate Results for Reference Standard from Antagonist Testing Hiyoshi



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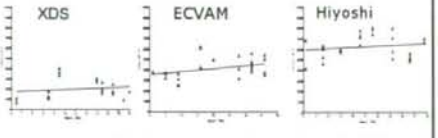
DMSO Linear Regression Analysis



	N¹	Intercept²	Slope	P-value (slope)³
XDS	25	1700	22	0.47
ECVAM	24	3600	30	0.07
HIYOSHI	26	6000	12	0.42

¹Number of plates tested (combined agonist and antagonist plates)
²Intercept values are reported as unadjusted relative light units
³Statistically significant from zero at p<0.05

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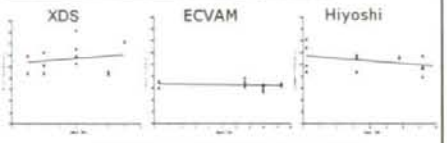
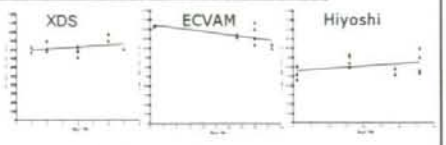
Ral/E2 Fold-Reduction Linear Regression Analysis



	N°	Intercept¹	Slope	P-value (slope)²·³
XDS	12	10.8	0.23	0.848
ECVAM	12	6.8	-0.01	0.654
HIYOSHI	14	12.0	-0.04	0.196

°Number of plates tested
¹Intercept values are reported as fold-reduction
²Statistically significant from zero at p<0.05

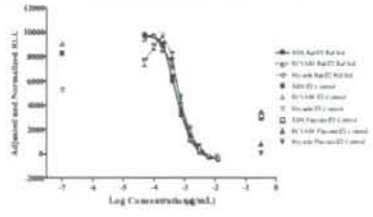
E2 Control Linear Regression Analysis



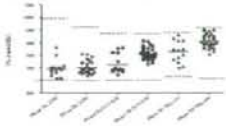
	N°	Intercept¹	Slope	P-value (slope)²·³
XDS	12	7844	116	0.296
ECVAM	12	10510	-58	0.031
HIYOSHI	14	5600	26	0.166

°Number of plates tested
¹Intercept values are reported as relative light units
²Statistically significant from zero at p<0.05
³Values in italics have p values that are less than 0.05

Comparison of Antagonist Reference Standard and Controls



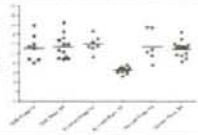
Interlaboratory Comparison of DMSO Control



Data points represent DMSO control RLU values from agonist and antagonist plates tested in Phase IIb. Solid horizontal lines represent the mean DMSO control RLU value for each data set. Dashed lines indicate the mean DMSO control RLU historical database value plus and minus 2.5 times the standard deviation from the mean. DMSO control RLU values cannot be less than zero.

	Plates Tested		Mean (RLU)		SD (RLU)		CV	
	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb
XDS	12	25	2261	2000	1646	1025	73%	51%
ECVAM	10	24	2820	4197	1835	1007	63%	24%
Hiyoshi	13	27	4446	8272	1769	1095	40%	17%

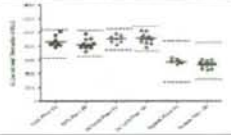
Interlaboratory Comparison of Ral/E2 Fold-Reduction



Data points represent Ral/E2 fold-reduction values from plates tested in Phase IIb. Solid horizontal lines represent the Ral/E2 fold-reduction value for each data set. Plates are rejected if the fold-reduction for the maximum Ral/E2 response is less than three.

	Plates Tested		Mean (RLU)		SD (RLU)		CV	
	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb
XDS	6	12	11.1	11.4	2.68	2.44	24%	21%
ECVAM	7	12	12.1	8.57	1.67	0.84	14%	10%
Hiyoshi	6	14	11.4	10.9	3.22	1.83	28%	16%

Interlaboratory Comparison of E2 Control



Data points represent E2 control values in adjusted RLU from plates tested in Phase IIb. Solid horizontal lines represent the mean E2 control value for each data set. Dashed lines indicate the mean E2 control historical database value plus and minus 2.5 times the standard deviation from the mean.

	Plates Tested		Mean (RLU)		SD (RLU)		CV	
	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb	Ph. IIa	Ph. IIb
XDS	3	12	8264	8259	783	711	9%	9%
ECVAM	7	12	8881	9175	554	726	6%	8%
Hiyoshi	6	14	5728	5270	347	478	6%	9%