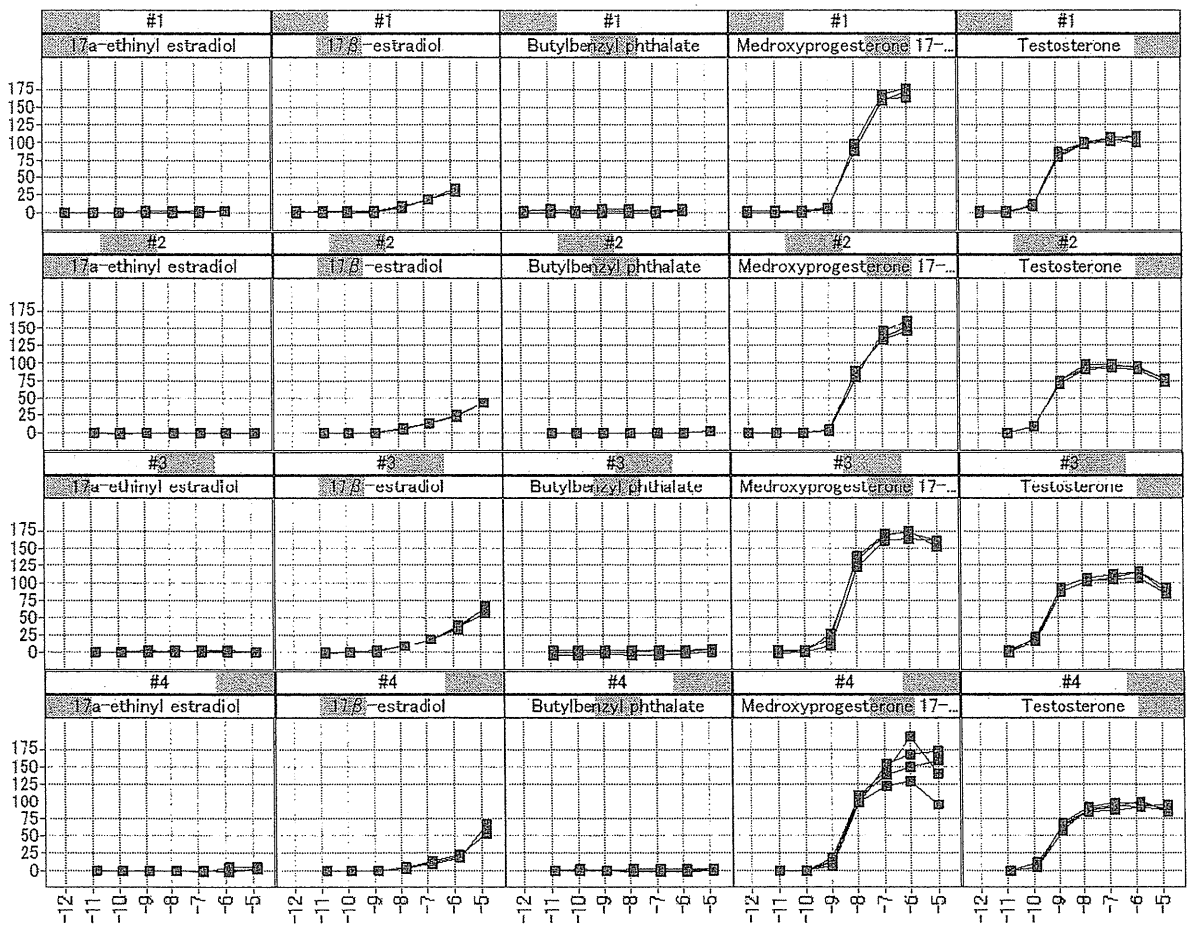

図 2　AR STTA バリデーションフェーズ 2 におけるアゴニストアッセイによるコード化合物測定結果の施設間比較

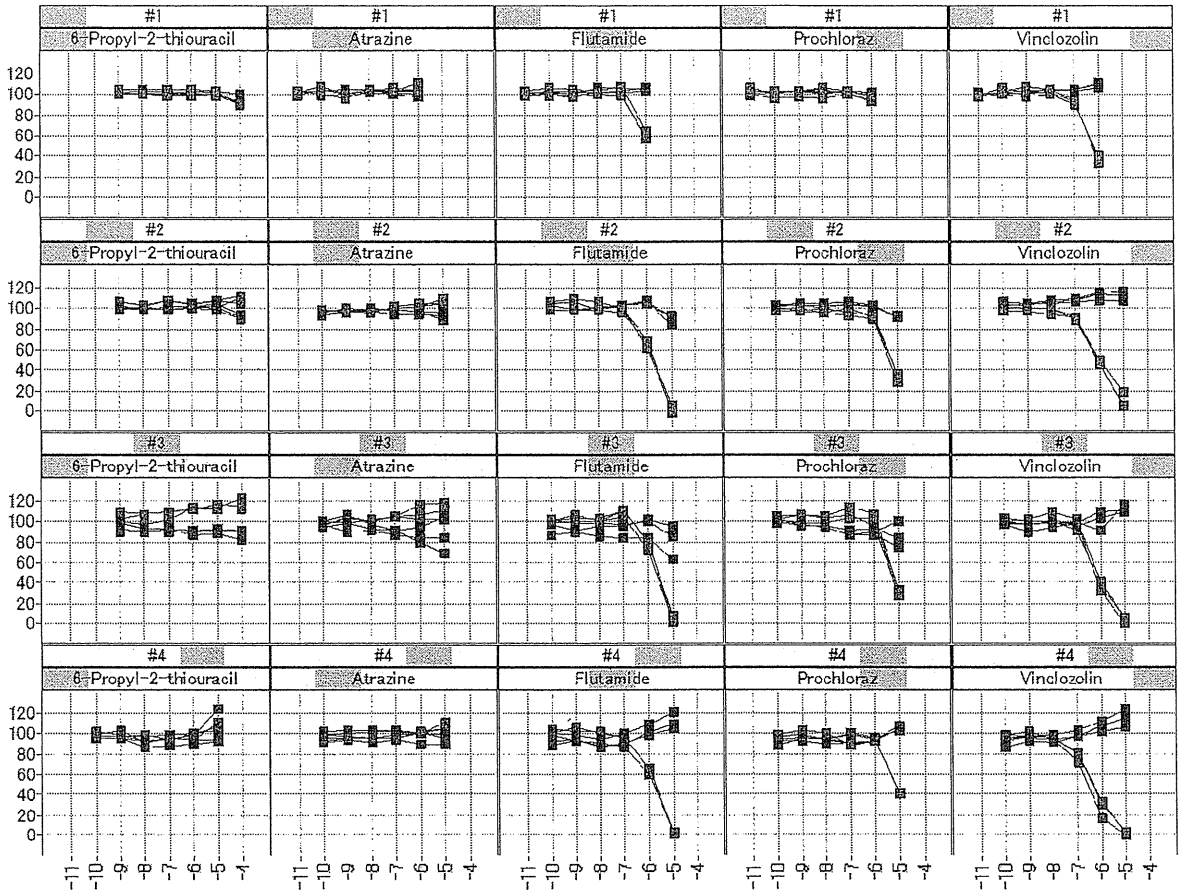

図 3　AR STTA バリデーションフェーズ 2 におけるアンタゴニストアッセイによるコード化合物測定結果の施設間比較

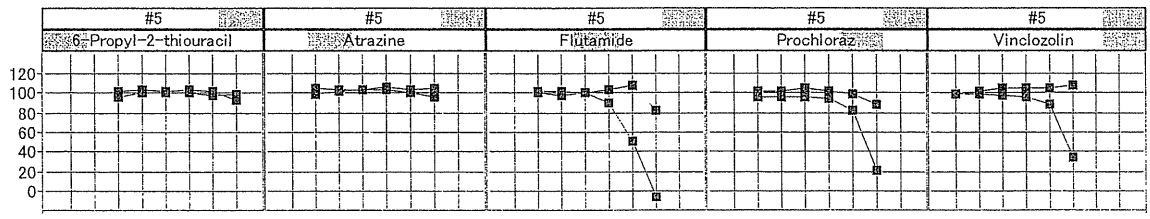

図 4　図 3 の施設#1 における、高濃度での追加測定結果

Addendum

2nd Validation Study For Androgen Receptor (AR) Mediated Stably Transfected Transcriptional Activation (AR-STTA) Assay to Detect Androgenic and Anti-androgenic Activities: AR EcoScreen™

(Version 141127)

Prepared by

Study management team of the 2nd validation study of AR STTA

2014

Index

1. SUMMARY

1. The AR STTA assay (AR EcoScreen^{TM}) is a trans-activation assay based on Chinese Hamster Ovary cells stably transfected with human AR and an AR response element fused to a luciferase reporter gene. This assay is designed to screen for substances that may induce (agonist) or inhibit (antagonist) AR-mediated transcription.

2. The validation report of this assay system has been submitted to OECD in 2010. However the Peer review panel report stated that a dedicated inter-laboratory study should be carried out, using the final test protocol to test substances covering a broad range of activity, especially including non-active substances and weak agonists and antagonists. This was an additional inter-laboratory validation study corresponding to the major Peer review comment for the validation report.

3. The additional validation study consisted of Phase-1 and Phase-2 studies. The Phase-1 study was to confirm the overall laboratory proficiency by testing the same lots of reference chemicals and to collect data to set a reference criteria for mestanolone which was the newly added reference chemical for the agonist study. The Phase-2 study was to provide the supplemental data according to previous Peer review comments of this assay and to evaluate the assay performance (within/between-laboratory reproducibility and predictive capacity) by testing 10 coded chemicals (each five for agonist and antagonist).

4. In the Phase-1 study, all laboratories passed the reference criteria within the minimum three runs. The inexperienced Korean laboratory yielded successful results for the additional reference chemical for the agonist assay, Mestanolone that met the tentative reference criteria decided based on the results obtained with three Japanese laboratories. In the in the Phase-2 agonist study, all laboratories yielded correct positive/negative outcomes corresponding to the candidate effects. Consequently, the Accuracy, Sensitivity and Specificity of the agonist assay were all calculated to be 100% in all laboratories. In addition, the CV% of LogPC10(M) and LogPC50(M) for positive chemicals were less than 5% and high reproducibility of this assay was confirmed.

5. In the Phase-2 antagonist study, the Accuracy, Sensitivity and Specificity for all four laboratories were calculated to be 95%, 92% and 100%, respectively, because of the false negative response in one chemical in one laboratory..

6. However the cause of the false negative response for the chemical was confirmed to be a dose-selection issue rather than a technical issue. In addition, the CV% of LogIC30(M) and

LogIC50(M) for positive chemicals in the additional trial were less than 4%, and high reproducibility of this assay was confirmed.

7. Therefore, the concordance of positive/negative outcomes of coded test chemicals were more than 80% for each of agonist and antagonist assay, and the high performance of this assay was confirmed.

8. The results of the additional validation study show that the original protocol is well established and robust, however the maximum dose selected by the solubility test described in the original protocol may occasionally affect the sensitivity of the assay. Therefore the following sentence should be including in the section of solubility test in the guideline.

9. "This solubility test is very important step to determine the maximum dose for the assay and it may affect the sensitivity of the assay. The highest concentration should be selected based on the cell viability rather than the avoidance of some precipitation in higher dose range. "

2. INTRODUCTION

10. Numerous chemicals found in the environment, as well as some synthetic chemicals may disrupt the endocrine functions of wildlife and humans. At the present time, there is a global concern regarding endocrine disruption effects resulting from chemical exposure, particularly those mediated by the estrogen receptor (ER) and androgen receptor (AR). To ensure the safety of chemicals, an effective procedure for screening chemicals for endocrine modulating activity has been pursued by regulatory agencies in several countries, including the United States Environment Protection Agency (US-EPA), Japan and Europe. The EDSTAC recommended that in vitro assays, such as receptor binding and reporter gene assays, be used to screen chemicals for hormone receptor agonist and antagonist activity as part of a tier 1 screening battery, then many efforts have been taken to develop reporter gene assay systems for evaluating ER and AR mediated effects of chemicals.

11. Several reporter gene assay systems are currently at, or will soon begin validation at national, European and international levels, but are not yet close to completion and full assessment of their validation status. Currently, "Stably Transfected Transcriptional Activation (TA) using HeLa-9903 cell line for detecting estrogenic activity of chemicals" has been adopted as OECD test guideline (TG 455) in 2009. Although the need for AR in vitro assays are also urgent, at the present time there are no in vitro screening assays for androgenic activity that have been peer reviewed for potential test guideline development, to enable use for OECD regulatory purposes.

12. We have developed the reporter gene assay system using the AR EcoScreen cell and compiled a validation report based on results from the pre-validation study with 40 chemicals and the inter-laboratory validation study performed with the four participating laboratories using the same 5 chemicals for both androgenic and anti-androgenic activities.

13. The validation report was submitted to OECD in 2010. However the Peer review panel report stated that a dedicated inter-laboratory study should be carried out, using the final test protocol to test substances covering a broad range of activity, especially including non-active substances and weak agonists and antagonists.

14. According to the peer review comment, we made a plan of the additional inter-laboratory validation study. And the additional validation study was conducted with four participating laboratories in 2013-2014.

3. OBJECTIVES

15. The aim of this study was to evaluate intra-laboratory repeatability and intra- and inter-laboratory reproducibility of Androgen Receptor (AR) EcoScreen protocol using additional chemicals according to the OECD peer review comments for the previously conducted 1st validation study.

4. VALIDATION DESIGN

16. The validation study for the stably transfected TA assay using AR-EcoScreen™ cell line to detect androgenic/anti-androgenic activities consisted of the Phase-1 and Phase-2 studies. Prior to starting the validation study, each laboratory conducted the proficiency test following the technical training.

ORGANIZATION

Schematic drawing of the organization for the additional validation was shown in Fig. 1.

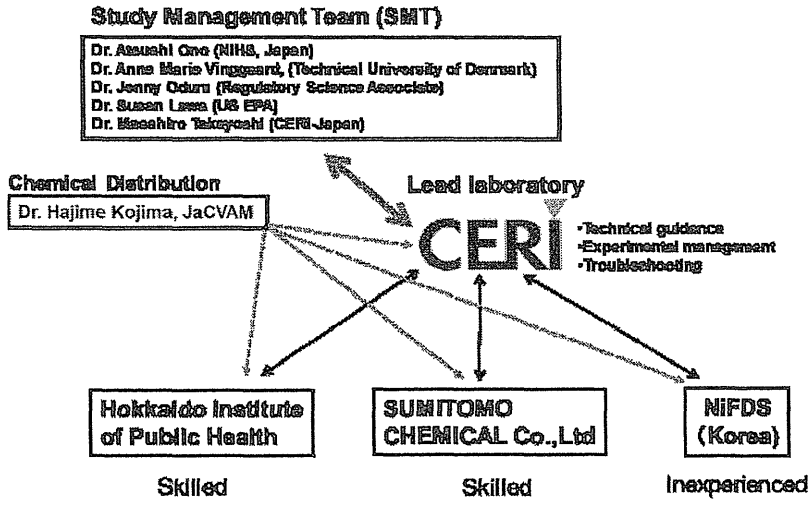

Fig. 1 Schematic drawing of the study organization

Sponsor

Ministry of Economy, Trade and Industory, Japan.

Supporters

Ministry of Health, Labour and Welfare, Japan.
National Institute of Health Sciences (NIHS)
Japanese Center for the Validation of Alternative Methods (JaCVAM)

Participating laboratories

The validation study is conducted by four participating laboratories as follows;

- Chemicals Evaluation and Research Institute (CERI, Lead Laboratory)
- Environmental Health Science Laboratory of Sumitomo Chemical Co. Ltd,
- Hokkaido Institute of Public Health
- National Institute of Food and Drug Safety Evaluation, Korea (NIFDS)

17. The lead laboratory representing the test method was responsible for providing the test method protocol and the necessary assay datasheets (MSExcel format) and worksheets (MSword format), etc. The lead laboratory was also responsible for providing, if necessary, new versions of the protocols during the entire validation trail. The lead laboratory and the other participating laboratory were contacted by the Project Coordinator for technical issues.

The Study management team (SMT)

SMT was organized with following members to support the validation process;

Dr. Atsushi Ono (NIHS, Japan)	Project Coordinator Quality assurance
Dr. Masahiro Takeyoshi (CERI-Japan)	Expertise of this assay Quality assurance
Dr. Anne Marie Vinggaard (Technical University of Denmark) Dr. Jenny Odum (Regulatory Science Associate) Dr. Susan Laws (US EPA)	Validation study expertise

* See Appendix 1 for detailed contact address

Chemical Distribution Management

Dr. Hajime Kojima (JaCVAM, Japan)

CHEMICALS AND OTHER MATERIALS

18. Reference chemicals and test chemicals were shipped according to proper regulatory procedures. Each participating laboratory was notified by Chemical Distribution Management when any reference chemicals, and test chemicals were shipped. Upon receipt, chemicals were stored in appropriate storage conditions as per recommendations provided by Chemical Distribution Management. Each participating laboratory notified the SMT Project Coordinator upon receipt.

19. The information with regard to the lot of serum and the list of the other materials used in the validation were announced by CERI prior to the start of validation study, and all laboratories obtained the same products and used for study with a very few exceptions, namely the dimethyl sulfoxide used as a vehicle by CERI in Phase-2 study.

Reference chemicals and vehicle

20. Reference chemicals and vehicle used in the validation study (Table 1) were distributed from Distribution Management (JaCVAM) prior to the start of Phase-1 study to Japanese participant laboratory and prior to the start of Phase-2 to NiFDS. Japanese participant laboratory conducted Phase-1 and 2 studies using distributed chemicals. NIFDS conducted Phase-1 study using chemicals obtaining locally (Korea) and conducted Phase-2 study using distributed chemicals. Solvent (DMSO, CAS: 67-68-5) was obtained from Sigma as product code of D8418, and the lot No. of DMSO used in the study was SHBB3758V excluding the Phase-2 study of CERI in which the lot No was SHBC3313V.

Table 1-1 List of reference chemicals used in the validation study

Chemical Name	CAS	MW	maker	Code	Lot No.
5α-Dehydrotestosterone	521-18-6	290.44	TCI	A0462	JN01
Mestanolone	521-11-9	304.47	APIN	27879m	212259
Di(2-ethylhexyl)phthalate	117-81-7	390.56	sigma	67261	BCBG7259V
Hydroxyflutamide	52806-53-8	292.21	LKT lab	H9718	26801402
Bisphenol A	80-05-7	228.29	sigma	239658	MKBF3852V

Table 1-2 Vehicle used in the validation study

Chemical name	CAS	MW	maker	Code	Lot Code
Dimethyl sulfoxide	67-68-5	78.13	sigma	D8418	SHBB3758V SHBC3313V *

* Product used for Antagonist assay in CERI Phase-2.

Test chemicals

21. Test chemicals have been selected based on the suggestion from the voluntary chemical selection team of OECD validation management group of non-animal (VMG-NA) by considering the following criteria.

+ ICCVAM recommendations: ICCVAM Evaluation of In Vitro Test Methods for Detecting Potential Endocrine Disruptors: Estrogen and Androgen Receptor Binding and Transcriptional Activation Assays
+ appropriate negative and positive effects on published AR Ecoscreen™ assay results
+ historical data of lead laboratory
+ availability
+ costs

22. Coded test chemicals (Table 2) were packaged so as to conceal their identities and shipped prior to starting Phase-2. Coded test chemicals, along with a sealed health and safety information package were shipped to the designated Safety Officer. The Safety Officer retained the safety information package and passed the coded test chemicals to the Study Director. The safety information package contained necessary information about the substance hazards and provided instructions for emergency actions. A disclosure key for identifying the test chemicals by code was also included in the package. Consequently, there was no occasion to open the safety information package in any participant laboratories.

Table 2 List of chemicals used in Phase-2 study

Chemical Name	CAS	MW	maker	Code	Lot No.	Expected result
Testosterone	58-22-0	288.42	sigma	46923	SZBA235XV	Ago /P
17β-estradiol	50-28-2	272.39	sigma	E8875	SLBC5955V	Ago /P
Medroxyprogesterone 17-acetate	71-58-9	386.53	sigma	46412	SZB8248XV	Ago /P
17α-ethinyl estradiol	57-63-6	296.41	sigma	E4876	071M1429V	Ago /N
Butylbenzyl phthalate	85-68-7	312.36	aldrich	308501	MKBH8959V	Ago /N
Flutamide	13311-84-7	276.21	sigma	F9397	SLBC6159V	Ant/P
Prochloraz	67747-09-5	376.67	sigma	45631	SZBA112XV	Ant/P
Vinclozolin	50471-44-8	286.11	sigma	45705	SZB7292XV	Ant/P
Atrazine	1912-24-9	215.69	sigma	45330	SZB8175XV	Ant/N
6-Propyl-2-thiouracil	51-52-5	170.23	sigma	P3755	BCBG1817V	Ant/N

Ago /P: Positive in agonist assay, Ago /N: Negative in agonist assay, Ant/P: Positive in antagonist assay, Ant/N: Negative in antagonist assay

Test chemical supply and allocation

23. Chemicals used in Phase-2 study were assigned according to the following Table 4;

Table 4 Chemical code and allocation of chemicals used in Phase-2 study

Chemical name	CERI	Sumitomo	Hokkaido	NiFDS
17β-estradiol	ARA31	ARA01	ARA16	ARA46
17α-ethinyl estradiol	ARA32	ARA02	ARA17	ARA47
Testosterone	ARA33	ARA03	ARA18	ARA48
Medroxyprogesterone 17-acetate	ARA34	ARA04	ARA19	ARA49
Butylbenzyl phthalate	ARA35	ARA05	ARA20	ARA50
Flutamide	ART36	ART06	ART21	ART51
Atrazine	ART37	ART07	ART22	ART52
Vinclozolin	ART38	ART08	ART23	ART53
Prochloraz	ART39	ART09	ART24	ART54
6-Propyl-2-thiouracil	ART40	ART10	ART25	ART55

5. PROTOCOL

24. In this validation study, the same protocol was used (ANNEX 1) in all laboratories. The draft protocol was written by the lead laboratory and was finalized by SMT.

25. The summary of the protocol is shown in Table 4.

Table 4 Summary of the AR STTA antagonist protocol

<table>
<tr><th>Study phase</th><th>Purpose</th><th>Procedures in brief</th></tr>
<tr><td rowspan="2">Proficiency test</td><td>a) Edge effects confirmation at each participating laboratory</td><td>a) Edge effects
(1) Expose 1nM 5α-Dehydrotestosterone (DHT) to all wells in a 96-well plate
(2) Check if the value of coefficient of variation (CV) value among all wells of luminescence intensity is less than 10%.
If yes, no edge effects are expected and all wells of 96-well plate can be used.
If no, edge effects are expected and the wells on the edge should not be used for further evaluation.</td></tr>
<tr><td>b) Confirmation of the technical transfer status at each participating laboratory by testing same stock of minimal reference chemicals used in the technical transfer meeting.</td><td>b) the technical transfer status
test the minimal reference chemicals (5α-Dehydrotestosterone and Di(2-ethylhexyl)phthalate (DEHP) for agonist assay, Hydroxyflutamide and DEHP for antagonist assay) used in the technical transfer meeting.</td></tr>
<tr><td>Phase-1</td><td>Confirm the overall laboratory proficiency by testing same lots of reference chemicals and to collect data to set a reference criteria for mestanolone.</td><td>Test "AR agonist, antagonist and negative chemicals"
<table>
<tr><th>Assay</th><th>Chemical Name</th><th>Expected effect</th></tr>
<tr><td rowspan="3">Agonist</td><td>5α-Dehydrotestosterone</td><td>Positive</td></tr>
<tr><td>Mestanolone</td><td>Positive</td></tr>
<tr><td>Di(2-ethylhexyl)phthalate</td><td>Negative</td></tr>
<tr><td rowspan="3">Antagonist</td><td>Hydroxyflutamide</td><td>Positive</td></tr>
<tr><td>Bisphenol A</td><td>Positive</td></tr>
<tr><td>Di(2-ethylhexyl)phthalate</td><td>Negative</td></tr>
</table></td></tr>
</table>

		In addition, data from the KFDA will be used to confirm the validity of performance criteria.
Phase-2	Test coded chemicals	Test the agonist and antagonist activities of coded 10 chemicals

6. VALIDATION STUDY PROCESS

Technical transfer meeting

26. Prior to starting the validation study, the technical transfer meeting was held at CERI for domestic two laboratories from October 9 to October 11, 2013.

27. The NIFDS staff had been technically trained in the technical transfer meeting held in CERI from October 16 to October 18, 2012.

Edge effect check

28. Edge effect check was conducted in all participating laboratories. Edge effect was checked by an assay plate which was uniformly seeded $9x10^3$ cell/well with 10nM Dihydrotestosterone (DHT). If the case that both CV% of RLU values among all wellsmeasured 24 h after stimulation were less the 10%, the edge effect was decided as negligible.

29. The results of edge effect check were given in Table 5. The CV% of RLU values among all well were less than 10% in all laboratories, therefore the edge effects were decided to be negligible.

Table 5 Results of edge effect test in each laboratory

	CERI	Sumitomo	Hokkaido	NiFDS		
				trial 1	trial 2	trial 3
AVG	3117.8	204832.8	4637.6	302467.5	287767.0	297763.7
SD	101.2	14081.0	195.0	18273.7	23220.0	20411.3
CV(%)	3.2	6.9	4.2	6.0	8.1	6.9

Proficiency test

30. The aim of the proficiency test was to confirm the technical transfer status at each participating laboratory by testing same stock of minimal reference chemicals used in the technical transfer meeting.

31. The proficiency test was absolved for the NIFDS, because the NIFDS staff had been technically trained in the technical transfer meeting held in CERI in 2012, and their proficiency was confirmed by the data submitted to CERI.

32. In the proficiency test, each laboratory, excluding NIFDS, tested the minimal reference chemicals (DHT and Di(2-ethylhexyl)phthalate (DEHP) for agonist assay, Hydroxyflutamide (HF) and DEHP for antagonist assay) used in the technical transfer meeting in their own laboratories with same plate assignment as in the technical transfer meeting.

33. In the event that at least one run of assay results met the performance criteria shown in Table 6, the laboratory was permitted to start the Phase-1 study.

Table 6-1 Performance criteria for reference chemicals in AR agonist assay

Fold-induction	>= 6.4		
PC10 value	Greater than 1 (fold-induction of VC) +2SD		
Chemical Name [CAS No.]	logPC10	logPC50	Test range
5α-Dehydrotestosterone (DHT) [521-18-6]	-9.87 ~-12.08	-9.00 ~ -11.03	$10^{-6} \sim 10^{-12}$M
Mestanolone [521-11-9]	to be confirmed	to be confirmed	$10^{-6} \sim 10^{-12}$M
Di(2-ethylhexyl)phthalate (DEHP) [117-81-7]	-	-	$10^{-5} \sim 10^{-10}$M

Table 6-2 Performance criteria for reference chemicals for AR antagonist assay

Fold induction of spike-in [Spike-in of 500 pM DHT]/[Vehicle Control]	>= 5.0		
PC_{ATG} inhibitory ratio	= <0.46		
Chemical Name [CAS No.]	log linearIC30	Log linearIC50	Test range
Hydroxyflutamide (HF) [52806-53-8]	-6.41 ~ -8.37	-6.17 ~ -7.80	$10^{-5} \sim 10^{-10}$M
Bisphenol A (BisA) [80-05-7]	-4.48 ~ -7.52	-4.29 ~ -7.05	$10^{-5} \sim 10^{-10}$M
Di(2-ethylhexyl)phthalate (DEHP) [117-81-7]	-	-	$10^{-5} \sim 10^{-10}$M

34. Results of the Proficiency test for agonist and antagonist assays were shown in Table 7. All results obtained in three domestic laboratories met the requirements for this test, and all passed the performance criteria.

Table 7-1 Results of the Proficiency test for agonist assay

		CERI		Sumitomo		Hokkaido	
		Result	Decision	Result	Decision	Result	Decision
Fold Induction		8.906	Pass	6.84	Pass	7.35	Pass
FI VC_Mean + 2SD		1.09	Pass	1.19	Pass	1.07	Pass
FI PC10		1.79		1.58		1.64	
DHT	log[PC10]	-10.71	Pass	-10.57	Pass	-10.85	Pass
	log[PC50]	-9.73	Pass	-9.41	Pass	-10.21	Pass
DEHP	log[PC10]	-		-		-	
	log[PC50]	-		-		-	

Table 7-2 Results of the Proficiency test for antagonist assay

		CERI		Sumitomo		Hokkaido	
		Result	Decision	Result	Decision	Result	Decision
Fold Induction		6.823	Pass	5.314	Pass	8.139	Pass
RTA of 10 nM DHT		123.57		137.57		122.52	
RTA of 0.1μM HF		3.32	Pass	4.24	Pass	7.33	Pass
RTA of 10 μg/mL CHX		-1.79		-5.07		-2.56	
HF	log[lin.IC30]	-7.36	Pass	-7.88	Pass	-7.18	Pass
	log[lin.IC50]	-6.95	Pass	-7.41	Pass	-6.77	Pass
DEHP	log[lin.IC30]	-		-		-	
	log[lin.IC50]	-		-		-	

Phase-1 study

35. The aims of the Phase-1 study were to confirm the overall laboratory proficiency by testing same lots of reference chemicals and to collect data to set a reference criteria for mestanolone. In addition, data from the NIFDS was used to confirm the validity of performance criteria.

36. In the Phase-1 study, the reference chemicals listed in Table 1-1 and Table 1-2 of the protocol were provided by Chemical Distribution Management, excluding NIFDS where the same lots of chemicals were obtained from their local distributors. Then each laboratory tested the Phase-1 chemicals according to the assay protocol at least three runs in triplicate.

37. The assay results were stored and locked in the Specified work sheet provided by CERI. Then

each laboratory submitted at least 3 sets of assay results meeting the all performance criteria shown in the assay protocol, to the Project Coordinator.

Agonist assay

38. The results of Phase-1 study for agonist assay in Japanese three laboratories were summarized in Table 8.

39. All FI (Fold induction) values for positive control in Japanese laboratories were over 7.40, LogPC10(M) and LogPC50(M) values for DHT were within the range required in performance criteria.

Table 8 Results of the Phase-1 study for agonist assay in Japanese laboratories

		FI	FI VC mean + 2SD	FI of PC10	DHT (Log PC10) (M)	DHT (Log PC50) (M)	Mestanolone (Log PC10) (M)	Mestanolone (Log PC50) (M)
CERI	1	8.38	1.12	1.74	-10.76	-9.81	-10.65	-9.62
	2	8.64	1.08	1.76	-10.66	-9.70	-10.56	-9.59
	3	8.68	1.14	1.77	-10.71	-9.75	-10.64	-9.65
Sumitomo	1	7.67	1.10	1.67	-10.64	-9.59	-10.47	-9.43
	2	7.35	1.08	1.64	-10.77	-9.82	-10.66	-9.60
	3	8.14	1.12	1.71	-10.69	-9.67	-10.57	-9.53
Hokkaido	1	7.71	1.07	1.67	-10.83	-10.10	-10.79	-9.87
	2	7.84	1.08	1.68	-10.83	-10.08	-10.81	-10.00
	3	7.40	1.08	1.64	-10.83	-10.11	-10.84	-10.08
For 3 labs.				MEAN	-10.75	-9.85	-10.67	-9.71
				SD	0.07	0.20	0.13	0.22
				MEAN+2SD	-10.60	-9.45	**-10.41**	**-9.26**
				MEAN-2SD	-10.89	-10.25	**-10.92**	**-10.15**

40. In addition, the results of LogPC10(M) and LogPC50(M) values for mestanolone in three Japanese laboratories passed the requirement of the performance criteria in three of three trials.

41. The aim of the Phase-1 study was to set the primary reference criteria for mestanolone as the mean logPCx±2SD with the data obtained in three Japanese laboratories. The ranges were calculated as -10.41~-10.92 for LogPC10(M) and -9.26~-10.15 for LogPC50(M), respectively.

42. The results of Phase-1study for agonist assay in NiFDS were shown in Table 9.

Table 9 Results of the Phase-1 study for agonist assay in NiFDS

		FI	FI VC mean + 2SD	FI of PC10	DHT (Log PC10) (M)	DHT (Log PC50) (M)	Mestanolone (Log PC10) (M)	Mestanolone (Log PC50) (M)
NiFDS	1	7.44	1.07	1.64	-10.82	-9.75	-10.69	-9.56
	2	6.91	1.05	1.59	-10.79	-9.80	-10.70	-9.60
	3	6.94	1.04	1.59	-10.64	-9.50	-10.49	-9.35

43. The results in NiFDS passed all the requirements of the performance criteria including mestanolone in three of three trials.

44. The PC values of mestanolone (mean logPCx±2SD) including the data from NiFDS were almost the same as the range calculated from the data of the three Japanese laboratories. The Project coordinator therefore decided that the performance criteria for Mestanolone in Phase-2 study should be as shown in Table 10.

Table 10 Performance criteria for reference chemicals in AR agonist assay for Phase-2 study

Fold-induction	**> = 6.4**		
PC10 value	Greater than 1 (fold-induction of VC) +2SD		
Chemical Name [CAS No.]	logPC10	logPC50	Test range
5α-Dehydrotestosterone (DHT) [521-18-6]	-9.87 ~-12.08	-9.00 ~ -11.03	$10^{-6} \sim 10^{-12}$M
Mestanolone [521-11-9]	**-10.41~-10.92**	**-9.26~-10.15**	$10^{-6} \sim 10^{-12}$M
Di(2-ethylhexyl)phthalate (DEHP) [117-81-7]	-	-	$10^{-5} \sim 10^{-10}$M

Antagonist assay

45. All Labs conducted three trials (Table 11). The fold induction of 2nd trial of NiFDS of Korea was 4.95. This value was lower than the performance criterion (>=5.0). However, the deviation was slight and all other performance criteria were met. Thus, the deviation was negligible and the result was judged as acceptable.

46. Consequently, all laboratories passed all of criteria in three of three trials, and all four laboratories passed the Phase-1 study.

Table 11 Results of the Phase-1 study for antagonist assay

		FI*	RTA 0.1uM HF	HF (logIC30) (M)	HF (logIC50) (M)	BisA (logIC30) (M)	BisA (logIC50) (M)
CERI	1	7.07	3.91	-7.36	-6.92	-5.76	-5.47
	2	7.29	2.81	-7.44	-6.99	-5.88	-5.56
	3	7.43	3.99	-7.41	-6.97	-5.78	-5.49
Sumitomo	1	5.44	4.02	-7.55	-7.10	-5.92	-5.58
	2	5.54	6.97	-7.28	-6.82	-5.74	-5.40
	3	6.00	2.09	-7.63	-7.19	-5.88	-5.56
Hokkaido	1	6.91	7.19	-6.93	-6.62	-5.53	-5.21
	2	6.56	4.39	-7.10	-6.72	-5.71	-5.42
	3	7.19	4.85	-7.17	-6.76	-5.61	-5.31
NiFDS	1	5.49	6.32	-7.59	-7.14	-6.00	-5.58
	2	4.95*	5.46	-7.78	-7.49	-6.13	-5.76
	3	5.05	7.24	-7.83	-7.40	-6.29	-5.74
		MEAN		-7.42	-7.01	-5.85	-5.51
		SD		0.27	0.27	0.21	0.16
		MEAN+2SD		-6.88	-6.48	-5.42	-5.18
		MEAN-2SD		-7.97	-7.54	-6.28	-5.83

*Value in red letter was deviated from the acceptance criteria.